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Evidence for an ecological cost of enhanced herbicide metabolism in *Lolium rigidum*

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Running title: ecological cost of herbicide resistance

Summary

1. In some cases, evaluation of resource competitive interactions between herbicide resistant vs. susceptible weed ecotypes provides evidence for the expression of fitness costs associated with evolved herbicide-resistance gene traits. Such fitness costs impact the ecology and evolutionary trajectory of resistant populations.

2. Neighbourhood experiments were performed to quantify competitive effects and responses between herbicide-susceptible (S) and resistant (R) *Lolium rigidum* individuals in which resistance is due to enhanced herbicide metabolism mediated by cytochrome P450.

3. In two-way competitive interactions between the S and R phenotypes, individuals of the S phenotype were the stronger effect competitors on both a per capita and per unit-size basis. The S phenotype also exhibited a stronger competitive response to wheat plants than did the R phenotype, displaying significantly greater (30%) above-ground biomass at the vegetative stage. When subjected to competition from wheat, R individuals produced significantly fewer reproductive tillers and allocated fewer resources to reproductive traits than individuals of the S phenotype.

4. The role of potential mechanisms underlying this resistance cost driven by traits such as plant size and tolerance to low resource availability, as well as the evolutionary implications of the results are discussed.

5. *Synthesis.* Evolved herbicide resistance due to enhanced-herbicide metabolism mediated by cytochrome-P450 in *Lolium rigidum* has been shown to be accompanied with an impaired ability to compete for resources. These results are consistent with the resource-based theory that predicts a negative trade-off between growth and plant defence.

Key-words: enhanced herbicide metabolism, fitness costs, herbicide resistance, plant defence, resource allocation, resource competition, trade-off

Introduction

Organisms with heritable resistance to environmental stresses may have an ecological disadvantage compared to susceptible organisms when the selective force or stress is absent. The resource-based allocation theory predicts that plants divert resources into different organs and functions in order to maximize their ecological success or adaptive strategy under environmental selection (Bazzaz *et al.* 1987, Lerdau & Gershenzon 1997). As environmental resources are limited, any increase in allocation to one organ or function implies a decrease in allocation to other sinks (Lerdau *et al.* 1997). This theory helps to understand the trade-off usually found in plants between growth and defence functions (Coley *et al.* 1985, Chapin III *et al.* 1993, Herms & Mattson 1994). Strong support for the existence of these trade-offs has been shown, for instance, in species from low-resource environments. Species adapted to these conditions display lower inherent growth rates, lower reproductive effort and a qualitatively and quantitatively higher investment in defence compounds (Grime 1977, Coley *et al.* 1985, Chapin III *et al.* 1993).

The evolution of herbicide resistance in plants provides an excellent model system to test the growth-defence trade-off predicted by the resource-based allocation theory (Bergelson & Purrington 1996, Coustau *et al.* 2000). Here we examine a case of where herbicide resistance is endowed by enhanced detoxification capacity. The herbicide resistance mechanism of enhanced rates of cytochrome P450 enzyme activity has been found in herbicide-resistant ecotypes of the major Australian weed *Lolium rigidum* and the European weed *Alopecurus myosuroides* (Owen *et al.* 2007, Hall *et al.* 1995, Délye *et al.* 2007, Preston

et al. 1996, Christopher *et al.* 1991). Thus far the only study examining for any resistance cost associated with the P450 metabolism herbicide resistance mechanism showed a physiological resistance cost manifest as decreased plant relative growth rate (RGR) in resistant *L. rigidum* (Vila-Aiub *et al.* 2005a). Decreased growth in P450-based resistant individuals should result in an ecological resistance cost under competitive conditions (Weiner 1990, Herms & Mattson 1992).

Competitive interactions between plants have two components: plant *effects* on the availability of resources and plant *responses* to changes in resource availability (Goldberg 1990). Effects on and responses to environmental resources are related to a plant's ability to suppress neighbouring plants (competitive effect) and to persist regardless of competitor presence (competitive response). Ultimately, the outcome of plant resource competition will result from differential resource uptake, resource loss and/or resource use efficiency among species (Goldberg 1990). We performed target-neighbourhood design experiments (Weiner 1982, Goldberg & Werner 1983) to determine competitive *effects* and *responses* of herbicide-susceptible and metabolism-based herbicide-resistant *L. rigidum* phenotypes that share a common genetic background (Vila-Aiub *et al.* 2005b). We interpret our results to determine if there is an ecological cost of enhanced metabolism-based herbicide resistance and to relate this cost to the resource-based allocation theory.

Methods

PLANT MATERIAL

The SLR31 *L. rigidum* population is well documented as being resistant to multiple herbicide modes of action (reviewed in Preston & Powles 2002). We have previously determined that the majority of individuals in SLR31 exhibits resistance due to enhanced rates of metabolism

of several herbicides mediated by the cytochrome P450 enzymatic complex (R phenotype) (Christopher *et al.* 1994, Preston & Powles 1998, Christopher *et al.* 1991) and that 10% of individuals are herbicide susceptible (S phenotype) (Vila-Aiub *et al.* 2005b). Random mating in controlled field conditions under relaxed selection (no herbicide) for several generations was allowed before a plant cloning technique was used to identify and isolate discrete lines of the S phenotype and the P450-based herbicide resistant (R) phenotype within this one SLR31 *L. rigidum* population (Vila-Aiub *et al.* 2005b). Both lines were grown and produced seed under the same experimental conditions. These phenotypic lines enable resistance cost comparisons of herbicide resistant vs. susceptible individuals within a single population in a relatively homogeneous genetic background.

EXPERIMENTAL DESIGN

A target-neighbourhood design evaluates the vegetative and/or reproductive performance of an indicator or target plant whose density is held constant under increasing densities and/or biomass of neighbour plants (Weiner 1982, Goldberg *et al.* 1983). This experimental design allows the assessment of the per capita and per unit-size competitive effects of different neighbour species on a single target species as well as the response of different target species to a single neighbour type. Various experiments were performed to test our working hypothesis that P450-enhanced herbicide metabolism is associated with an impaired capacity to compete for resources. Experimental designs aimed to determine (1) competitive effects and responses of the S and R phenotypes when competing between each other (Fig. 1A), and competitive responses of the S and R phenotypes competing within a wheat crop at the (2) vegetative (Fig. 1A) and (3) reproductive stage (Fig. 1B).

Seeds of uniform weight of the S and R phenotypes were germinated in plastic trays containing 0.7% (w/v) agar solidified water at 25 °C / 15 °C with a 12 h light/dark cycle, coinciding the warm phase with the light cycle. Individual seedlings of approximately 2 cm height were transplanted into pots (25 cm diameter x 23 cm height) containing potting mix (50% peatmoss and 50% river sand) according to the planting patterns shown in Fig. 1. Pots were arranged in a completely randomized design and placed outdoors under prevailing field conditions during the normal growing season for this species. Macronutrient concentrations in the potting mix were determined as follows (mg kg^{-1}): NO_3 499, NH_4 2, P 99, K 466, S 323 and organic C 5.88. A slow-release fertilizer (Macrocofe Blue Plus) (12 g per pot) and liquid fertilizer were applied during the tillering phase. Pots were constantly maintained at water field capacity.

In experiments assessing the competitive responses of the phenotypes to increasing density and biomass of wheat plants (see below), transplanting of the *L. rigidum* target seedlings (S and R) and seeding of the neighbour wheat seeds was performed on the same day. Rapid germination and emergence of wheat seeds coincided with the resumption of growth of the transplanted seedlings, ensuring that all experiments evaluated size-symmetric competition between target (*L. rigidum*) and neighbour (wheat) plants (Goldberg 1990).

EXPERIMENT 1: COMPETITIVE EFFECTS AND RESPONSES OF S AND R PHENOTYPES (VEGETATIVE STAGE)

Herbicide-susceptible and P450-based resistant phenotypes were evaluated as target and neighbour plants, resulting in a 2 x 2 matrix of comparisons. Six densities (0, 20, 40, 100, 200 and 480 plants m^{-2}) of neighbouring plants were sown and spatially arranged as shown in Fig. 1A, with each neighbour equidistant from the target plant. Above-ground biomass of target

and neighbour plants was harvested 93 days after transplanting, oven-dried for 72 h at 70 °C and then weighed. There were 10 replicates for each treatment where a target plant was grown in the absence of neighbours, and six replicates for the four pairwise target-neighbour comparisons, giving a total of 140 independent experimental units.

EXPERIMENT 2: COMPETITIVE RESPONSES OF S AND R PHENOTYPES TO WHEAT (VEGETATIVE STAGE)

The competitive response of the S and R phenotypes was evaluated (Fig. 1A) during the vegetative stage when plants were subjected to increasing competition from wheat plants (0, 20, 40, 100, 200 and 480 plants m⁻²). Above-ground vegetative biomass of S and R target plants and neighbour wheat (cv. Westonia) plants was determined 60 days after transplanting as previously described. Treatments in which target plants had no neighbours were replicated 10 times, and there were six replicates for all other target-neighbour combinations, giving 80 independent experimental units.

EXPERIMENT 3: COMPETITIVE RESPONSES OF S AND R PHENOTYPES TO WHEAT (REPRODUCTIVE STAGE)

Estimation of reproductive biomass in the S and R *L. rigidum* cross-pollinated phenotypes was achieved after growing five target plants of each of the S and R phenotypes subjected to increasing wheat densities (0, 40, 80, 120, 200 and 600 plants m⁻²) (Fig. 1B). There were six replicates for each treatment. Prior to flowering, each of the 72 experimental units was encased in a pollen-proof enclosure to prevent cross-pollination between neighbouring treatments. At maturity, seed heads were harvested from target (S, R) and wheat plants and the number of reproductive tillers (spikes) was counted. Above-ground vegetative biomass for

both target and wheat plants was determined as previously described. Seed heads were threshed and total seed mass was recorded. The number of seeds produced per target plant (S_n) was estimated as:

$$S_n = \frac{TS_w \times 50}{S_w} \quad \text{eqn 1}$$

where TS_w denotes the total seed weight produced per plant and S_w represents the mean weight of 50 seeds per plant ($n = 3$). Individual seed weight (ISW) was determined from the average weight of 50 seeds. Harvest index (HI) (%) was calculated as the ratio of seed mass to total above-ground biomass (seed mass + vegetative biomass + chaff). The vegetative biomass and reproductive traits (reproductive tillers, seed mass, seed number) for the S and R plants are expressed as the mean of the five target plants. The competitive responses of the phenotypes were assessed after comparison of regression slopes which describe the response of reproductive target traits (number of spikes, seed mass, seed number, ISW, HI) to increasing competition from wheat.

REGRESSION ANALYSIS AND STATISTICAL ANALYSIS

To standardize for differences in productivity (experiment 1), data for biomass production of target plants in the presence of neighbours were expressed as a percentage of dry matter production for that phenotype in the absence of competition (Goldberg & Scheiner 2001).

Per unit-size and per-individual competitive effects and responses were analysed using a hyperbolic non-linear model to describe the response of the target plants to increasing

density and/or biomass of neighbour plants (Weiner 1982, Goldberg *et al.* 1983, Goldberg & Fleetwood 1987):

$$G = \frac{a}{(1 + b x)} \quad \text{eqn 2}$$

where G represents the biomass of the target plant at neighbour density or biomass x , a is the biomass of the target plant in the absence of competitors (neighbours) ($x = 0$) and b the slope of the regression. The model was fitted by least-squares regression analysis using SigmaPlot software (version 6.0; SPSS Science). The variance in growth of the target plant explained by the density and biomass of neighbours (R^2 of the regression model) indicates the importance of resource competition relative to other factors affecting target plant performance (Goldberg *et al.* 1987).

The relative per capita and per unit-size competitive effects and responses of the S and R phenotypes were established after comparison of regression slopes (b parameter) by one-way analysis of variance (ANOVA). Steep slopes denote strong competitive effects but weak competitive responses (Weiner 1982, Goldberg *et al.* 1983, Goldberg *et al.* 1987). For experiment 2, the hyperbolic model was fitted after log-transformation of data ($y = \log [x + 1]$).

When analysing data from experiment 3, comparisons of the means of slope estimates (b parameter) between the two phenotypes were not statistically possible without violating the ANOVA homoscedasticity assumption. For this reason, the mean competitive responses of the S and R target phenotypes were calculated (as proposed by Goldberg *et al.* 1987, and Weigelt & Jolliffe 2003):

$$CR_A = \sum \frac{G_{Amix}}{G_{Amono}} (1 / n) \quad \text{eqn 3}$$

where CR_A represents the mean competitive response of species A, G_{Amix} denotes the performance (biomass production) of species A in competition across all wheat density treatments, $G_{A mono}$ is the mean biomass acquired by species A in the absence of competition and n is the number of replicated treatments ($n = 30$). This index reflects the changes in reproductive traits of the S and R target species averaged over the entire range of wheat density and biomass. Rankings of competitive responses of the S and R phenotypes to wheat at the reproductive stage were established by ANOVA of mean estimates provided by equation 3.

The magnitude (%) of resistance costs was estimated as $([1 - (\text{resistant biomass} / \text{susceptible biomass}) \times 100])$ for individual target plants averaged over all neighbour densities.

Results

EXPERIMENT 1

COMPETITIVE EFFECTS OF THE S AND R PHENOTYPES AS NEIGHBOURS

When grown as spaced plants without resource competition, individuals of the S phenotype produced significantly ($P = 0.001$) more aerial vegetative biomass (12.6 ± 0.7 g [SE]) than R individuals (8.8 ± 0.6 g).

The hyperbolic model adequately explained ($P < 0.0001$) variations in growth responses of target plants to increasing densities and biomass of neighbouring plants (Fig. 2, 3). Competitive effects of the two phenotypes were evaluated by comparing estimates of

regression slopes: the steeper the slope, the greater the competitive effect of neighbour plants (comparisons within each row in Table 1). As neighbours the S phenotype had a greater competitive effect on R target plants on a per capita basis than if R plants were the neighbour plants (Table 1; Fig. 2 C vs. D). When the competitive effect was evaluated on S target plants, no difference in competitive effect was found between S and R neighbours: both phenotypes displayed a similar ability to reduce the growth of S target plants (Table 1; Fig. 2 A vs. B). Differences in competitive effects between phenotypes which are solely the result of differences in plant size should not be apparent when effects are adjusted by size or neighbour weight (Goldberg *et al.* 2001). This did not occur as S neighbours also showed a greater capacity to reduce the growth of R target plants on a per unit-size comparison (Table 1; Fig. 3). These results indicate that the comparisons of competitive effects between the S and R phenotypes on a per plant basis were also manifested on a per unit-size basis.

COMPETITIVE RESPONSES OF THE S AND R PHENOTYPES AS TARGETS

Regardless of neighbour identity, analysis of overall competitive responses revealed that target R plants showed a higher capacity to continue growing despite the presence of competing neighbours than target S plants (comparisons within each column in Table 1). This stronger competitive response observed in the R phenotype was found in both per capita and per unit-size comparisons (Table 1) and was attributed to the fact that R plants exhibited significantly stronger competitive responses (lower regression slopes) than target S plants in competition with conspecific R neighbours (Table 1; Fig. 2 and 3 B vs. D). There were no differences in the competitive responses of S vs. R phenotypes to the presence of S neighbours (Table 1; Fig. 2 and Fig. 3 A vs. C).

EXPERIMENT 2: COMPETITIVE RESPONSES OF THE S AND R PHENOTYPES TO WHEAT (VEGETATIVE STAGE)

No difference ($P = 0.40$) in aerial biomass production of the S (6.8 ± 0.5 g) and R (7.4 ± 0.6 g) target plants was found in the absence of crop competition. However, when subjected to competition from wheat, significant differences were found in the competitive responses of the two phenotypes (Table 2; Fig. 3). The hyperbolic model provided a good fit ($P < 0.0001$) of the response of S and R target plant biomass to increasing density and above-ground vegetative biomass of wheat plants (per-unit size competitive responses are shown, Fig. 4). Under competition with wheat, herbicide susceptible individuals were stronger response competitors because S plants under increasing wheat competition showed significantly higher biomass production than R target plants (Table 2). The rankings of competitive response between the S vs. R phenotypes were consistent on a per capita and per unit-size basis (Table 2).

EXPERIMENT 3: COMPETITIVE RESPONSES OF THE S AND R PHENOTYPES TO WHEAT (REPRODUCTIVE STAGE)

When grown alone in the absence of competition, there were no significant differences ($P > 0.05$) in number of reproductive spikes, seed mass or seed number of the S vs. R phenotypes (data not shown). However, when grown in the presence of competition from increasing wheat plant density, S individuals showed a significantly stronger mean competitive response than the R individuals for all reproductive traits except individual seed weight, ISW (Table 3). Despite no difference in mean ISW, individuals of the S target phenotype exhibited a linear increase in ISW and harvest index (HI) when subjected to increasing competition from wheat. No such significant response of these reproductive parameters to increasing wheat

competition was found for the R phenotype (data not shown), suggesting that increased harvest index and seed weights were additional key indicators of a strong reproductive response to competition of the S phenotype in comparison to the R phenotype.

Discussion

The results of this study provide clear evidence for a resource-based cost associated with the evolution of herbicide resistance by enhanced P450-associated herbicide metabolism in a major weed species. Herbicide-resistant individuals display an impaired ability to suppress the growth of herbicide susceptible individuals (i.e. competitive effect) and a significantly lesser capacity of vegetative growth and reproductive allocation under competition from wheat plants (i.e. competitive response) when compared to herbicide-susceptible individuals.

This ecological cost associated with enhanced plant metabolism-based herbicide resistance is in agreement with the resource-based allocation theory which predicts a negative correlation between plant growth and resistance to environmental stresses (Lerdau *et al.* 1997, Bazzaz *et al.* 1987, Coley *et al.* 1985). For instance, inducible and constitutive plant chemical defences to deter insect herbivory or pathogenic bacteria have been shown to be associated with reduced plant growth, survival and reproduction (Tian *et al.* 2003, Zavala *et al.* 2004, reviewed in Strauss *et al.* 2002). Furthermore, the expression of fitness costs derived from enhanced insecticide- metabolism resistance mechanism has been also found in resistant insects (Daly 1993, Boivin *et al.* 2003).

The results also reveal that under intense resource competition the herbicide resistance cost is magnified. Individual target plants produced (on average for the S and R phenotypes) 7% less above-ground biomass when competing with neighbouring individuals of the S phenotype than when competing with the R phenotype (Fig. 2). When competing with wheat,

an increase in the magnitude of the resistance cost was observed in individuals of the R phenotype: 30% and 23% at the vegetative and reproductive stage (seed number), respectively. These estimates represent a significant increase in the resistance cost as the intensity of competition increased, coinciding with the prediction that the expression and magnitude of resistance costs become more apparent when environmental resources are limited (Herms *et al.* 1992, Van Dam & Baldwin 2001).

The mechanism driving the expression of resistance costs associated with P450-based resistance is unknown. However, if P450-enhanced herbicide metabolism resistance is due to over-expression of P450 enzymes (Feyereisen 1999) an energy drain leading to higher plant construction costs and allocation of resources that otherwise might be available to other plant functions or growth processes is likely.

THE ROLE OF PLANT SIZE

The ability of plants to reduce the growth of neighbours (competitive effect) and continue to grow despite the presence of neighbours (competitive response) is largely dependent on the capacity to capture resources (Goldberg 1990). If plant size is the major trait involved in conferring an absolute measure of resource uptake, it is expected that large plants will capture more resources than small plants, and thus, larger plants will be stronger effect and response competitors (Goldberg 1990). Such size-driven process is largely manifest as differences in biomass and/or height between competing individuals influence a plant's potential for light interception and creates a continuous positive feedback where large individuals acquire a disproportionate share of the contested resource (Weiner 1990, Schwinning & Weiner 1998).

In agreement with our previous study (Vila-Aiub *et al.* 2005a), in the absence of competition, differences in plant growth between the S and R phenotypes are evident at the

vegetative stage (the S phenotype produced 30% more aerial biomass than the R phenotype) but not at the reproductive stage. The ranking of competitive effects based on the ability to suppress the growth of target species coincided with the rankings of potential maximum plant size between the S and R phenotypes (Table 1). Thus larger neighbouring S plants more strongly suppressed the growth of target R plants than when the neighbour plants were the smaller R plants. This result agrees with other studies in which differences in competitive effects between plant species were driven by differences in plant sizes (Goldberg 1987, Goldberg *et al.* 1987, Miller & Werner 1987, Goldberg 1996). When compared to individuals of the S phenotype, individuals of the R phenotype are likely to acquire resources at lower rates given their inherently reduced RGR and lower net assimilation rate (NAR) (Vila-Aiub *et al.* 2005a). Notwithstanding the previous results, it is acknowledged that no difference in plant size was found between the S and R phenotypes in absence of competition in experiment 2, and this probably reflects differences in the growth period length between experiment 1 (93 days) and 2 (60 days).

Despite the differential size between S and R individuals, no difference in competitive effect was observed on target S plants: respectively large and small neighbour S and R plants displayed the same competitive effect (Table 1). Both these results suggest that intraspecific competition among S individuals was less intense than when there was interspecific competition between the S and R phenotypes.

Accepting that the capacity to capture resources is strongly associated with plant size, it has been predicted that competitive effects and responses will be positively correlated as larger plants will also show a stronger competitive response compared to smaller plants (Goldberg 1990). When grown in competition with wheat, individuals of the S phenotype were clearly stronger response competitors than individuals of the R phenotype, which

exhibited greater reductions in both vegetative and reproductive biomass (Table 2, 3 and Fig. 5). Results also show that under increasing competition with wheat, S plants possess the ability to allocate proportionally more resources to reproductive structures (HI) (Table 3).

THE POTENTIAL ROLE OF LOW RESOURCE TOLERANCE

There are some results that lead us to consider other factors in addition to plant size to provide the S phenotype with a resource uptake advantage over the R phenotype. That plant size alone determined the differential competitive effects on a per capita basis was not confirmed as S individuals continued to show a higher competitive effect than R individuals on a per weight basis (Table 1).

Individuals of the S phenotype were the strongest response competitors against wheat, but the weakest overall response competitors when competing with both conspecifics and R neighbour plants (Table 1). Moreover, pairwise comparisons revealed that R individuals displayed the strongest competitive response when competing with conspecifics (i.e. weak intraspecific competition) (Table 1) (see *ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS*). Additionally, differences in the competitive response of the S and R phenotypes to wheat were not only significant on a per capita but also on a per unit-size basis (Table 2). Taken together, this evidence suggests that differential tolerance to low resource levels may have driven differential competitive responses between the S and R phenotypes (Goldberg 1990). This potentially greater ability of the S phenotype to continue to grow under low resource levels might only be expressed where resource competition is intense, for example, when a wheat crop is present. The competitive effect of wheat on *L. rigidum* target plants was considerably greater than that of other *L. rigidum* phenotypes, as evidenced by the fact that mean target plant weight was markedly lower when competing with 480 wheat plants m⁻²

(highest density) ($0.13 \text{ g target g}^{-1} \text{ neighbour wheat}^{-1}$) than when competing with the same density of *L. rigidum* neighbours ($0.40 \text{ g target g}^{-1} \text{ neighbour}^{-1}$; averaged over the S and R phenotypes as neighbours). These differences in target biomass suggest that the reduced competitive response ability of the R phenotype might only be expressed under intense competitive conditions or significantly resource-limited environments.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

Understanding of fitness costs associated with herbicide resistance genes and their moderation by weed-weed and weed-crop interactions is essential to predict the evolutionary trajectories of herbicide-susceptible and resistant weed species under different ecological conditions. If the impaired resource competitive responses associated with the herbicide-resistant phenotype leads to reduction in plant fitness then selection against those individuals expressing an enhanced herbicide resistance metabolism is expected. This inherent fitness cost will thus contribute to maintain genetic polymorphism associated with herbicide resistance (Antonovics & Thrall 1994).

Despite their impaired ability when competing with the crop and herbicide-susceptible plants, herbicide-resistant plants showed a weak intraspecific competition denoted by the capacity to continue growing in the presence of conspecifics (Table 1). Thus, spatially patchy aggregations of resistant individuals may provide a refuge from which resistance may amplify in a field. It has been shown that intraspecific spatial aggregation may act as a strategy for promoting the survival of weaker competitors (R) that otherwise may be displaced by the superior competitor (S) (Stoll & Prati 2001).

Aside from the implication for herbicide resistance evolution, our study is significant as it demonstrates how intense human-derived selection over just a few generations can select

for altered life-history strategies, based on differential patterns of resource allocation to growth, defence and reproduction. It is now widely acknowledged that evolutionary change is able to take place over ecological timescales (Carroll *et al.* 2007, Hairston Jr *et al.* 2005, Thompson 1998) and that this process can be accelerated by human activity (Palumbi 2001). Our study suggests that weedy and other invasive plant species may be able to rapidly adapt to environmental change through changes in resource allocation. This observation may be particularly timely as we consider the potential impact of climate change on the distribution and management of these species.

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Table 1. Comparisons of competitive effects between neighbour phenotypes and competitive responses between herbicide-susceptible (S) and resistant (R) target phenotypes. Values represent the mean estimate of slopes (parameter b) derived from significant regressions ($y = a / 1 + bx$). Competitive effect estimates of neighbours on each target phenotype are compared within each row. Competitive response estimates of target phenotypes to each neighbour phenotype are compared within each column. Overall competitive response estimates result after evaluation of target response averaged over both the S and R phenotypes as neighbours. Values in parenthesis denote standard error of the mean. P values after ANOVA

		Neighbour phenotype (Per capita)		
Target phenotype	Overall competitive response	S	R	P
S	0.032 (0.004)	0.030 (0.005)	0.036 (0.006)	0.34
R	0.016 (0.003)	0.020 (0.004)	0.012 (0.002)	0.02
P	0.002	0.20	0.0001	
		Neighbour phenotype (Per unit size)		
Target phenotype	Overall competitive response	S	R	P
S	0.26 (0.03)	0.21 (0.03)	0.28 (0.06)	0.09
R	0.17 (0.03)	0.22 (0.04)	0.12 (0.02)	0.03
P	0.04	0.65	0.0006	

Table 2. Comparisons of competitive responses between herbicide-susceptible (S) and resistant (R) target *Lolium rigidum* phenotypes to neighbour (wheat) plants. Values represent the mean estimate of slopes (*b* parameter) derived from significant regressions ($y = a / 1 + bx$). Competitive response coefficients of target phenotypes to wheat are compared within each column. Values in parenthesis denote standard error of the mean. *P* values after ANOVA analysis

Target phenotype	Neighbour (wheat)	
	Density (per capita)	Biomass (per unit size)
S	0.008 (0.0009)	0.039 (0.004)
R	0.016 (0.002)	0.060 (0.006)
<i>P</i>	<i>0.001</i>	<i>0.012</i>

Table 3. Competitive responses (number of reproductive spikes, seed mass, seed number, individual seed weight (ISW) and harvest index (HI)) of herbicide-susceptible (S) and resistant (R) *Lolium rigidum* target phenotypes to wheat competition. Competitive response coefficients are estimated after competition index (equation 3). Competitive responses of target phenotypes to wheat are compared within each column. Values are mean target plant traits expressed as a percentage of the mean for that trait in the absence of competition. Values in parenthesis denote standard error of the mean. *P* values after ANOVA

Target phenotype	Spikes	Seed mass	Seed number	ISW	HI
S	73.2 (3.7)	80.7 (4.7)	74.9 (4.4)	108.8 (2.1)	118.7 (3.9)
R	60.2 (3.2)	62.7 (4.2)	57.7 (3.8)	109.6 (2.6)	105.4 (2.8)
<i>P</i>	0.01	0.006	0.005	0.81	0.007

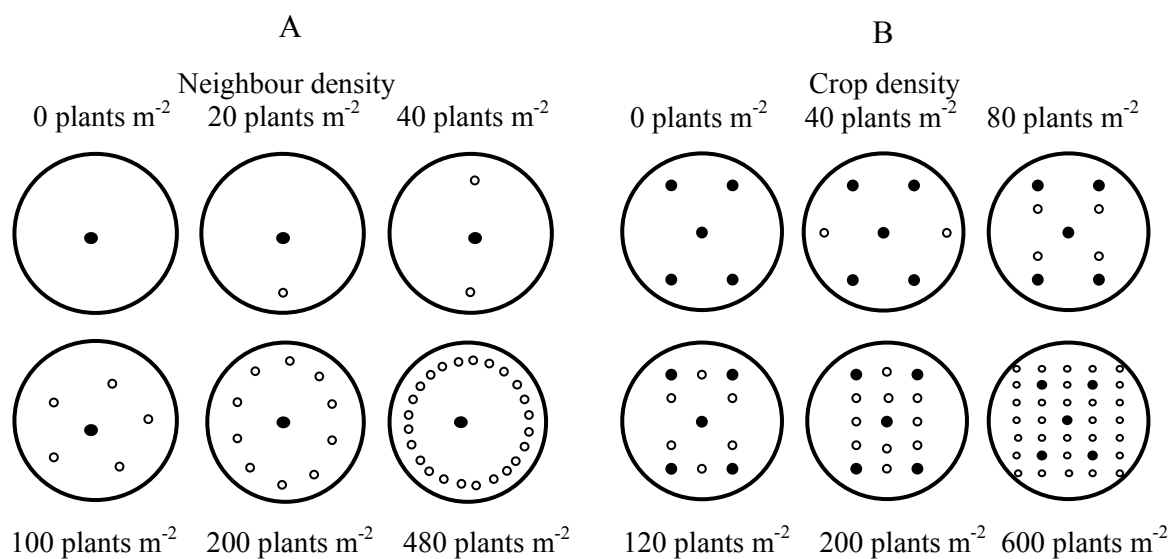


Fig. 1. Overview of experimental designs (target-neighbourhood model) to assess: (A) competitive effects and responses of the herbicide-susceptible (S) and resistant (R) phenotypes in competition with each other or with wheat during vegetative growth (experiments 1 and 2), and (B) competitive responses of the S and R phenotypes when competing with a crop (wheat) at the reproductive plant stage (experiment 3). Closed (●) symbols represent target plants (S or R) at a constant density: 20 plants m^{-2} (A) and 100 plants m^{-2} (B). Open (○) symbols represent neighbour plants (S, R or wheat in A, or wheat in B).

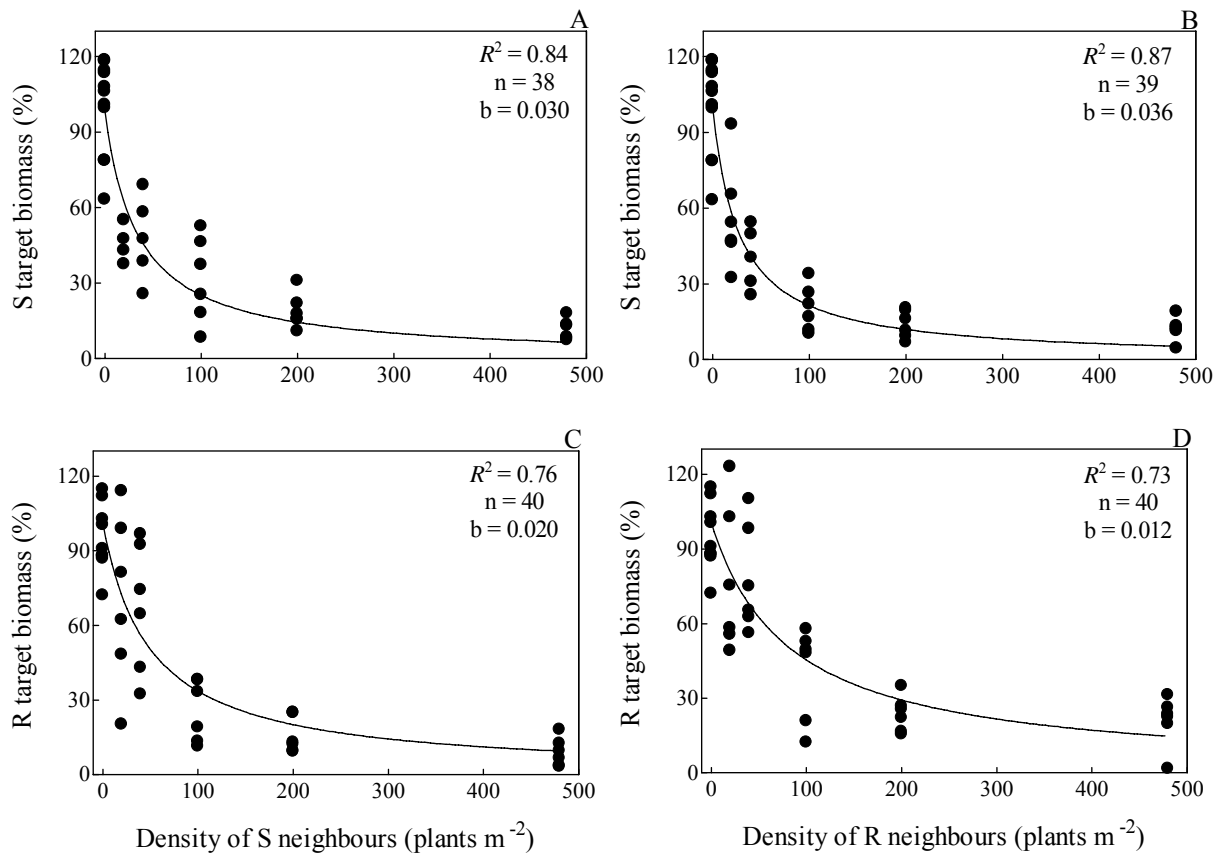


Fig. 2. Relationship between the variations of the above-ground biomass attained by herbicide-susceptible (S) and resistant (R) target plants (as percentage of plant biomass without neighbours) and increasing densities of S and R neighbour plants. Coefficients of determination (R^2) after regression (all data sets $P < 0.0001$) to fit the hyperbolic model ($y = a / (1 + bx)$). The effect of increasing density of different neighbour phenotypes on growth of a target phenotype (per capita competitive effects) and the response of different target phenotypes to increasing density of a neighbour phenotype (per capita competitive responses) is possible after comparison of regression slopes (b parameter of hyperbolic model, see also Table 1).

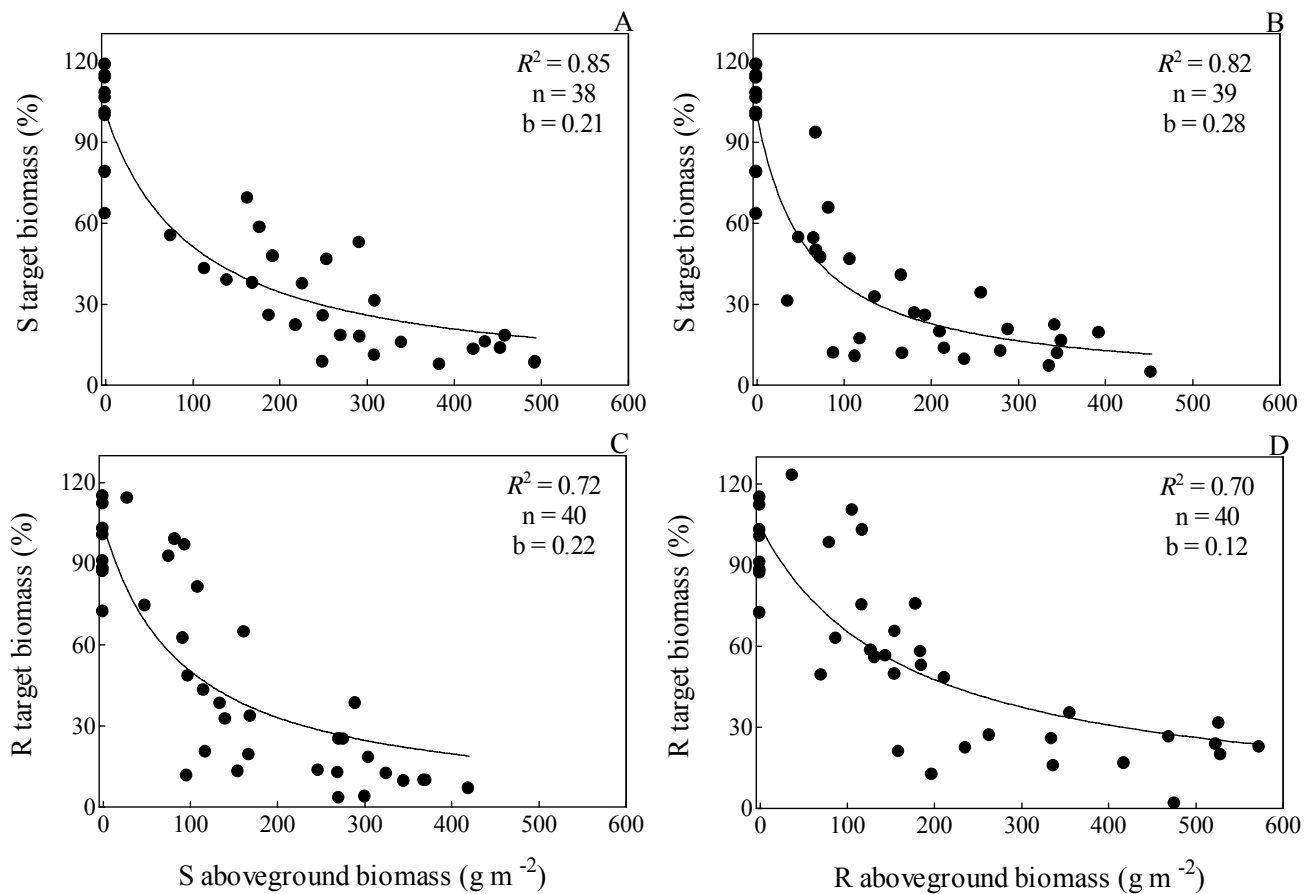


Fig. 3. Relationship between the variations of the above-ground biomass attained by herbicide-susceptible (S) and resistant (R) target plants (as percentage of plant biomass without neighbours) and increasing plant biomasses of S and R neighbour plants. Coefficients of determination (R^2) after regression (all data sets $P < 0.0001$) to fit the hyperbolic model ($y = a / 1 + bx$). The effect of increasing biomass of different neighbour phenotypes on growth of a target phenotype (per unit-size competitive effects) and the response of different target phenotypes to increasing biomass of a neighbour phenotype (per unit-size competitive responses) is possible after comparison of regression slopes (b parameter of hyperbolic model, see also Table 1).

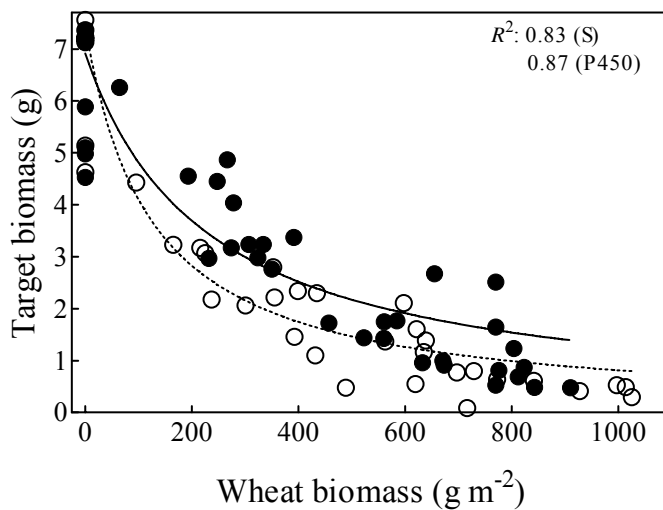


Fig. 4. Response of herbicide-susceptible (S, ●) and resistant (R, ○) target *Lolium rigidum* phenotypes to increasing total above-ground vegetative biomass of wheat neighbour plants. Coefficients of determination (R^2 , all data sets $P < 0.0001$) after regression to fit hyperbolic model ($y = a / 1 + bx$) are presented. Comparison of regression slopes (S, —; R, ---) determines hierarchies in overall per unit-size competitive responses of target plants to wheat neighbour plants (see Table 2).