

Combined effects of wheat competition and 2,4-D amine on phenoxy herbicide resistant *Raphanus raphanistrum* populations

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Received 10 September 2008

Revised version accepted 23 October 2008

Summary

Phenoxy herbicides are integral to the control of *Raphanus raphanistrum* populations in Australian crop production systems, but the development of phenoxy resistant *R. raphanistrum* populations poses a major threat to the sustainability of these systems. In dose–response pot studies, phenoxy herbicide resistant *R. raphanistrum* populations, WARR12 and WARR20, suffered large biomass reductions following treatment with recommended or higher application rates of phenoxy herbicides. This indicates the presence of a weak resistance mechanism where treated plants, although surviving, are affected by these herbicides. Subsequently, the competitive ability of 2,4-D amine treated or untreated WARR12 and WARR20 populations with wheat was assessed using a target-neighbourhood experiment. The combination of wheat competition and 2,4-D amine application resulted in

control of the resistant WARR12 population, but not the WARR20 population. Wheat crop competition alone resulted in large (>40%) biomass reductions of WARR12 and WARR20 populations. However, the application of the recommended rate of 2,4-D amine caused a large (>75%) reduction in WARR12 biomass, but had a reduced effect on WARR20 biomass. These studies possibly explain the largely successful control of *R. raphanistrum* populations being achieved with phenoxy herbicides in cropping systems across the Western Australia wheatbelt. However, the results also indicated that the strategy of combining crop competition with phenoxy herbicides for the control of this weed is likely to be an effective option in the short-term only.

Keywords: herbicide resistance, dose–response, *Triticum aestivum*, crop-weed competition, 2,4-D, MCPA, wild radish.

WALSH MJ, MAGUIRE N & POWLES SB (2009). Combined effects of wheat competition and 2,4-D amine on phenoxy herbicide resistant *Raphanus raphanistrum* populations. *Weed Research* **49**, 316–325.

Introduction

Raphanus raphanistrum L. (wild radish) is an annual weed prevalent in Australian dryland cropping systems. The adverse economic importance of *R. raphanistrum* is attributed to its ability to reduce crop yield and grain quality (Cheam & Code, 1995). This weed is a vigorous competitor for water, nutrients and light, resulting in

substantial yield reductions for wheat (*Triticum aestivum* L.) (Reeves *et al.*, 1981; Cousens *et al.*, 2001), lupin (*Lupinus angustifolius* L.) (Hashem & Wilkins, 2002) and oilseed rape (*Brassica napus* L.) (Blackshaw *et al.*, 2002). *Raphanus raphanistrum* is an extremely successful weed due to its characteristics which include, germination under a wide range of environmental conditions, a highly flexible life cycle, prolific seed production and a

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long-lived seed bank (Cheam & Code, 1995). In particular, the considerable genetic diversity (Bhatti, 2004; Madhou *et al.*, 2005) within this weed species has enabled *R. raphanistrum* to be successful across several agro-ecosystems of the Australian dryland crop production region. Consequently, *R. raphanistrum* is rated the most problematic dicotyledonous weed of Australian dryland crop production systems (Alemseged *et al.*, 2001).

Recently, the problematic nature of *R. raphanistrum* has been further exacerbated by the widespread evolution of herbicide-resistant populations of this weed. The propensity for the development of herbicide resistance in *R. raphanistrum* is due to the combination of genetic diversity and high-density populations in crops across the Australian cropping regions. This has led to evolved resistance to several of the highly effective selective herbicides commonly used for the control of *R. raphanistrum* populations. *Raphanus raphanistrum* has been documented with resistance to acetolactase (ALS) inhibiting herbicides (Walsh *et al.*, 2001) the phytoene desaturase inhibiting herbicide diflufenican (Walsh *et al.*, 2004) and the photosystem II (PSII)-inhibiting herbicides metribuzin and atrazine (Hashem *et al.*, 2001; Walsh *et al.*, 2004). Despite over five decades of use of the phenoxy herbicide 2,4-D for *R. raphanistrum* control in crop production systems, it is only recently that 2,4-D resistant populations have been identified (Walsh *et al.*, 2004). Phenoxy herbicide resistance in *R. raphanistrum* was initially identified in two populations from the Western Australian (WA) wheatbelt in 2001 (Walsh *et al.*, 2004). A survey of the WA wheatbelt in 2003 revealed that 60% of randomly collected *R. raphanistrum* populations displayed some level of resistance to the phenoxy herbicide 2,4-D amine (Walsh *et al.*, 2007). Given the current widespread occurrence of resistance, it is likely that phenoxy herbicide resistance has been present, but undetected, in WA crop fields for some time.

Documented cases of phenoxy herbicide resistance remain relatively rare, despite this herbicide chemistry being one of the most widely used worldwide. Globally, phenoxy herbicides have been used for the selective control of broad-leaved weeds in cereal production systems for six decades, yet presently only 35 phenoxy herbicide-resistant biotypes have been identified worldwide (Heap, 2008). This is low in comparison to the extensive resistance evident to ALS and PSII inhibiting herbicides, which have been in use for substantially shorter periods. Resistance to phenoxy herbicides is mostly low-level resistance (Coupland, 1994) and, therefore, a weak resistance mechanism has restricted the rate of evolution of resistant populations, as well as preventing their early detection. Plants with a weak resistance mechanism, although surviving herbicide treatment,

may have a reduced capacity for biomass and reproductive output. There are likely to be further reductions in the biomass and reproductive output of these already impeded plants under strong competition from a crop. The combination of these stress factors may in fact result in phenoxy herbicide-resistant *R. raphanistrum* plants having a negligible effect on crop yield.

In our screening of phenoxy resistant *R. raphanistrum* populations, we have observed that although plants survive phenoxy treatment there is a marked lowering in biomass production. However, because of the need to grow treated *R. raphanistrum* plants to maturity to confirm resistance, the effects of these herbicide treatments on biomass production have not been documented (Walsh *et al.*, 2004; 2007). In particular, the effects of phenoxy herbicide treatments on the biomass of resistant populations in the few weeks following treatment have not been investigated. Typically, this period coincides with the early stages of cereal crop biomass production when weed competition has the maximum impact on crop yield (Cousens *et al.*, 2001). Therefore, the initial aim of this study was to determine the effects of phenoxy herbicide treatment on the biomass of phenoxy herbicide-resistant *R. raphanistrum* populations in the few weeks following herbicide treatment. If plants are markedly affected by phenoxy herbicide treatment, then it is possible that crop competition effects will be greater on herbicide treated plants. Subsequently, a second aim was to evaluate the combined effects of wheat competition and phenoxy herbicides on the biomass and reproductive output of herbicide-resistant *R. raphanistrum* populations.

Materials and methods

Phenoxy herbicide dose–response studies

Two 2,4-D amine resistant *R. raphanistrum* populations, (WARR12 and WARR20) originated from cropping fields of the WA wheatbelt. The WARR12 population was collected from a cropping field near Nabawa (Lat. 28.52°S, Long. 48.81°E) in November 2001 with the WARR20 population collected in 2002 from a cropping field in the Wongan Hills region (Lat. 30.88°S, Long. 116.51°E). The susceptible *R. raphanistrum* population (WARR7) was collected in 1999 from a reserve at Yuna (Lat. 28.34°S, Long 115.01°E), where there had been no known herbicide application (Walsh *et al.*, 2004). Following collection, *R. raphanistrum* seeds were removed from their pod segments using a modified gristing mill. Seeds were then stored at 20°C under laboratory conditions until used in subsequent screening studies.

The resistance status of the WARR12 and WARR20 populations to 2,4-D amine was confirmed following

screening in 2004 and 2005 using procedures described by Walsh *et al.* (2007). Based on population survival at the recommended rate of 2,4-D amine (0.5 kg a.i. ha⁻¹), these populations were found to be similarly resistant, as the only previously documented populations WARR5 and WARR6 (Walsh *et al.*, 2004). WARR12 and WARR20 plants surviving this screen were grown to maturity in isolation, to ensure only cross pollination occurred within each population. Seeds were collected from mature plants in December 2005 and stored under laboratory conditions until used in these studies.

To establish the effects of phenoxy herbicide treatment on the biomass of resistant populations, dose–response studies were conducted comparing the response of these populations with that of the known susceptible population WARR7. The three *R. raphanistrum* populations were screened in four dose–response studies, one for each of the four phenoxy herbicides, 2,4-D amine (Amicide 625[®], 625 g a.i. L⁻¹), 2,4-D ester (Estericide 800[®], 800 g a.i. L⁻¹), MCPA amine (MCPA 500[®], 500 g a.i. L⁻¹) and MCPA ester (L.V.E. MCPA[®] 500 g a.i. L⁻¹) all supplied from Nufarm Limited. Experiments were commenced in May 2006 by planting 50 seeds of each population to a depth of 1 × 30 × 45 × 15 cm deep polystyrene trays that were lined with 2 cm of gravel and filled with potting mix (25% moss peat, 25% sand and 50% mulched pine bark). After planting, the trays were then placed in the outside plant growth area at UWA, Crawley campus. Fertiliser was applied to the trays on a weekly basis as a complete liquid fertiliser [N 19% (NH₂ 15%, NH₄ 1.9%, NO₃ 2.1%), P 8%, K 16%, Mg 1.2%, S 3.8%, Fe 400 mg kg⁻¹, Mn 200 mg kg⁻¹, Zn 200 mg kg⁻¹, Cu 100 mg kg⁻¹, B 10 mg kg⁻¹, Mo 10 mg kg⁻¹]. Pots were irrigated as required (field water capacity) to supplement rainfall throughout the duration of the experiment. The plants grew outdoors during the normal growing season for this species and closely resembled field grown plants.

Herbicide treatments were applied at the two-true leaf stage using a dual nozzle cabinet sprayer with a delivery rate of 98 L ha⁻¹ (200 kPa, 4 km h⁻¹) and Teejet[®] 11001 nozzles. Five application rates of each of the phenoxy herbicides were applied to all three populations (Table 1). The resistant populations, WARR12 and WARR20, were exposed to the same application rates (0, 0.25, 0.5, 1.0, 2.0 and 4.0 kg a.i. ha⁻¹ for each phenoxy herbicide) while WARR7, the susceptible population, was exposed to lower rates (0.0625, 0.125, 0.25, 0.5 and 1.0 kg a.i. ha⁻¹ for each phenoxy herbicide). There were three replicates of each treatment. The recommended rate for each of the phenoxy herbicides is 0.5 kg a.i. ha⁻¹.

Plant mortality assessments were made 22 days after herbicide treatment application. Mortality was assessed by visual ratings of the centre growth point of the rosette of each plant. If new green growth was present at the growing point, plants were determined to be alive. At this time, above ground biomass of surviving plants was determined by cutting plants at ground level, oven drying for 48 h at 70°C, then weighing. Plant biomass data were converted to a percentage of untreated control for presentation.

Target-neighbourhood experiment

A target-neighbourhood design (Fig. 1) with six replicates was used to assess and compare the interaction effects of herbicide application and wheat competition on the biomass of the two resistant (WARR12 and WARR20) and the susceptible WARR7 *R. raphanistrum* populations. This design was used to examine the variation in vegetative and reproductive performance of the target species (*R. raphanistrum*) in response to increasing densities of the neighbourhood species wheat (*T. aestivum* L.) (Weiner, 1982; Goldberg & Werner, 1983; Goldberg & Fleetwood, 1987). The target-neighbourhood design was established in a pot experiment

Table 1 Biomass as a proportion of untreated control of plants from WARR7 (S), WARR12 (R) and WARR20 (R) *Raphanus raphanistrum* populations following treatment with increasing application rates of four phenoxy herbicides

Rate	2,4-D amine			2,4-D ester			MCPA amine			MCPA ester		
	WARR7	WARR12	WARR20	WARR7	WARR12	WARR20	WARR7	WARR12	WARR20	WARR7	WARR12	WARR20
kg a.i. ha ⁻¹	Biomass (% untreated control)											
0.06	56.3			50.1			50.1			44.5		
0.13	38.2			48.7			48.7			46.0		
0.25	37.0 c	75.1 b	100.0 a	41.5 c	55.0 b	72.9 a	41.5 b	52.4 a	54.0 a	34.9 b	57.1 a	66.3 a
0.5	37.8 c	52.6 b	96.6 a	40.4 c	52.8 b	61.6 a	40.4 b	55.3 a	52.0 ab	25.6 b	59.0 a	57.5 a
1.0	30.0 b	49.1 ab	73.7 a	31.0 c	45.0 b	64.2 a	31.0 b	45.5 a	49.5 a	31.9 a	55.5 a	51.3 a
2.0		39.7 a	68.9 a		34.7 b	55.8 a		35.8 a	38.0 a		45.9 a	41.9 a
4.0		38.7 a	58.3 a		32.7 a	35.1 a		34.6 a	41.5 a		38.0 a	48.7 a

Values within a column followed by the same lower case letter are not significantly different at $P = 0.05$ level of significance.

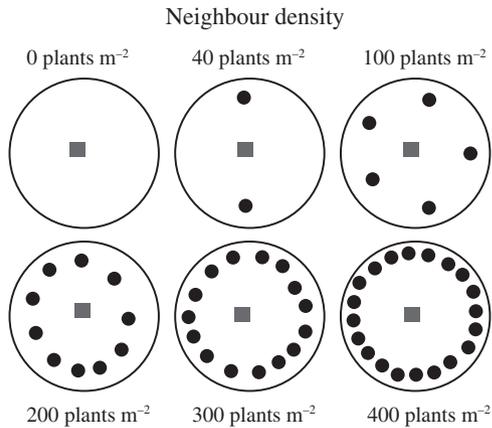


Fig. 1 Overview of experimental design of nearest neighbour experiment showing wheat planting densities.

examining the competitive responses of three *R. raphanistrum* populations to six wheat densities (0, 40, 100, 200, 300 and 400 plants m^{-2}), established by planting 0, 2, 5, 10, 15 or 20 wheat seeds in each pot (Fig. 1).

Raphanus raphanistrum seeds were planted in May 2006 in polystyrene trays filled with gravel and potting mix, described earlier. At the same time, wheat (*T. aestivum* L. cv. Wyalkatchem) seeds were planted into round plastic pots (25 cm diameter \times 23 cm height) also filled with potting mix over gravel. Seeds were planted to a depth of 1 cm in these pots at six densities using a seeding template to ensure uniform plant spacing and uniform distances from each plant to the centre of the pot. Six replicates of each wheat density were planted for each of the three *R. raphanistrum* populations evaluated. At 14 days after planting, target *R. raphanistrum* seedlings at the first fully expanded leaf stage were transplanted into the centre of each wheat-containing pot. Wheat seedlings were at the two-leaf stage at this time and replacement wheat seedlings at the same growth stage grown in separate polystyrene boxes were also transplanted into the pots where wheat failed to germinate. Pots were kept in an outside area at UWA, Crawley campus, where they were arranged in a randomised block design. Fertiliser was applied to the pots on a weekly basis as a complete liquid fertiliser [N 19% (NH_2 15%, NH_4 1.9%, NO_3 2.1%), P 8%, K 16%, Mg 1.2%, S 3.8%, Fe 400 mg kg^{-1} , Mn 200 mg kg^{-1} , Zn 200 mg kg^{-1} , Cu 100 mg kg^{-1} , B 100 mg kg^{-1} , Mo 10 mg kg^{-1}]. Pots were irrigated as required (field water capacity) to supplement rainfall throughout the duration of the experiment.

When *R. raphanistrum* plants had reached the two true leaf stage (14 days after planting), 0.5 kg a.i. ha^{-1} 2,4-D amine treatment was applied. This herbicide treatment was applied using a dual nozzle (Teejet® 11001) cabinet sprayer with a delivery rate of 98 L ha^{-1} (200 kPa, 4 km h^{-1}) as described earlier.

When wheat plants had reached anthesis (August), above ground biomass of the wheat and *R. raphanistrum* was determined by cutting plants at ground level from three of the six replicates before oven drying for 48 h at 70°C and weighing. At wheat maturity (November), *R. raphanistrum* and wheat plants in the remaining three replicates were harvested for the determination of biomass, using the procedures described above. After drying, the spikes were removed from wheat plants which were then hand threshed and the seed collected and weighed. The seed pods were collected from the dried *R. raphanistrum* plants and pod segments counted. Samples of 100 pod segments were collected from each treatment for each population and hand dissected to remove intact seeds. Viability was determined by placing seeds on agar plates in a germination cabinet (25°C 12-h light period, 15°C 12-h dark period). After 4 weeks, any seeds that had either germinated or did not decay were deemed to be viable. The proportion of viable seed in these sub-samples was then used to determine viable seed number from pod segment counts.

Standard error values were generated for *R. raphanistrum* and wheat biomass data at anthesis and maturity, as well as wheat grain yield and *R. raphanistrum* seed production, to show the variation around the mean of three replicates. An ANOVA (GENSTAT, ver. 8.0; VSN International, Ltd., Hemel Hempstead, UK, www.vsn.co.uk) was conducted where LSD values ($P = 0.05$) were used to make within and between population treatment effect comparisons between sprayed and unsprayed levels of biomass, wheat yield and seed production averages at each wheat density.

Results

Phenoxy herbicide dose–response studies

Three weeks after the application of phenoxy herbicides, the biomass of phenoxy herbicide-resistant *R. raphanistrum* plants (WARR12 and WARR20) was reduced by each of the four phenoxy herbicides. The application of the recommended rate of phenoxy herbicides (0.5 kg a.i. ha^{-1}) to the susceptible WARR 7 population resulted in large biomass reductions of around 60–70% (Table 1). However, substantial biomass reductions of 40–50% also resulted from the application of these treatments to the resistant WARR12 and WARR20 populations. The exception was WARR20 which was mostly unaffected by this rate of 2,4-D amine. The lowest application rate of 2,4-D amine (0.25 kg ha^{-1}) produced 23% and 0% reductions in resistant WARR12 and WARR20 plant biomass respectively. However, at higher application rates (4.0 kg a.i. ha^{-1}), biomass was much lower with 60% and 40% reductions in WARR12

and WARR20 plant biomass respectively. In contrast, the lowest application rates of the three other phenoxy herbicides (2,4-D ester, MCPA amine and MCPA ester) resulted in large plant biomass reductions of between 30% and 50% for both populations. Subsequently, plant biomass levels of the WARR12 and WARR20 populations were consistently reduced by about 60% following the highest application rates of each of the four phenoxy herbicides.

The WARR20 population was more tolerant of 2,4-D amine and ester herbicides than the WARR12 population. Plant biomass levels of the WARR20 population were always higher than those of the WARR12 population for all application rates of 2,4-D amine and 2,4-D ester (Table 1). Although WARR20 plant biomass levels were generally only 10–20% higher at most application rates, these differences were large enough to be significant ($P < 0.05$) in many instances. Therefore, even at 3 weeks after treatment, differential responses to herbicide treatment were evident between the two phenoxy resistant populations.

Target-neighbourhood experiment

The combined effects of 2,4-D amine and wheat competition reduced the biomass of *R. raphanistrum* target plants of WARR7 (susceptible) and WARR12 (resistant) populations, but not the WARR20 (resistant) population (Table 2 and Figs 2 and 3). Biomass of plants from the WARR7 and WARR12 populations were reduced by the individual effects of both 2,4-D amine application and wheat plant density (Table 2). However, there were only two instances when the biomass of these populations was significantly reduced by the interaction effect of these treatments, at anthesis for WARR7 and at maturity for WARR12. In contrast, the difficulty in controlling the WARR20 population

was indicated by the lack of effect of 2,4-D amine or wheat density treatments on the biomass and seed production of this population (Table 2). Seed production of *R. raphanistrum* was less affected than biomass, with only the 2,4-D amine treatment reducing the seed production of the WARR7 and WARR12 populations. The individual effects of 2,4-D amine treatment and increased wheat plant density also resulted in increases in biomass at anthesis and maturity for neighbouring wheat plants growing in competition with target *R. raphanistrum* from the WARR7 and WARR12 populations (Table 2). The only significant increase in wheat biomass due to the interaction effect of these treatments occurred at maturity for wheat plants grown in competition with WARR12 and WARR20 populations respectively.

Increasing competition from neighbouring wheat plants in combination with 2,4-D amine treatment led to the control of the phenoxy herbicide-resistant WARR12 *R. raphanistrum* target plants. WARR7 target plants were well controlled by 2,4-D amine (0.5 kg a.i. ha⁻¹) and, therefore, there was no additional effect of increasing plant density on these susceptible plants (Fig. 2A). However, 2,4-D amine treatment did not control resistant WARR12 and WARR20 target plants. In the absence of wheat competition, WARR12 plants survived the recommended rate of 2,4-D amine, however, they did suffer very large losses in biomass production of *c.* 90% (Figs 2B and 3B). The biomass of WARR12 plants was also reduced by competition alone from neighbouring wheat plants where the three highest wheat plant densities of 200, 300 and 400 plants m⁻² resulted in an *c.* 50% reduction in plant biomass (Figs 2B and 3B). At these wheat plant densities, WARR12 plants were killed by 2,4-D amine treatment. The biomass of *R. raphanistrum* plants from the WARR20 population was not reduced by the

Table 2 ANOVA on effects of 2,4-D amine, wheat plant density and their interaction effects on the biomass and yield of *Raphanus raphanistrum* and wheat

Population	Variable	Biomass					
		Anthesis		Maturity		Yield	
		<i>R. raphanistrum</i>	Wheat	<i>R. raphanistrum</i>	Wheat	<i>R. raphanistrum</i>	Wheat
WARR7	2,4-D amine	***	*	***	***	***	*
	Wheat density	*	**	*	**	NS	NS
	2,4-D amine × wheat density	*	NS	NS	NS	NS	NS
WARR12	2,4-D amine	***	*	***	***	***	NS
	Wheat density	**	***	***	**	NS	NS
	2,4-D amine × wheat density	NS	NS	*	*	NS	NS
WARR20	2,4-D amine	NS	NS	NS	NS	NS	NS
	Wheat density	NS	***	NS	***	NS	**
	2,4-D amine × wheat density	NS	NS	NS	*	NS	NS

*** $P < 0.0001$; ** $P = 0.01$; * $P = 0.05$.

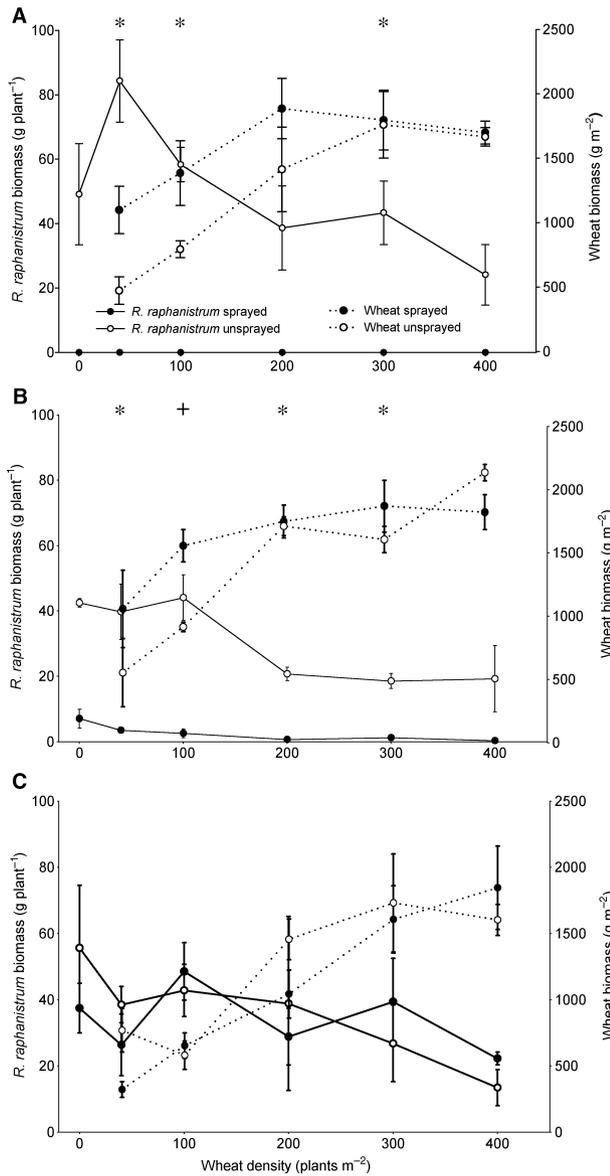


Fig. 2 *Raphanus raphanistrum* and wheat biomass at anthesis of wheat grown as the neighbour population for three target *Raphanus raphanistrum* biotypes, WARR7 (A), WARR12 (B) and WARR20 (C) subjected to increasing wheat plant densities and were untreated or treated with 2,4-D amine (0.5 kg a.i. ha⁻¹). Capped bars represent standard error values showing variation around the mean of three replicates. '*' and '+' indicate significant differences ($P < 0.05$) in *R. raphanistrum* and wheat biomass, respectively, at individual wheat plant densities.

application of the recommended rate of 2,4-D amine (Figs 2C and 3C, Table 2). The effect of wheat competition on this population was also not significant, although there was a general decline in plant biomass with increasing wheat plant densities.

Wheat biomass levels at anthesis were only reduced by *R. raphanistrum* competition when wheat plant densities were low. As expected, wheat biomass values per unit area increased at higher plant densities (Fig. 2).

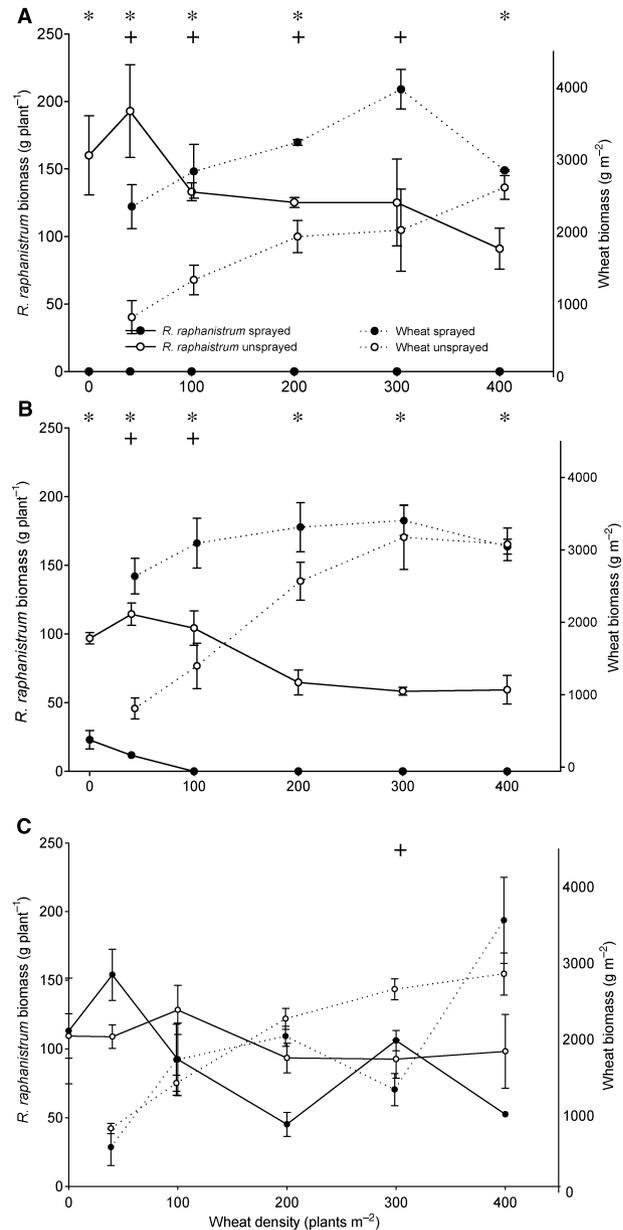


Fig. 3 *Raphanus raphanistrum* and wheat biomass at wheat maturity of wheat grown as the neighbour population for three target *Raphanus raphanistrum* biotypes, WARR7 (A), WARR12 (B) and WARR20 (C) subjected to increasing wheat plant densities and were untreated or treated with 2,4-D amine (0.5 kg a.i. ha⁻¹). Capped bars represent standard error values showing variation around the mean of three replicates. '*' and '+' indicate significant differences ($P < 0.05$) in *R. raphanistrum* and wheat biomass, respectively, at individual wheat plant densities.

However, increases were only recorded when planting densities were increased from 40 and 100 plants m⁻² and no differences were observed between the three highest plant densities (200, 300 and 400 plants m⁻²). At wheat planting densities of 40 and 100 plants m⁻² there were reductions in wheat biomass between 2,4-D amine treated versus untreated for wheat growing in competition with WARR7 and WARR12, but not WARR20

plants (Fig. 2). At these lower wheat plant densities, the observed decrease in wheat biomass between 2,4-D amine sprayed and unsprayed treatments reflects the effect of *R. raphanistrum* plant competition on wheat biomass. In the absence of 2,4-D amine treatment, the competition from WARR7 and WARR12 plants resulted in a wheat biomass reduction of *c.* 50%. However, 2,4-D amine had a reduced effect on WARR20 plants and therefore, with no differences in plant biomass between treated and untreated, the competitive effects of *R. raphanistrum* plants on wheat plants could not be determined.

Reductions in wheat biomass levels due to *R. raphanistrum* competition were larger and more evident at wheat maturity than at wheat anthesis. The trend for decreasing biomass of target *R. raphanistrum* plants in response to increasing wheat plant densities observed at anthesis was also evident at wheat maturity (Fig. 3). However, over the period between wheat anthesis and maturity, *R. raphanistrum* competition effects were likely to have been greater as a result of the considerable growth of *R. raphanistrum* plants over this period. These plants had *c.* 2.5- to threefold increases in biomass compared with 1.5- to twofold increases in wheat biomass over the period between wheat anthesis and wheat maturity. Where 2,4-D amine treatment affected the biomass of WARR7 and WARR12 plants preventing competition effects, there were larger increases (twofold) in wheat biomass levels. These increases were particularly evident at the lowest planting densities (40 and 100 plants m^{-2}) (Fig. 3A and B). However, without herbicide treatments, competition from target WARR7 and WARR12 *R. raphanistrum* plants resulted in lower increases in wheat biomass (1.5-fold) than those observed with herbicide treatment. Consequently, there were larger differences in wheat biomass levels between herbicide treated versus untreated for the three lowest plant densities for WARR12 and the four lowest densities for WARR7 populations.

In the absence of 2,4-D amine treatment, increasing wheat competition reduced seed production of target *R. raphanistrum* plants. However, as a result of the highly variable data, the overall effect of wheat competition on wild radish seed production was not significant ($P > 0.05$) (Table 2). It was evident though, that at higher wheat densities there were markedly lower levels of seed productions in target wild radish plants (Fig. 4). In particular, competition from wheat plant densities of 200 plants m^{-2} caused reductions of 50% and 30% in the seed production of WARR7 and WARR12 plants respectively (Fig. 4A and B). At the highest wheat plant densities (400 plants m^{-2}), wild radish seed production of all three populations was reduced by between 30% and 60%.

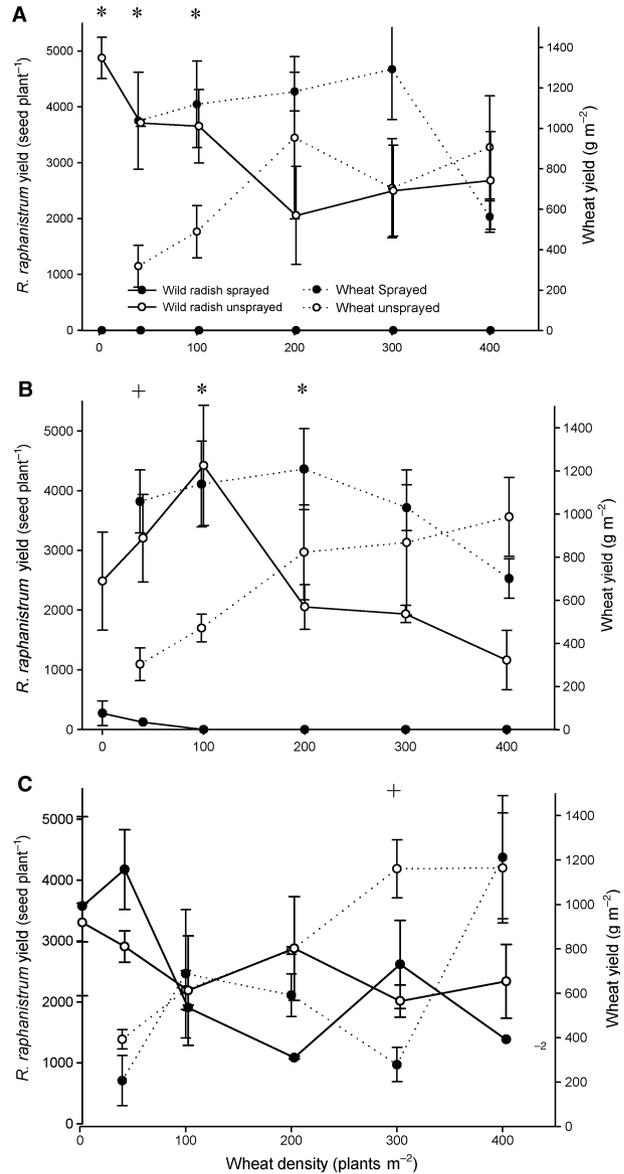


Fig. 4 Viable seed production of *Raphanus raphanistrum* target plants and grain yield of wheat grown as the neighbour population for three target *R. raphanistrum* biotypes, WARR7 (A), WARR12 (B) and WARR20 (C) subjected to increasing wheat plant densities and were untreated or treated with 2,4-D amine (0.5 kg a.i. ha^{-1}). Capped bars represent standard error values showing variation around the mean of three replicates. ** and + indicate significant differences ($P < 0.05$) in *R. raphanistrum* and wheat biomass, respectively, at individual wheat plant densities.

Wheat yields were reduced by the effects of *R. raphanistrum* competition at low planting densities only. Similar to wheat biomass yields, wheat grain yields increased with increasing wheat plant densities with maximum yields recorded at planting densities of 200 plants m^{-2} or higher (Fig. 4). In the presence of *R. raphanistrum* competition, there were large increases in wheat yields at the higher wheat planting densities. However, where *R. raphanistrum* plants were

controlled by 2,4-D amine (WARR7 and WARR12), wheat yields were markedly higher at low plant densities and only minor increases occurred at higher wheat plant densities. Therefore, *R. raphanistrum* competition caused a similar pattern of reductions in wheat biomass and grain yields.

Discussion

This study and our previous work (Walsh *et al.*, 2004, 2007) clearly establishes that populations of *R. raphanistrum* have evolved phenoxy herbicide resistance. These studies found that while both WARR12 and WARR20 are resistant, the WARR20 population displayed a higher level of resistance than the WARR12 population. However, it must be emphasised that plants in both these populations exhibit only a moderate level of phenoxy herbicide resistance. As was observed here, the application of phenoxy herbicides severely affected biomass and reproductive output of resistant plants from the WARR12 and WARR 20 populations. Currently, the mechanism endowing this moderate level of resistance in *R. raphanistrum* is unknown. In fact, both the mode of action of phenoxy herbicides and the basis for resistance to phenoxy herbicides is poorly understood. Of the cases of phenoxy herbicide resistance that have been studied, an altered target site has been demonstrated in a number of weed biotypes (Peniuk *et al.*, 1993; Zheng & Hall, 2001; Goss & Dyer, 2003; Abdallah *et al.*, 2006). However, non-target-site resistance to phenoxy herbicides has also been demonstrated in several weed biotypes as well (Barnwell & Cobb, 1989; Lutman & Heath, 1989; Coupland *et al.*, 1990, 1991; Weinberg *et al.*, 2006). For most of these resistant biotypes, a similar moderate levels of phenoxy herbicide resistance have been observed including *Galeopsis tetrahit* L. (Hemp nettle) (Weinberg *et al.*, 2006), *Kochia scoparia* L. (*Kochia*) and *Daucus carota* L. (wild carrot) (Whitehead & Switzer, 1967).

Phenoxy herbicides continue to be used for *R. raphanistrum* control across the large WA wheatbelt, despite the widespread occurrence of resistance to these herbicides within *R. raphanistrum* populations. Our recent survey of the WA wheatbelt determined that c. 60% of *R. raphanistrum* populations possessed some level of resistance to 2,4-D amine (Walsh *et al.*, 2007). However, despite this high frequency of resistance, phenoxy herbicides continue to be used across this region, frequently achieving satisfactory control of *R. raphanistrum* populations. Therefore, this situation is rather unique in that despite the evolution of phenoxy herbicide resistance in *R. raphanistrum*, these biotypes are often controlled by phenoxy herbicides. As seen

from these studies, it appears that the application of phenoxy herbicides in combination with wheat crop competition will control some phenoxy herbicide-resistant *R. raphanistrum* populations. Despite being resistant to phenoxy herbicides, the application of 2,4-D amine to WARR12 plants growing in competition with high wheat plant densities (≥ 100 plants m^{-2}) led to the control of these plants (Fig. 3B). The impact of enhanced wheat crop competition on *R. raphanistrum* survival has been demonstrated previously (Eslami *et al.*, 2006; Walsh & Minkey, 2006). Reductions in *R. raphanistrum* plant biomass in response to wheat competition effects at higher plant densities has also been reported previously (Walsh & Minkey, 2006). The control of the phenoxy resistant WARR12 population through the combined effects of crop competition and 2,4-D amine, possibly explains the satisfactory level of control currently being achieved in WA wheat systems. However, there is a likelihood that continued reliance on this strategy will lead to the selection of more resistant *R. raphanistrum* populations. This may already be evident in the higher level of tolerance to 2,4-D formulations exhibited by the WARR20 population, which was not controlled by this strategy. Although the more robust level of phenoxy herbicide resistance displayed by the WARR20 population is currently rare, the continued reliance on phenoxy herbicides will most likely increase the frequency of these biotypes.

Despite a long history of use in Australian agricultural systems, phenoxy herbicide resistance was only recently identified in *R. raphanistrum* populations (Walsh *et al.*, 2004). However, given that 60% of *R. raphanistrum* populations in Western Australian crop production systems currently have some level of 2,4-D amine resistance (Walsh *et al.*, 2007), it is likely that resistance has remained undetected for some time. Dose-response and target-neighbourhood studies conducted here clearly showed that although resistant, *R. raphanistrum* plants have a weak resistance mechanism and were severely affected by phenoxy herbicide treatments, suffering large reductions in biomass and seed production. Our studies suggest that this weak resistance mechanism in combination with intense crop competition may have delayed the evolution of, as well as detection of resistance in *R. raphanistrum* populations in Australian cropping fields.

Crop competition is likely to play an ever increasing role in the future management of herbicide-resistant *R. raphanistrum* populations in Australian dryland crop production systems. The potential for higher crop seeding rates and consequently increased wheat crop competition was shown here to lead to the suppression of the biomass and seed production of *R. raphanistrum* (Figs 2–4). Similarly, Walsh and Minkey (2006)

concluded that *R. raphanistrum* seed production in particular was reduced by up to 35% when the conventional wheat seeding rate of 60 kg ha⁻¹ was increased to 180 kg ha⁻¹. In this study, comparable changes in wheat density (i.e. 100–300 plants m⁻²) resulted in 25% and 30% reductions in biomass and seed number respectively for the three *R. raphanistrum* populations examined (Figs 3 and 4). This competition effect on *R. raphanistrum* is not limited to wheat. Blackshaw *et al.* (2002) showed that a 2-week delay in the emergence of *R. raphanistrum* relative to that of oilseed rape, reduced *R. raphanistrum* biomass and seed yield by as much as 60%. Therefore, crop competition can have substantial effects on the biomass and reproductive ability of *R. raphanistrum* populations. Although crop competition, resulting from practical crop plant densities, will not control *R. raphanistrum* populations, competition effects perfectly complement other incomplete control strategies.

Acknowledgements

The authors would like to thank all WAHRI staff and students who were involved in the conduct, analysis and review of this research. We are grateful to the GRDC for providing the funding for this research.

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