

## Effects of wheat crop density on growth of ryegrass

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### ABSTRACT

Laboratory bioassay was conducted to assess the effects of wheat (*Triticum aestivum*) crop density on weed control of annual ryegrass (*Lolium rigidum*). Increasing the density of allelopathic wheat strongly ameliorated its competitiveness and significantly inhibited the growth of annual ryegrass. The increased wheat density steadily decreased the total root length and total root surface area of ryegrass, however, the root diameter of ryegrass increased. The roots of annual ryegrass were thinner than wheat. Interactions between the wheat and annual ryegrass greatly influenced the annual ryegrass root length and diameter within 0.180-0.225 mm and within 0.225-0.270 mm. In addition, root distribution and dominance in different diameter class was wheat density dependent and uneven. The increased root diameter and decreased root length and surface area of rye grass may be due to strong allelopathic effects of wheat (significant quantity of allelochemicals were produced at middle and high densities). This might led to the suppression of annual ryegrass growth.

**Key words:** Annual ryegrass, *Lolium rigidum*, mixed planted, phenolic acids, root exudates, *Triticum aestivum*, wheat.

### INTRODUCTION

Weeds cost over \$4 billions annually to Australian farmers and have evolved the herbicide resistance that threatens environmental sustainability and the viability of crop production (13). Australia is facing severe challenges of herbicide options for effective control of certain crop weeds, hence, alternative means such as allelochemicals are urgently required. The allelochemicals produced by plants may control weeds in fields as biological herbicides (1, 6).

Wheat is major cereal crop worldwide, it has potential for weed control owing to its allelopathic impact and capability to produce and exude allelochemicals into fields (3, 25). Wheat residues significantly reduces the weed density and biomass (14). Wheat crop suppresses the growth of annual ryegrass (24) wheat extracts inhibited the seed germination and seedling growth of *Amaranthus retroflexus*, *Stellaria media* and *Digitaria ciliaris* (11). Allelochemicals such as phenolic compounds are often associated with allelopathic effects of wheat (3). Phenolic acids [*p*-hydroxybenzoic, vanillic, and *trans*-ferulic acids] are negatively related to root length of annual ryegrass (24). Wheat produces 238.4-559.7 mg Kg<sup>-1</sup> of shoot dry matter and 551.4-611.8 mg Kg<sup>-1</sup> of root dry matter

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DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), an effective allelochemical (11, 23). Generally, single allelochemical is not responsible for the allelopathy effects of wheat (5), hence, total phenolic acids may be the simple index to measure its allelopathy effects. Furthermore, allelochemicals are important in interspecies interactions (3). Allelopathic effects of rice are strengthened due to increasing total amounts of phenolic compounds produced (9). The root length of perennial ryegrass is reduced with increasing wheat density (7). In lentil/weed interactions studies, the weed suppression is more with increasing the lentil density in field conditions (12). Therefore, density-dependent crop allelopathy may have agronomic potential in integrated weed management in fields.

To better understand the mechanism of weed control, it is necessary to evaluate the root growth and morphological changes in wheat/weed interactions. One crop affects another crop through the excreted allelochemicals (20) and inhibited the radicle, coleoptile and hypocotyl growth in mixed culture condition (15). However most studies in crop/weed interactions, are focussed on root length. For example, the root growth of annual ryegrass is inhibited (35-87 %) by wheat than without wheat (24) and other root morphology parameters (root diameter, root volume) were little studied.

This study aimed to: (i) Assess the effectiveness of density-dependent crop allelopathy in weed control and (ii) Examine the associated root mechanisms in wheat-annual ryegrass interaction as a model system.

## MATERIALS AND METHODS

### I. Plant Material, Seed Sterilization and Pregermination

Wheat (*Triticum aestivum* L. cv. 'Cranbrook') and annual ryegrass (*Lolium rigidum*) were test plants in bioassay. Wheat and Annual rye grass seeds were sterilized (2.0 % sodium hypochlorite solution for 15 min and rinsed by sterilized distilled water 5 times), then soaked in sterilized distilled water for 24 h in growth chamber [25 °C and 13 h light/11 h dark]. Wheat seeds were germinated in Petri dishes (9 cm dia.) for 24 h. The rye grass seeds were germinated in Petri dishes (9 cm dia.) for 72 h in growth chamber [25 °C and 13 h light/11 h dark]. The fluorescent light intensity in the cabinet was  $3.56 \pm 0.16 \times 103$  lux.

### II. Density-Dependent Wheat Allelopathy Bioassay

Wheat and annual ryegrass seedlings with radical length 0.5 cm were transplanted into 50 mL 0.3 % agar in 600 mL beaker at the same time. The plants were planted in following combinations (Table 1). Co-grown for 14 days and then harvested. Seedlings were arranged in beaker as shown in Figure 1.

Table 1. Treatment combinations in bioassay

Density	Treatment details	Density	Treatment details
Sole Annual ryegrass	0W/10R	Density 20	20W/10R
Density 1	1W/10R	Density 50	50W/10R
Density 3	3W/10R	Density 80	80W/10R
Density 5	5W/10R	Density 110	110W/10R
Density 10	10W/10R	Density 140	140W/10R
Density 15	15W/10R		

W: Wheat, R: Annual ryegrass.

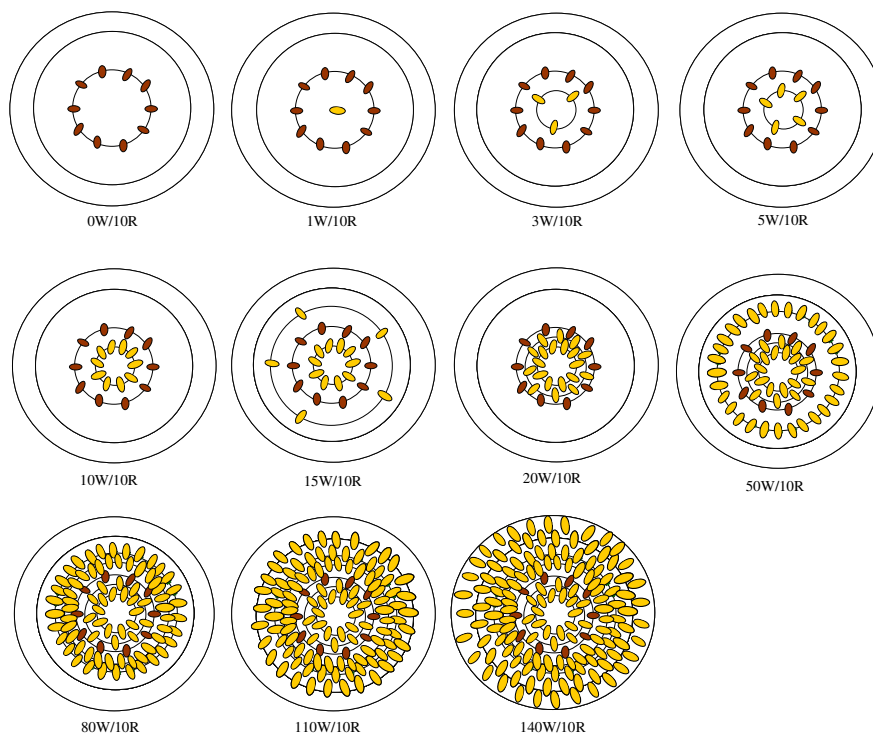




Figure 1. Seedlings arrangement in beaker. W : Wheat; R : Annual ryegrass, Numbers : wheat and annual ryegrass density in each beaker,  Wheat seedlings,  Ryegrass seedling

**Measurements:** Agar medium was collected after wheat seedlings were uprooted and mixed with 20 mL distilled water (used to wash roots to remove the residual agar on roots). All agar-water mixtures were filtered through 0.22  $\mu\text{m}$  filter paper, then 10 ml was extracted to measure the total phenolic acids by Folin-Ciocalteu colorimetric method (16).

First, the wheat and annual ryegrass plants were separated. Then plant was partitioned into root and shoot. Root morphology data (root average diameter, total root length, root volume, and root surface area) was measured by WinRhizo 2005 by root scanning. All plants samples were dried in oven 60  $^{\circ}\text{C}$  for 4-days and weighed.

Specific root length (cm/mg) was calculated as under:

$$\text{Specific Root Length (cm mg}^{-1}\text{)} = \text{Total root length (cm)} / \text{Root dry biomass (mg)}$$

**Statistical Analysis:** Data were subjected to analysis of variance using the SAS software (15). Mean values were compared by least significant difference (LSD) at 5 % level.

## RESULTS

### Wheat dry biomass

Compared with density 1, shoot dry biomass of wheat was increased by density 3 but was decreased by density 110 and density 140 (Figure 2A). Shoot dry biomass of wheat in density 5, density 10, density 15, density 20, density 50 and density 80 remained similar to density 1. Root dry biomass of wheat in density 3 was maximum and was minimum in density 110 and density 140. Compared with density 1, root dry biomass of wheat in density 5, density 10, density 15, density 20, density 50 and density 80 was steadily decreased with increasing wheat density.

### Annual ryegrass Inhibition (%)

Inhibition of annual ryegrass shoot was greatest in density 10 (173 %) and lowest in density 140 (25.5 %) (Figure 2B). Compared with sole annual ryegrass, inhibition percentage of annual ryegrass shoot was significantly increased by density 3, density 10, density 15 and density 20 while was significant decreased by density 80, density 110 and density 140. Inhibition of annual ryegrass root was maximum in density 1 (130.8 %) and minimum in density 140 (13 %). Compared with sole annual ryegrass, inhibition percentage of annual ryegrass root was 130.8 %, 123.7 % and 116.5 % in density 1, density 3 and density 5, respectively. When wheat density was increased to 50, over half annual ryegrass root was inhibited (inhibition of annual ryegrass root was 45.5 %).

### Wheat root morphology

Compared with density 1, specific root length of wheat was decreased by density 5, density 10, density 15, density 20, density 50 and density 80 (Table 2). Difference in specific root length of wheat among density 5, density 10, density 15, density 20, density 50 and density 80 was not significant. Root length of wheat was decreased with increase in wheat density (Table 2). Compared with density 1, root length of wheat was reduced (50 %) at density 10, density 15 and density 20, 69 % and 76 % by density 50 and density 80, and 83 % and 88 % by density 110 and density 140. Similar trend was observed in root surface area of wheat. Average root diameter of wheat steadily increased with increasing wheat density, which was 0.233-0.294 mm for density 1, density 3 and density 5, 0.328 mm for density 10 and density 20, and was 0.410-0.440 mm for density 50, density 80, density 110 and density 140 (Table 2). Root volume of wheat changed little at different densities except at density 110 and density 140 (Table 2).

Most root length of wheat had root diameter within  $0.000 < L \leq 0.400$  mm (Figure 3A). With wheat density increasing, root length of wheat distributed in root diameter class of  $0.000 < L \leq 0.200$  mm was steadily decreased from 72 % in density 1 to only 17 % in density 140, while root length of wheat distributed in root diameter class of  $0.200 < L \leq 0.400$  mm was significantly increased from 26 % in density 1 to 67 % in density 15 and from 58 % in density 50 to 52 % in density 140. Root length of wheat distributed in root diameter class of  $L > 0.400$  mm was only 2 % to 9 % in density 1, density 3, density 5, density 10 and density 15, however it was increased to 20 %-31 % from density 50 to density 140.

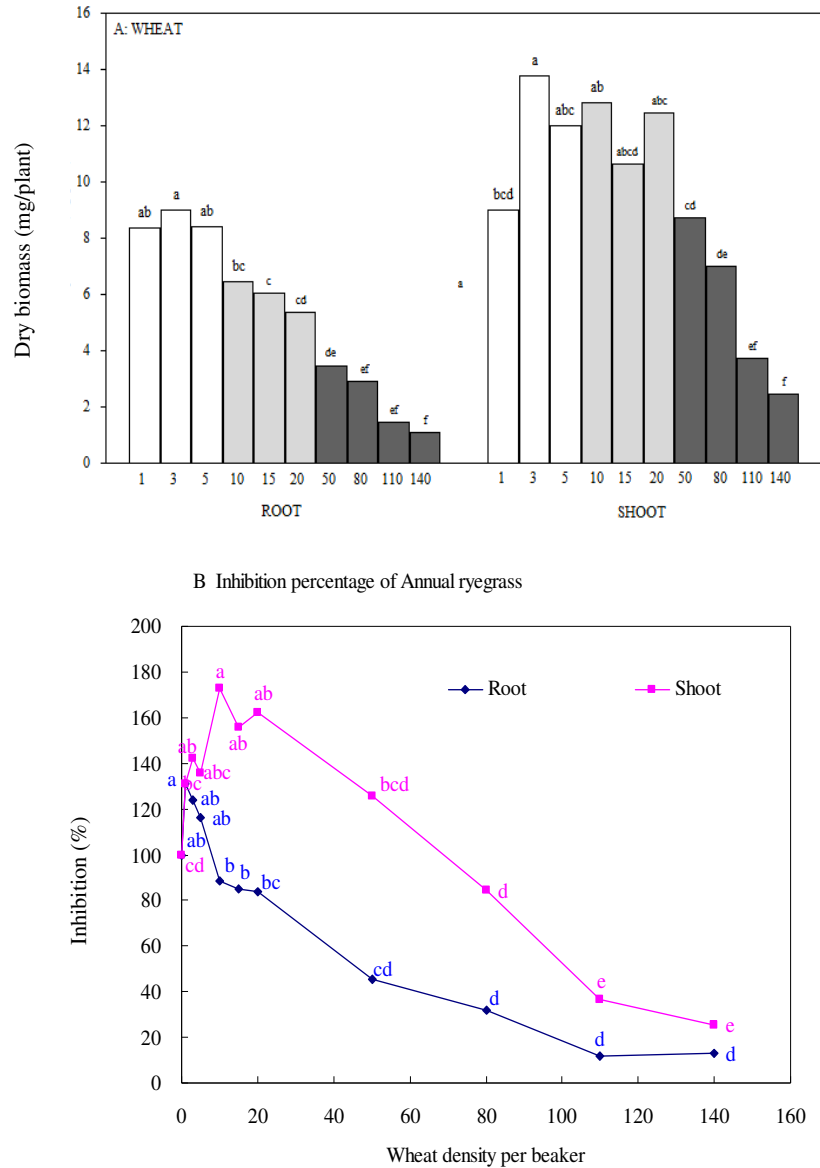


Figure 2. Density dependent allelopathy on root and shoot dry biomass of wheat (A) and inhibition (%) of annual ryegrass (B). Column with different letters indicate a significant difference at  $P < 0.05$  among different wheat density.

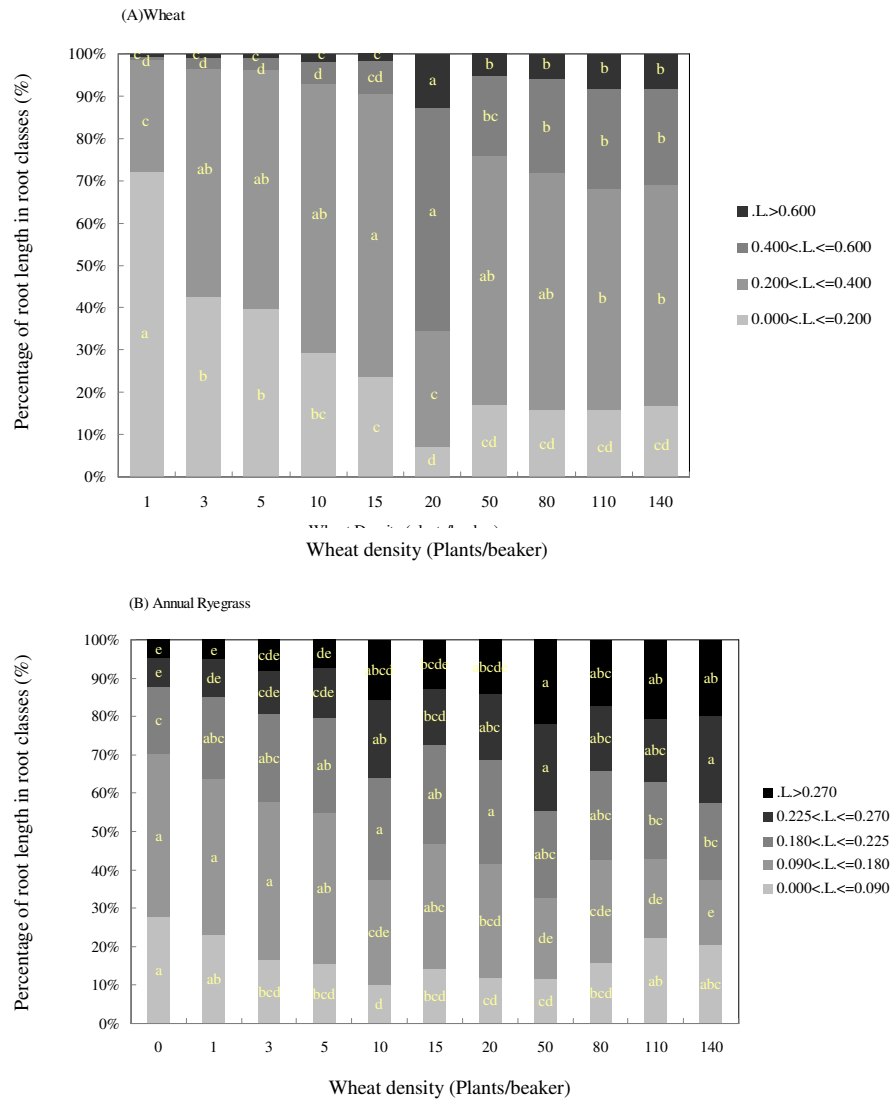


Figure 3. Density dependent on percentage of root length (cm) occupied by total root length (cm) in root classes of wheat (A) and annual ryegrass (B). Values with different letters indicate a significant difference at  $P < 0.05$  in different wheat density; numbers between  $L$ . means average root diameter, they means percentage of root length between root diameters occupied by total root length (%).

### Annual ryegrass Root morphology

Specific root length of annual ryegrass changed little among different wheat density treatments (Table 2). Compared to sole planted annual ryegrass, total root length of annual ryegrass was decreased by density 10, density 15, density 20, density 50, density 80, density 110 and density 140. Compared to sole annual ryegrass, total root length of annual ryegrass was reduced 35-46 % by density 10 and density 20, 73 % by density 50 and density 80, and 85 % by density 110 and density 140. Compared with sole annual ryegrass, root surface area of annual ryegrass was significantly increased by density 1, while decreased by density 50, density 80, density 110 and density 140 (Table 2). The root diameter of annual ryegrass was influenced by wheat density, it was 0.178 mm for sole planted annual ryegrass, 0.188-0.209 mm for density 1, density 3 and density 5 which was similar to sole annual ryegrass, 0.249-0.277 mm for density 10 to density 140 which was significant higher than sole planting annual ryegrass (Table 2). The root volume of ryegrass changed little in various densities except in density 110 and density 140 (Table 2).

Compared with sole annual ryegrass, annual ryegrass mixed with wheat significantly changed the root length distribution of annual ryegrass in different root diameter class (Figure 3B). Most root length of annual ryegrass distributed in root diameter class of  $0.000 < L \leq 0.225$  mm. For annual ryegrass sole planted, 88 % root length of annual ryegrass was distributed in root diameter class of  $0.000 < L \leq 0.225$  mm; for annual ryegrass mixed planted, 85 %-57 % root length of annual ryegrass was distributed in root diameter class of  $0.000 < L \leq 0.225$  mm. Compared with sole annual ryegrass, density 3, density 5, density 10, density 15, density 20, density 50 and density 80 significantly decreased the root length distribution in root diameter class of  $0.000 < L \leq 0.090$  mm. Density 10, density 20, density 50, density 80 and density 140 significantly decreased root length distribution of annual ryegrass in root diameter of  $0.090 < L \leq 0.180$  mm. Only density 5, density 10, density 15 and density 20 increased root length distribution of annual ryegrass in root diameter class of  $0.180 < L \leq 0.225$  mm, it was 24 %-26 % higher than sole planting annual ryegrass. Root length distribution of annual ryegrass in diameter class of  $0.225 < L < 0.270$  mm was significantly increased by density 10, density 15, density 20, density 50, density 80 and density 140. Root length distribution of annual ryegrass in diameter within  $L > 0.270$  mm only was increased by density 50 and density 80 (Figure 3B).

### Root exudates

Total phenolic contents were  $0 \text{ mg L}^{-1}$  in sole annual ryegrass. Wheat/annual ryegrass mixed planting increased the exudation of total phenolic contents (Figure 4). Amounts of exudation of total phenolic contents in density 5 were 5 times higher than in sole annual ryegrass. For density 10 and density 20, total phenolic contents excreted by wheat were  $11.4 \text{ mg L}^{-1}$  and  $27.8 \text{ mg L}^{-1}$  respectively, which were 11 to 27 times higher than sole annual ryegrass. Exudation of total phenolic contents with wheat densities from 50 to 140 was up to 141-418 times than sole annual ryegrass.

Table 2. Effects of wheat density on root morphological parameters of wheat and annual ryegrass

Crop or Weed	Wheat Density (plants/ha)										
	0	1	3	5	10	15	20	50	80	110	140
Wheat	Specific root length (cm/mg) 0.0650	7.52a	6.24abc	5.94bc	4.8c	5.27bc	5.41bc	5.96bc	5.59bc	6.65ab	6.32abc
	Total root length (cm/plant) <0.001	62a	56ab	49b	4.53a	31c	29c	19d	15de	10de	7e
	Root surface area (cm <sup>2</sup> /plant) <0.001	4.52a	5.07a	4.53a	3.16bc	3.43b	5.13a	2.42cd	2.07de	1.37ef	0.96f
	Average root diameter (mm) <0.001	0.233d	0.290cd	0.294cd	0.328c	0.343c	0.566a	0.414b	0.439b	0.440b	0.440b
	Root volume (cm <sup>3</sup> /plant) <0.001	0.0263bcd	0.0369b	0.0333bc	0.0294bc	0.026bcd	0.0733a	0.0248cd	0.0228	0.0153de	0.0107e
Annual Ryegrass	Specific root length (cm/mg) 0.0650	2.59abcd	2.18bcd	2.32abcd	1.97cd	1.96cd	1.79d	1.88cd	2.46abcd	2.98ab	3.09a
	Total root length (cm/plant) <0.001	12.9ab	15.9a	12.8b	8.5c	8.1c	6.8cd	3.9de	3.6e	1.8e	2e
	Root surface area (cm <sup>2</sup> /plant) <0.001	7.19bc	9.37a	8.26ab	6.55bc	5.67c	5.15cd	3.36de	2.97e	1.59e	1.66e
	Average root diameter (mm) <0.001	0.178e	0.188de	0.208cde	0.249abc	0.232bcd	0.242abc	0.284a	0.257ab	0.283a	0.277ab
	Root volume (cm <sup>3</sup> /plant) <0.001	0.0320ab	0.0447a	0.0437a	0.0430a	0.0403a	0.0323ab	0.0230bc	0.0200bc	0.0110c	0.0113c

Values with different letters indicate a significant difference at P<0.05 in different wheat density; italics in column 2 indicate P value.

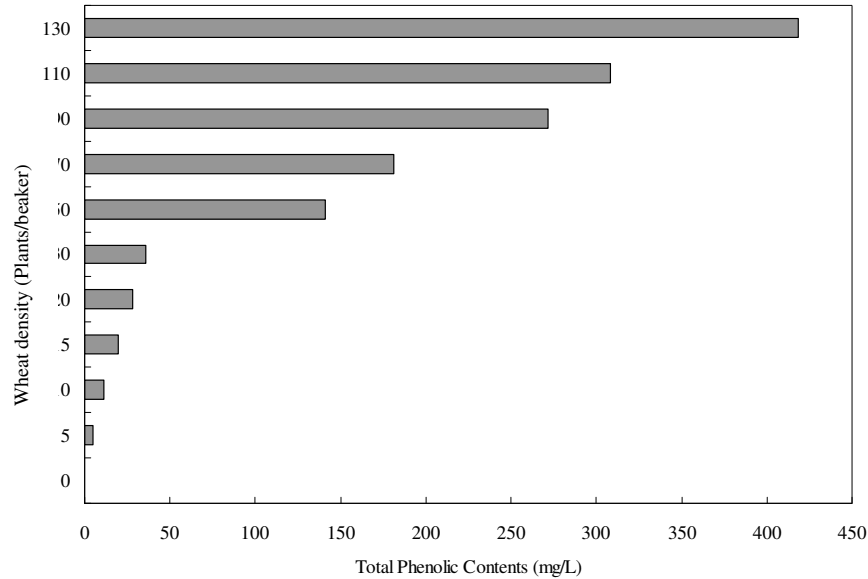


Figure 4. Effects of wheat densities on total phenolic contents in agar

## DISCUSSION

Density-dependent allelopathy played major role in wheat/annual ryegrass interactions under intermediate density (density 3, density 10, density 15 and density 20). Shoot dry biomass of annual ryegrass was increased by densities 3, density 10 and density 20 compared with sole planted annual ryegrass. The root dry biomass of annual ryegrass changed little in density 1 to density 20. Contrarily previous study showed that root and shoot dry biomass of annual ryegrass was decreased by low density of wheat and annual ryegrass (18). At density 50 and density 80, root dry biomass of annual ryegrass was decreased, while its shoot dry biomass remained similar to sole planted annual ryegrass. Shoot dry biomass of wheat was not influenced by wheat density (except at density 110 and density 140), but its root dry biomass was decreased with increasing wheat density. In wheat/annual ryegrass interactions, the roots of wheat and annual ryegrass were more sensitive than shoot. Weidenhamer *et al.* (19) demonstrated that individual plant weight was slowly decreased or increased, when allelopathy was main factor until resource competition became the main factor due to increasing density, and the maximum dry weight was obtained at intermediate density. Our results suggested that allelopathy worked at low and intermediate densities (until wheat density increased to 110 and 140 per beaker).

The wheat produced following allelochemicals (identified from the water-agar medium): *p*-Hydroxybenzoic acid, Vanillic acid, *cis-p*-Coumaric acid, Syringic acid, *cis*-Ferulic acid, *trans-p*-Coumaric acid, *trans*-Ferulic acid (phenolic acids) and DIMBOA, and their concentration was changed by wheat accessions (21). In different wheat growing

seasons, the above phenolic acids and 4 benzoxazinones (HBOA, DIBOA, HMBOA, DHBOA) were identified from water-agar medium (5). Previous study showed that amounts of DIMBOA+DIBOA, total phenolic contents and Phenolics+ BOAs all significantly decreased the root length of annual ryegrass and the inhibition was enhanced by their increased concentration (5). Phenolic acids are effective allelochemicals excreted from the wheat and they worked jointly (5, 24). Now it is possible to measure the total phenolic contents as one simple index, to evaluate the allelopathic effects in bioassay. Allelopathic effects are highly related to plant species densities (19). The increasing densities of wheat seedlings in water-agar medium (0, 1, 4, 8, 12, 15, 20 and 25 wheat seedlings vs 12 annual ryegrass seedlings) enhanced the magnitude of inhibition, as the allelochemicals released from wheat into water-agar medium increased with higher density (22). The root length of perennial ryegrass was reduced with increasing wheat density (7). The increasing amounts of total phenolic contents produced by wheat (at higher density) strengthened the allelopathic effects of wheat. When the concentration of total phenolic contents increased to  $141 \text{ mg L}^{-1}$  in density 50, root growth of annual ryegrass was strongly inhibited, though shoot growth remained similar to sole planted annual ryegrass. At increased density of density 110 and density 140, concentration of total phenolic contents were 300-400 times over sole planted annual ryegrass root, hence, the growth of annual ryegrass nearly stopped and shoot dry biomass was decreased. This was similar to results in alfalfa/barnyard grass on early seedling growth, where the root growth was reduced earlier than shoot with increased allelochemicals concentration (4). However, inhibition did not occur when wheat and annual ryegrass were sown at the same time by equal-compartment-agar method (22). In this bioassay, we changed the seedlings arrangement in water-agar growth medium and the new seedlings arrangement was similar to actual conditions. Allelopathic activity of crop seedlings stages offers great potential for further weed suppression in field conditions (2,22). So the increased wheat allelopathic effects by changing the density could suppress the weed growth, when they grew together simultaneously.

Allelochemicals excreted by wheat accompanied with root morphology changes, probably was the main mechanism of weed suppression in crop/weed interactions. Our results showed that annual ryegrass steadily reduced the total root length, while increased the root diameter with higher amounts of total phenolic contents. When interspecies neighbour existed, roots of *Coreopsis tripteris* and *Solidago altissima* became coarser, while those of *Andropogon gerardii* remained similar under field conditions (8). Annual ryegrass maintained same root surface area at density 1 to density 20 and low dose of allelochemicals (produced by wheat) increased the shoot dry biomass of annual ryegrass. At density 50 or more, amounts of total phenolic contents were increased and the root surface area of annual ryegrass was greatly decreased. This reduction together with total decrease in root length suggested that root growth of annual ryegrass was strongly inhibited by wheat at high density. Research on maize/Italian ryegrass showed that Italian ryegrass monocropped or associated with the maize formed a dense root in the soil, which probably greatly modified the maize crop and influenced the root density of maize under field conditions (10). Study on *Vallisneria spiralis* showed that root morphology and root distribution were greatly influenced by plant density (26). Thus the modified crop allelopathic ability due to changes in crop density, inhibited the root growth and reduced

the competitive ability of annual ryegrass was contributed to the weed suppression in wheat/annual ryegrass interactions.

Most wheat root were distributed in root diameter class of 0.200<.L.<0.400 mm and was influenced little by wheat density, wheat mainly changed the root length distribution in root diameter class of 0.000<.L.<0.200 mm and .L.>0.400 mm with density changing. Roots of annual ryegrass were thinner than wheat, and annual ryegrass only increased coarse root (L.>0.270 mm) at high wheat density (density 50 and density 80). Competitiveness of annual ryegrass was mainly due to the belowground interactions, thinner and fibrous roots may enhance the competitiveness of annual ryegrass (18). Xie *et al.* also showed that formation of fine root was good at increasing adaption of *Vallisneria natans* in competitive conditions (26). Results here further suggested that root length of annual ryegrass distributed in root diameter class of 0.180<.L.<0.225 mm and 0.225<.L.<0.270 mm played major role in wheat/annual ryegrass interaction.

## CONCLUSIONS

Density-dependent wheat allelopathy plays major role in wheat/annual ryegrass interactions under low and intermediate densities. Crop density greatly influenced the total phenolic contents excretion, and root morphology and the root response were more sensitive than shoot in test crops. Roots of annual ryegrass were thinner than wheat and root diameter class [0.180<.L.<0.225 mm and 0.225<.L.<0.270 mm] of annual ryegrass played major role in wheat/annual ryegrass interactions. The changes in the wheat crop density suppressed the annual ryegrass growth and development. The density-dependent crop allelopathy may have agronomic potential in integrated weed management programmes for effective weeds control in fields.

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