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Abstract

Aims The allelopathic activity of canola (*Brassica napus*) germplasm was investigated using the important Australian weed, annual ryegrass (*Lolium rigidum*) as the target species.

Methods Three different canola plant densities (10, 20, and 30 seedlings/beaker) of each of 70 world-wide genotypes were tested *in vitro* in close proximity to annual ryegrass seedlings.

Results The allelopathic activity of canola, as measured by reduction in annual ryegrass root and shoot growth, increased with canola crop seedling densities. Density did not consistently influence shoot length of annual ryegrass. Greater shoot length suppression was observed in genotype cv. Rivette and BLN3343C00402. The Australian genotype cv. Av-opal and the breeding line Pak85388-502 suppressed root length of ryegrass more than other genotypes, even at low densities. At the lowest density, the least allelopathic genotypes were cv. Barossa and cv. Cescaljarni-repka, although they became more allelopathic at higher density. An overall inhibition index was calculated to rank each of the canola genotypes. There were significant differences between canola genotypes in their ability to inhibit root and shoot growth in ryegrass.

Conclusion Considerable genetic variation exists among canola genotypes for their allelopathic effects on annual ryegrass. Further investigation is required to determine the allelopathic mechanisms, particularly to identify the responsible allelochemical(s) and the gene(s) controlling the trait. This research suggests that highly allelopathic canola genotypes can be potential for controlling weeds such as annual ryegrass in integrated weed management programs.

Keywords Canola, weeds, allelopathy, and allelochemicals

Introduction

Canola (*Brassica napus* L.) has become an important oilseed crop worldwide and a profitable break crop for grain growers in Australia. This enhanced production can be partially attributed to the increased need for diversity in farming systems, and demand for higher quality oils for human consumption. Accordingly, the global canola area has grown rapidly over the past 20 years, rising from the sixth to the second most important oilseed crop (AOF 2013; Canola Council of Canada 2013). Weeds are a restrictive factor that significantly reduces the grain yield and quality of canola (Lemerle et al. 2012). Grass weeds including annual ryegrass, vulpia (*Vulpia myuros*) and wild oat (*Avena spp*), are the most important weeds of canola crops in Southern Australia (Lemerle et al. 2001). The *Brassicaceae* weeds, such as wild radish (*Raphanus raphanistrum*) are also prevalent and their heavy infestation can reduce canola yield up to 90% (Blackshaw et al. 2002). The management of such weeds is a challenge due to the limited availability of post-emergent selective herbicides for use in conventional canola cultivars and the low efficacy against these 'canola-like' weeds (Preston and Baker 2009).

The introduction of herbicide-tolerant cultivars allows growers to manage many of their most difficult weeds (Harker et al. 2000). However, prolonged and widespread use of these herbicides increases the risk of herbicide resistance in Australian annual ryegrass including to glyphosate (Heap 2013; Pratley et al. 1999). The intensive and repeated use of a single herbicide (or those with the same mode-of-action) also facilitates the shifting of the weed population from susceptible to tolerant species (Green 2009). In addition, herbicide-resistant crop seed left behind at harvest and in the following season can become volunteers which are difficult to control. Left uncontrolled, these plants eventually set seed and increase the incidence of herbicide-resistant weeds, causing escalating economic loss.

The combination of herbicides and associated resistant cultivars is the most cost-effective and widely used weed control method in canola (Beckie et al. 2006). Canola growers have few alternatives to current weed management systems, because the likelihood of new herbicide modes-of-action becoming available is limited (Pratley et al. 1998). There has been speculation about the factors limiting new herbicide appearance (Duke 2012). However, low commodity prices, herbicide-induced crop injury, herbicide residue concerns, and public concern about the environmental and human health effect of herbicides are forcing growers to consider non-chemical alternative options (Blackshaw et al. 2008). One feasible option, as a supplement to synthetic herbicides, is canola competition and

allelopathy to increase the weed suppression by the crop. Crop competition occurs in communities when two or more plant seeks a common resource within limited spaces (Harper 1977). Furthermore agronomic factor, crop density, can influence the competitive effects of weeds and reduce the need for herbicides (O'Donovan 1994). The manipulation of canola agronomy by choice of canola genotype and by increasing crop density has been shown to reduce weed impacts in Australia (Lemerle et al. 2012). Increasing seeding rate has a major effect on crop/weed interaction (Hume 1985) and allelochemical concentrations are a function of the density of the allelopathic crop (Belz and Hurle 2005). This suggests that density may be an important factor in enhancing canola allelopathic activity .

Allelopathy, as first described by Molisch (1937), is the stimulatory or inhibitory impact of any biochemical interaction between plants (Rice 1984). Different plant species have been reported to have allelopathic activity that could be utilised in agricultural or ecological systems (Rice 1984). Where the allelopathic effect is inhibitory, the term phytotoxicity is commonly used. *Brassica spp* have received attention because of their allelopathic activity via their residues and plant extracts especially when used as cover crops (Haramoto and Gallandt 2005; Norsworthy et al. 2011). The development of crops with the capability to exert allelopathic effects on crop weeds through root exudates is another option (Olofsdotter et al. 2002). Research has shown that in wheat (Wu et al. 2000a) and rice (Dilday et al. 1994; Seal et al. 2004) the degree of allelopathic effect differs between crop cultivars. The production of allelochemicals is also influenced by environmental conditions (Quader et al. 2001). It is especially important to evaluate the allelopathic potential of a crop or its cultivars under field conditions but it is difficult to eliminate the influence of competition when assessing the allelopathic potential of a crop in the field (Wu et al. 2000b). The standardized laboratory assay is a rapid and an inexpensive procedure for screening the allelopathic potential of large numbers of crop genotypes against target weed species (Wu et al. 2000b). Further, the interaction between crop and weed is critical at the seedling stage. Where weed species can be allelopathically suppressed by crop plants during the seedlings establishment period, the crop will gain competitive advantage over weeds. Due to the economic importance of canola and the diversity of cultivars grown throughout the world, research is required to determine the potential of crop allelopathy for weed management in canola. The objectives of this study are to (i) evaluate the role of canola density on its allelopathic impact and (ii) evaluate canola seedling allelopathy for the suppression of annual ryegrass in 70 diverse *Brassica* genotypes.

Methods and Materials

Plant materials

Seeds of 60 *Brassica napus* genotypes and ten genotypes from closely-related *Brassica* species were selected for the bioassay screening. All seed was obtained from the National *Brassica* Germplasm Improvement Program, located at NSW Department of Primary Industries, Wagga Wagga, Australia. The *Brassica* genotypes originated from Australia, Asia, Europe and Canada. The genotypes were chosen to try and maximise the genetic diversity present in the set of 70 genotypes. Seed of annual ryegrass was obtained commercially. Agar (technical grade) was purchased from Sigma Aldrich (St. Louis, USA).

Sterilisation and germination

Brassica seeds were surface-sterilised by soaking in 2% sodium hypochlorite (NaOCl) for 5 minutes, then rinsed six times in sterilised distilled water. The seeds were transferred to a petri dish with one sheet of Whatman No. 1 filter paper, moistened with 5 ml sterilised distilled water, and sealed with parafilm. The surface-sterilised seeds of *Brassica* and ryegrass were kept in a 12-hour light/12-hour dark, 20°C/15°C controlled environment.

General bioassay and growing conditions

The equal-compartment-agar-method (ECAM), described previously by Wu et al. (2000b) and based on the plant box method and relay seedling technique, was used for the bioassay screening. This technique provides a rapid, simple, inexpensive method for the initial screening of the allelopathic potential of a large number of genotypes against a target weed species under laboratory conditions. Glass beakers (600 ml, 12 cm depth, 8 cm diameter) containing 30 ml of 0.3% agar-medium (no nutrients, 1.3 cm depth) were autoclaved. The preliminary experiment with a single genotype showed canola density played a major role in its allelopathic activity in suppressing annual ryegrass root growth and diameter (Asaduzzaman et al. 2012). Hence for each *Brassica* genotype, 10, 20, or 30 uniform seedlings per beaker were chosen and aseptically transplanted from the germination dish onto one half of the agar surface, with the embryo up. The beaker tops were sealed with parafilm to prevent contamination and evaporation from the agar surface, and were placed in a controlled growth incubator with a daily 12-hour light/12-hour dark, 20°C/15°C cycle. After the *Brassica* plants had been left to grow for six days, 15 pre-germinated seeds of annual ryegrass were aseptically sown on the other half of the agar surface, at a distance of 4 cm from the *Brassica* seedlings. A piece of pre-autoclaved white paperboard was inserted down the centre of the beaker with the lower edge of the paperboard

ending 1 cm above the agar surface. The beaker was divided this way to minimize competition for space and light between the *Brassica* and annual ryegrass seedlings. The roots of the *Brassica* could freely enter the annual ryegrass section of the agar volume, so that any allelochemicals produced and released by the *Brassica* seedlings could diffuse throughout the entire agar medium to influence the annual ryegrass root growth. After the ryegrass was sown, the beaker was again wrapped with parafilm and placed back in the growth cabinet for a further seven days co-growth. The receiver species, annual ryegrass, was also grown alone as a control. After seven days, each annual ryegrass seedling was carefully removed from the agar to avoid root breakage, and the root and shoot lengths were measured.

Experimental design and statistical analysis

A randomized complete block design with four replications was used for the experiment described above. For each genotype, 4 x 4 (1 control + 3 density) = 16 experimental units were arranged spatially using DiGGer design software in R (Coombes 2002). A total of 35 separate experiments were needed to test all 70 *Brassica* genotypes while all experimental condition was identical. Raw data for the root and shoot length of annual ryegrass for the different densities of each *Brassica* genotype were used separately for statistical analysis. Data (expressed as the percentage of the control root and shoot growth) were subjected to analysis of variance using Genstat v13 (VSN International, Hemel Hempstead, UK) and the treatments means compared using the least significance difference (LSD) at a 5% level of probability. Plots of residual versus fitted values were examined for all traits to ensure that the assumptions of analysis of variance were met.

The density-response data were further subjected to the analysis of whole-range assessment proposed by An et al. (2005). Whole-range assessment is a simple method for analyzing allelopathic density-response data. It considers the overall effect or response across the whole range of application rates instead of assessing the effect of each individual rate on the test species. The approach used here was to calculate the inhibition area of the ryegrass

Compared with the control (100%) over the whole range of genotype densities on the X axis. Thus inhibition area = $\int_{CT}^{100}[100 - f(C)]dC$ where C is the allelochemicals concentration or equivalent and CT is the threshold concentration for causing inhibition in annual ryegrass. Overall biological activity across the whole range of concentrations or equivalent is then summarised, calculated and presented by a single value "inhibition index" which is defined as the percentage of the inhibition area to the total area, therefore inhibition index = (inhibition area/total

area) x 100, where the total area as defined as $\int_0^{100} 100dC$. WESIA software developed by Liu et al. (2007) was used to compute the inhibition area and calculation of 'inhibition index', which was defined as the percentage of the maximum area inhibited. The inhibition index gives a relative indication of the biological activity for each genotype. Genotypes with strong allelopathic activity will have high index values whereas low values indicate weak or no activity. The calculated inhibition index of each *Brassica* genotype for root and shoot of annual ryegrass was also analysed with Genstat v13.

Results

Effect of density

The suppression of annual ryegrass shoot length was variable (data not shown). However, there were significant interaction effects between densities and genotypes (Table 1). The higher shoot length inhibition of annual ryegrass was recorded at increased density of Rivette (8) and BLN3343-C00402 (18). Minimum inhibition of shoot length was observed at the low density of genotypes Tarcoola-141 (61) and Maintainer-gsr-ms-501 (28). In contrast, root growth of annual ryegrass was considerably reduced at higher densities of canola when compared with the control. The results of the bioassay of the root length of annual ryegrass for the 60 *B. napus* and 10 non-*napus* genotypes were plotted in a density-response curve. For illustration simplicity only the four most allelopathic and the four least allelopathic genotypes are presented (Fig. 1). Most *Brassica* genotypes significantly reduced the root growth of annual ryegrass with increased density. However, this trend was not apparent in three *napus* genotypes, Cescaljarni-repka (69), Lantern (67) and Barossa (70), and two non-*napus* genotypes, Kaga (46) and Hosin (25). At densities of 10 and 20 seedlings per beaker, these genotypes still permitted the elongation of the annual ryegrass roots but inhibited root length at density 30 seedlings per beaker. Interestingly, at a density of ten seedlings per beaker, the weakest genotype Barossa slightly stimulated annual ryegrass root growth.

All genotypes significantly reduced the root but not shoot growth of ryegrass at a density of 30 seedlings per beaker compare with the control. At this highest density, the least phytotoxic genotype Cescaljarni-repka reduced by only about 15% the root length, whereas Av-opal (1) and Pak85388-502 (2) controlled root growth by 73% and 70% respectively. The combined density results of all genotypes were processed in the WESIA software to calculate the overall allelopathic effect of each genotype.

Genotypic effects on the root and shoot growth of annual ryegrass

The allelopathic inhibition indices of *Brassica* genotypes against annual ryegrass roots ranged from 55% to 8% depending on genotype (Table 2). Annual ryegrass shoot inhibition, due to the phytotoxic activity of canola genotypes was less than that against ryegrass roots, although there was significant variation between genotypes and that ranged from 24% to 1% (Table 2). In Fig 2, deviation of data from the solid 1:1 line shows a weak relationship between root and shoot inhibition of annual ryegrass but significant correlation was present. Several genotypes showed strong inhibition of root length but were less active against shoot growth. However, the most allelopathic genotypes, such as Av-opal, Pak85388-502, Rivette (8) and Rainbow (11) showed stronger suppression of both root and shoot growth than the less allelopathic genotypes. The genotype BLNC004001 (19) had the highest index value of 24% for annual ryegrass shoots followed by Rainbow. These were statistically similar to both Av-opal and PAK85388-502 which showed the greatest allelopathic activity on root growth. The lowest shoot inhibition index of 1% was seen in Maintainer-gsr-ms-501(18) and was statistically similar to the two least-allelopathic genotypes with regard to root elongation, Barossa and Cescaljarni-repka.

Of the 70 *Brassica* crop genotypes, nine were strongly allelopathic or phytotoxic, significantly inhibiting the root growth of ryegrass with an inhibition index of more than 45%. By contrast, three genotypes were very weakly allelopathic, with considerably less inhibition of ryegrass root growth and an inhibition index of less than 15%.

Both *napus* and non-*napus Brassica* species showed similar variation in their phytotoxicity regarding root and shoot growth of ryegrass (Fig. 3). However, of 10 non-*napus* genotypes, the inter-specific progeny from crosses *B. napus* x *B. juncea* (3), *B. napus* x *B. juncea* (4), and *B. juncea* x *B. carinata* (5), demonstrated greater phytotoxic activity. These three genotypes were among the eight most phytotoxic groups of the 70 in the bioassay. The representative of the species *B. carinata (*15) was found to have a medium phytotoxic effect with an inhibition index of 43% for root growth and 9% for shoot growth. *B. rapa accession* (46*)* and a progeny from a cross of *B. napus* x *B. carinata* (27) were comparatively less inhibitory to the shoots but more inhibitory to the roots of annual ryegrass.

In this bioassay genotypes originating from five different continents were used (Fig. 4) and broad range of phytotoxic differences between genotypes were shown even though they originated from same continent. The Australian genotypes were well represented in the most phytotoxic group with regards root and shoot inhibition. However, although Av-opal, an Australian genotype, was the most phytotoxic, another Australia-originating genotype, Barossa, was the least phytotoxic. All Asian genotypes showed medium phytotoxic potential and genotypes originating from unknown sources demonstrated a wide range of allelopathic phytotoxicity, as did the Australian genotypes

Discussion

A broad range of phytotoxic potential exists within this *Brassica* germplasm. This work, together with other published studies in rapeseed (Uremis et al. 2009a), rice (Dilday et al. 1994; Seal et al. 2004), and wheat (Wu et al. 2000a), suggests that there is a potential genetic basis to allelopathy. Several attempts have been made to understand the genetic basis of phytotoxicity and to locate genetic markers governing the production of allelochemicals (Dilday et al. 1998; Niemeyer and Jerez 1997). The present study determined that there is substantial variation in allelopathic phytotoxic activity in seedlings of canola genotypes, thereby providing a sufficient gene pool for the development of allelopathic canola cultivars to suppress weeds, and to track-down the genes controlling the allelochemicals responsible. Canola allelopathy could be a quantitative trait because it is normally distributed across the tested genotypes. However, this needs validation using a segregating population from a cross between extreme parents. Identifying crop cultivars with allelopathic potential is the first step in the process of incorporating the genes involved into competitive, high-yielding cultivars and ultimately becoming a tool in an integrated weed management program.

Brassica genotypes showed similar patterns in the density-response curve for annual ryegrass root growth and there was a density by genotype interaction ($P<0.01$). This indicates that the agronomic trait of density has a role in canola and other *Brassica* allelopathy, with higher densities inducing greater inhibition for both weakly and strongly allelopathic genotypes. We confirmed that root elongation of the target species was more affected, than shoot length, by increasing canola density, as found in rice (Navarez and Olofsdotter 1996 ; Seal et al. 2004) and in wheat (Li et al. 2011). At a high density of *Brassica* seedlings per beaker, the degree of annual ryegrass root inhibition by all genotypes was significant when compared with the control. At this density the concentration of allelochemicals had extended well beyond the threshold concentration required to produce an effect. Olofsdotter et al. (2002) noted that

any compound can be toxic if applied at a high enough dose. A differential allelopathic potential was apparent at the lower density of ten canola seedlings per beaker in Barossa, Cescaljarni-repka and Urvashi. There is a contrary stimulatory effect at very low concentrations of allelochemicals that could complicate any analysis at these low levels (Rice 1974; Streibig 1988).

Williamson and Weidenhamer (1990) indicated that the toxicity of allelochemicals depends on their bioactive concentration which is determined by the concentration of allelochemicals at a given point and the flux of toxin in and out of the system. From Fig. 1, it is apparent that there is a genotypic effect in addition to a concentration effect and non-*napus* genotypes had the potential to be more toxic if used at even higher densities. It is also possible that, if the set of allelochemicals is much the same for all canola genotypes, then the genotypic variation in phytotoxicity is due to the amount of allelochemicals they exude. Both the composition and concentration of these exudates determines their effect. It has yet to be shown whether the differential allelopathic effect is due to different mixtures of exudate compounds or to varying concentrations of the same compounds. The chemical composition of allelochemicals of the canola that impacted on annual ryegrass needs to be determined.

In the present research, some of the tested genotypes were non-*napus* and derived from interspecific hybrids of closely related *Brassica* species, for example *B. napus* × *B. juncea* and *B. juncea* × *B. carinata*. It was speculated that non-*napus Brassica* species may be generally more phytotoxic because of their high glucosinolate content. However, the results of this study do not support that finding because, despite low glucosinolate content (<30 μmol/g oil-free meal), phytotoxicity was high in some of the canola-quality *napus* genotypes. This implies that *napus* canola does not necessarily have lower allelopathic activity than non-*napus,* or vice versa. If adapted *napus* genotypes have the same phytotoxic potential as more distant relatives, there is more potential to breed for allelopathy without undesirable side-effects. Similar findings have also been reported by Uremis et al. (2009b), demonstrating that the allelopathic activity of *Brassica* species is not attributable to glucosinolate content, and varies between species.

The similar range of allelopathic activity in genotypes originating from different continents suggests that there is no geographical influence on the degree of allelopathy, with a range of strong and weak allelopathic genotypes found within countries and possibly spread worldwide.

In the current bioassay the phytotoxic effects on receiver plants are due to allelochemicals via chemicals exuded into the agar medium by *Brassica* roots. While such laboratory studies can identify key genotypes that possess outstanding phytotoxic potential, the question remains as to whether ECAM results predict field performance. In Australia, in an allelopathy study of rice, Seal et al. (2008) found good conformity between laboratory (using ECAM) and field outcomes. Rice cultivars ranked as allelopathic in the bioassay also performed well in the field and tended to produce lower weed dry biomass. A relatively high correlation co-efficient (r= 0.84**) was reported when the field outcomes were compared with the ECAM bioassay data (Seal et al. 2008).

The identification and quantification of distinct categories of phytotoxic compounds and their complex biochemical pathways through metabolomic approaches is essential. The identification and mapping of genes influencing the production of such chemicals is also an important future direction for canola allelopathy research. Identifying genotypes of varying allelopathic activity allows for the quantification of the biochemical cost of allelopathy (Meiners et al. 2012) by comparing growth rates in the presence and absence of competition, which will provide opportunities to understand the underlying trade-offs related to allelopathy.

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List of Tables and Figures

Table 1. Genotype, density and genotype x density effects from ANOVA of root and shoot length (as percent of control) of annual ryegrass in the presence of 70 diverse *Brassica* genotypes

Character	Component	df	ms	*P*
Root	Genotype	69	1535.3	<0.01**
	Density	3	133971.2	<0.01**
	Genotype x Density	207	285.4	<0.01**
	Residual	837	121.3	
Shoot	Genotype	69	735.2	<0.01**
	Density	3	12155.4	<0.01**
	Genotype x Density	207	167.4	<0.01**
	Residual	837	104.9	

Table 2. Name and overall root allelopathic index of 70 diverse *Brassica* genotypes tested against annual ryegrass under laboratory conditions

Number	Name	*Brassica* species	Root Inhibition Index (%)	Shoot Inhibition Index (%)
1	Av-opal	*napus*	55	21
2	Pak85388-502	*napus*	52	20
3	Roy98310	*napus x juncea*	52	24
4	Roy47-99P1	*napus x juncea*	52	15
5	JC134	*juncea x carinata*	51	20
6	Sardi603	*napus*	49	16
7	Atr-beacon	*napus*	49	17
8	Rivette	*napus*	47	23
9	44C76	*napus*	46	9
10	Bau-m-58-501	*napus*	45	21
11	Rainbow	*napus*	45	24
12	BLN 4143	*napus*	44	21
13	Surpass 400	*napus*	44	12
14	Ag-outback	*napus*	44	5
15	ATC94044-1	*carinata*	43	9
16	RP004	*napus*	43	10
17	Dong-hae-18-501	*napus*	43	12
18	BLN3343-C00402	*napus*	43	24
19	BLN3343-C00401	*napus*	42	24
20	Tarcoola-1	*napus*	42	7
21	Monty	*napus*	42	10
22	Skipton	*napus*	41	15
23	Charlton	*napus*	41	10
24	44C73	*napus*	41	10
25	Hosin	*rapa*	40	8
26	Sardi607	*napus*	39	7
27	Surpass400-NCB4	*napus x carinata*	38	14
28	Maintainer-gsr-ms-501	*napus*	38	1
29	Taiwan-2-501	*napus*	38	13
30	Eureaka	*napus*	38	15
31	Wesway	*napus*	38	7
32	BLN1990	*napus*	37	13
33	Azuma-501	*napus*	37	3
34	Vinnickij-501	*napus*	37	7
35	Buk-wuk-13-501	*napus*	37	5
36	Iiwao-natane-502	*napus*	36	10
37	Ukraine-c-501	*napus*	36	5
38	Teri-oo-r9903	*napus*	36	15
39	Tarcoola-191	*napus*	36	10
40	Tarcoola-22	*napus*	35	12
41	Seetha	*napus*	35	8
42	Tarcoola-21	*napus*	34	7
43	Rafal-502	*napus*	34	6

44	Cb-telfer	*napus*	33	10
45	BLN 3614	*napus*	32	11
46	Kaga	*rapa*	32	5
47	Ag-spectrum	*napus*	32	6
48	Austria-3-501	*napus*	30	12
49	A-19890	*napus*	30	5
50	Atr-cobbler	*napus*	29	6
51	Zhongyou-za-no8	*napus*	29	7
52	Purler	*napus*	29	8
53	Topas	*napus*	28	6
54	BLN 4135	*napus*	26	19
55	Ag-emblem	*napus*	26	13
56	Drakkar	*napus*	25	6
57	Chon-nam	*napus*	24	7
58	Av-jade	*napus*	22	9
59	BLN 4139	*napus*	22	7
60	Hurricane-TT	*napus*	22	3
61	Tarcoola-141	*napus*	21	2
62	Mutu-98-1	*napus*	19	5
63	NU-41737-502	*juncea*	18	10
64	WA050085	*napus*	17	8
65	X-06-06-3725	*napus*	17	3
66	Maluka	*napus*	16	8
67	Lantern	*napus*	16	3
68	Urvashi	*juncea*	11	11
69	Cescaljarni-repka	*napus*	10	5
70	Barossa	*napus*	8	7
Mean inhibition index			35	10
Inhibition index LSD (5%)			10	9

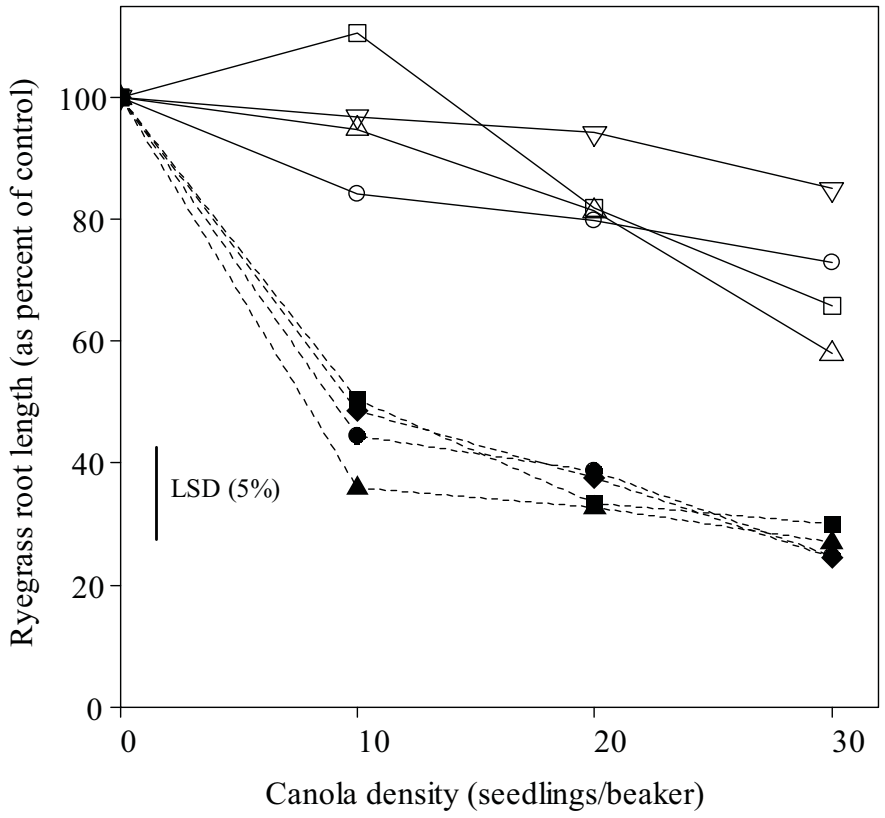

Fig. 1 Effect of canola density and genotype on ryegrass root length for the eight most extreme genotypes. Open symbols are the least allelopathic genotypes, closed symbols are the most allelopathic. □ = Barossa, ▽ = Cescaljarni-repka, Δ = Urvashi, O = Lantern, ■ = Pak85388-502, ◆ = JC134, ● = Roy47-99P1, and ▲ = Av-opal.

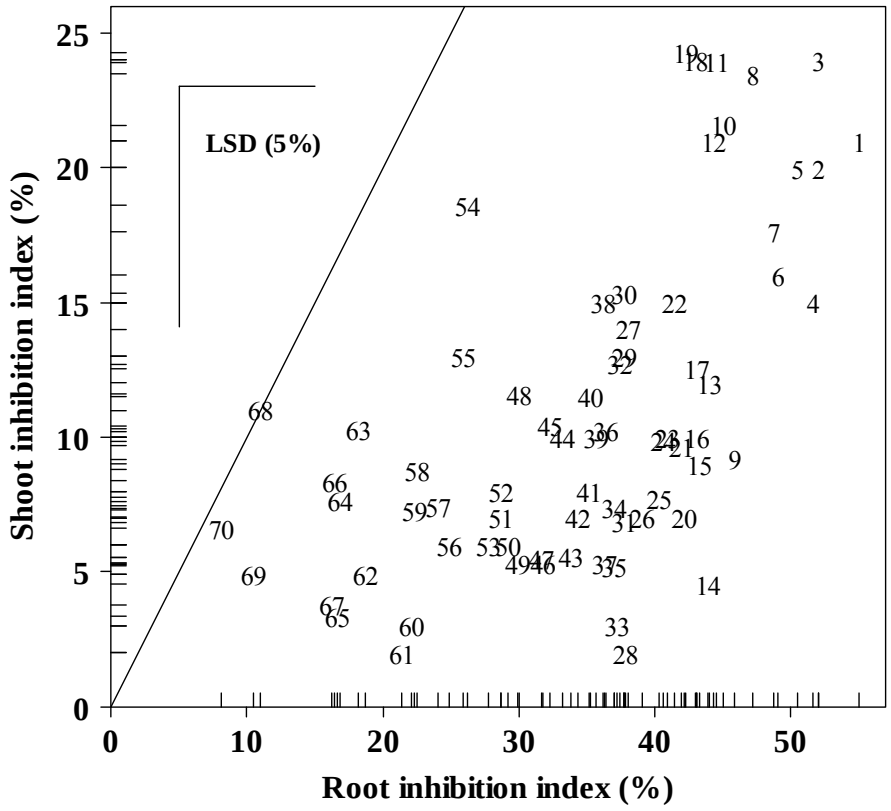

Fig. 2 Annual ryegrass root and shoot inhibition indices (%) for 70 *Brassica* genotypes ($r = 0.37$*, $P = 0.002$; r is Pearson's correlation co-efficient). The solid line is the 1:1relationship between the root and shoot indices. Numbers refer to the genotypes listed in Table 2.

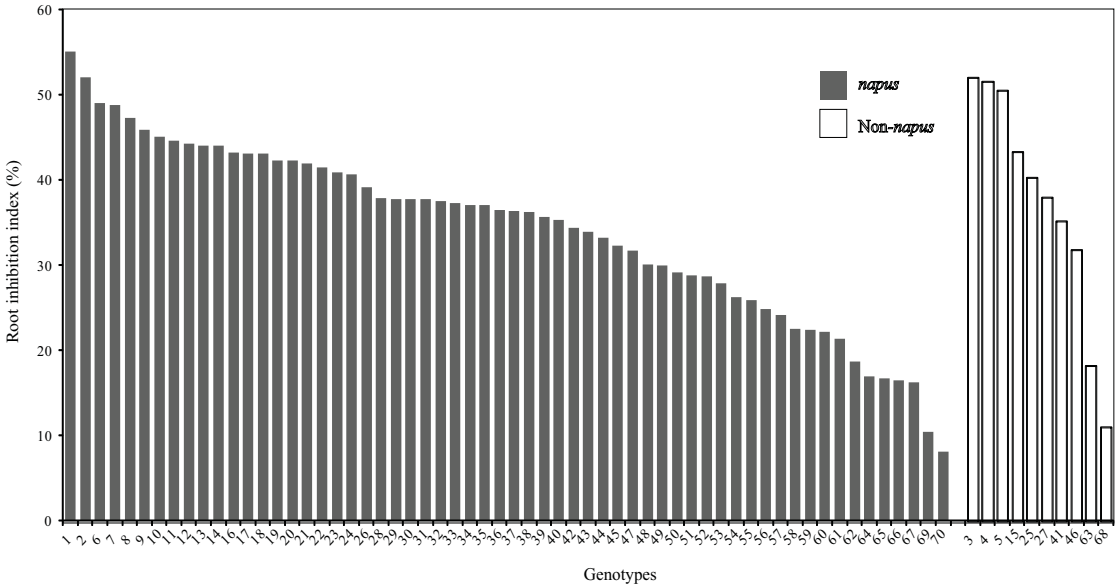

Fig. 3 Comparisons of annual ryegrass root inhibition indices (%) of *napus* and non-*napus Brassica* genotypes as listed in Table 2 (LSD = 10; *P<0.05**).

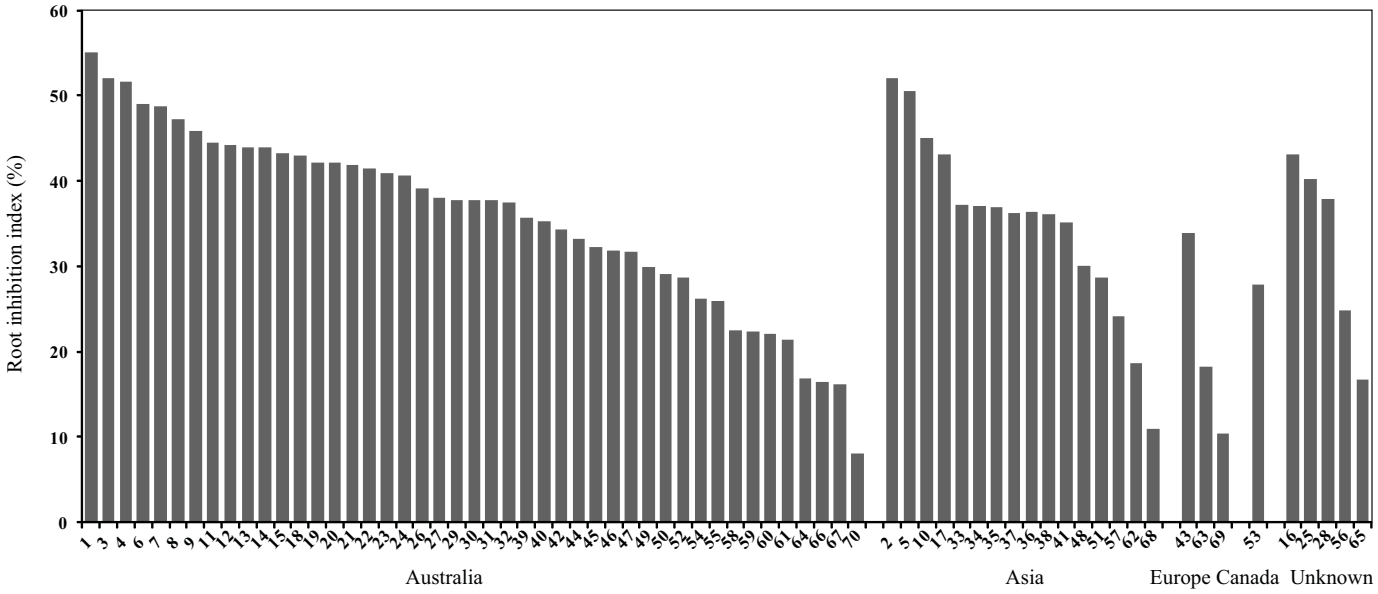

Fig. 4 Comparisons of annual ryegrass root inhibition indices (%) between *Brassica* genotypes from different continents (LSD = 10; *P<0.05**).