

How reproductive allocation and flowering probability of individuals in plant populations are affected by position in stand size hierarchy, plant size and CO₂ regime

Caroline Brophy^{1,*}, David J. Gibson², Peter M. Wayne³ and John Connolly¹

¹ School of Mathematical Sciences, Environmental and Ecological Modelling Group, University College Dublin, Belfield, Dublin 4, Ireland

² Department of Plant Biology, Center for Ecology, Southern Illinois University, Carbondale, IL 62901-6509, USA

³ Harvard Medical School Osher Research Center, Landmark Center Suite 22A, 401 Park Dr, Boston, MA 02215, USA

*Correspondence address. Department of Mathematics, National University of Ireland, Maynooth, Co. Kildare, Ireland. Tel: +353-1-708-4641; Fax: +353-1-708-3913; E-mail: caroline.brophy@maths.nuim.ie

Abstract

Aims

We investigate the effect of position within a size-structured population on the reproductive allocation (RA) and flowering probability of individual plants of *Sinapis arvensis*. We also assess the effects of plant size and changing level of CO₂ on both responses.

Methods

Sinapis arvensis L., (field mustard), an annual agricultural weed, was grown in monoculture at six densities under ambient and elevated CO₂ in a study with 84 stands. Individual aboveground biomass and reproductive biomass were measured. Varying density produced a wide range of mean plant sizes across stands and size hierarchies within stands. Many (~40%) individuals had zero reproductive biomass. Employing a novel modelling approach, we analysed the joint effects of position in stand size hierarchy, plant size and CO₂ on RA and flowering probability of individuals.

Important Findings

We found a strong effect of position within the size hierarchy of individuals in a population: for an individual of a given size, greater size

relative to neighbours substantially increased RA and flowering probability at a single harvest time. There was no other effect of plant size on RA. We found a positive effect of elevated CO₂ on RA regardless of position within the size hierarchy. These observed patterns could impact doubly on the reproductive biomass (*R*) of small individuals. First, because RA is not affected by size, smaller plants will have smaller *R* than larger plants; and second, for smaller plants lower down in a population size hierarchy, their RA and hence *R* will be further reduced. These results suggest that size relative to neighbours may be independent of and more important than direct abiotic effects in determining RA. Further studies are required to evaluate how these observed patterns generalize to other populations in non-experimental conditions.

Keywords: asymmetric competition • neighbour effects • non-reproducing plants • stand effects • *Sinapis arvensis*

Received: 10 July 2008 Revised: 2 October 2008 Accepted: 7 October 2008

INTRODUCTION

Understanding the factors governing reproductive allocation (RA) in plants is a key element in understanding species dynamics in populations and communities (Reekie and Bazzaz 2005). The role of plant size as a determinant of reproductive

output in isolated plants is well documented (Mc Connaughy and Coleman 1999; Weiner 2004). The relationship between the RA and size of an individual plant over time (its RA trajectory) may change with density (Weiner 2004) or with varying environmental conditions (He and Bazzaz 2003) and may not be linear (Cheplick 2005). There have been many

investigations of the effects of single factors on the relationship between RA and size for plant species, at the individual (e.g. Damgaard *et al.* 2002) and stand level (e.g. Wayne *et al.* 1999).

In the experiment described in Wayne *et al.* (1999), plants of *Sinapis arvensis* (field mustard), an agricultural weed, were grown in competition and in isolation. Measurements were recorded at the individual plant level although only data at the stand level have previously been published. In this paper, we use the results from this experiment to determine how the RA of *S. arvensis* is affected by its position within the stand size hierarchy. In addition, we investigate how plant size and CO₂ also affect the RA of *S. arvensis*. Few studies have previously examined the combined effect of several factors on the RA of individual plants. We also investigate how these multiple factors affect the flowering probability of individuals of *S. arvensis*.

Density is well known to affect reproduction either at the stand level (He and Bazzaz 2003; van Kleunen *et al.* 2006; Wayne *et al.* 1999) or at the individual plant level (He and Bazzaz 2003). Variation in density will produce stands varying in their mean plant size and will also produce within stand size hierarchies of individuals, perhaps allowing the estimation of an allometric relationship between RA and size (Weiner 2004). However, density may also affect the characteristics of RA trajectories (Weiner 2004): the relationships between RA and mean plant size may not be the same if the plant sizes observed are produced by competition rather than by age. Competition between individuals is frequently asymmetric and produces asymmetrically sized distributions (Weiner and Thomas 1986). In addition to this effect on size, does position in the within-stand size hierarchy affect RA? If allelic frequencies are related to the size of plants in the hierarchy, such an asymmetric response could have a double effect on the future genetic makeup of populations. If RA were unaffected by plant size (i.e. same proportion of biomass allocated to reproduction regardless of size), smaller plants would contribute less reproductive material to subsequent generations (i.e. have lower fitness); but if in addition to this, smaller individuals in a stand are disadvantaged by having a lower RA, this second, asymmetric, effect will further reduce their fitness.

The effect of CO₂ on reproductive output has been broadly documented at the stand level (Ackerly and Bazzaz 1995; He and Bazzaz 2003; He *et al.* 2005; Jablonski *et al.* 2002; Stinson and Bazzaz 2006; Wayne *et al.* 1999) and in recent years has been reported at the individual plant level (He and Bazzaz 2003; He *et al.* 2005) but the results are varied. In a study of 48 wild species, on average, elevated CO₂ reduced RA by 15% (Jablonski *et al.* 2002). Changing level of CO₂ may also affect the relationship between RA and position within a size-structured population.

There are many differences in how reproductive output is measured and in how RA is calculated (Gibson 2002; He *et al.* 2005; Jablonski *et al.* 2002; Reekie and Bazzaz 2005; Stinson and Bazzaz 2006). In this paper, we define RA as the proportion of aboveground plant biomass attributed to

reproductive biomass. Analytic methods available to date for characterizing RA have limited the types of issues that can be usefully addressed. Allometric regression (Harper 1977; Sletvold 2002; Sugiyama and Bazzaz 1998) allows the estimation of RA trajectories under different biotic and abiotic environments. In a linear allometric model relating RA to plant size, significant effects of additional factors in the model imply changes in the RA trajectories (Weiner 2004). In many studies, the data on reproduction are collected at a single destructive harvest (Gibson *et al.* 1999). Allometric regression models derived from such data provide a description of how RA is affected by plant size and other factors at that time. While such allometric approaches can provide insight into developmental effects on RA, static allometric data are not equivalent to sequential measures of individual plants' RA within developing stands. As such, caution must be used when using allometric relationships derived from data at a single time point to interpret the dynamic nature of reproduction–size relationships. The use of allometric regression for analysing RA becomes problematic when some individuals in a population do not produce reproductive structures. Methods proposed for dealing with plants with zero reproductive output (Méndez and Karlsson 2004; Schmid *et al.* 1994; Sletvold 2002; Sugiyama and Bazzaz 1998; Thompson *et al.* 1991) are discussed in Brophy *et al.* (2007). They describe a framework for analysing RA which allows multiple questions to be addressed and includes methods for eliciting biologically useful information from the patterns of non-reproduction. This framework consists of a two-component mixture model; the first component is a truncated regression model and the second component is a logistic regression model. This framework allows all RA values (non-zero and zero) to be modelled simultaneously but incorporates zero values in an appropriate way and allows for multiple reasons for zero RA in plants. RA and flowering probability can be predicted from the model.

We implement this framework in analysing the individual plant level data produced at a single point in time from a study on *S. arvensis* carried out under two CO₂ regimes and at a range of plant densities (Wayne *et al.* 1999). We separate the effect of position within the size hierarchy on RA from the effects of CO₂ and size of the individual. We address the following hypotheses in respect of the RA of individual plants:

- (i) the position of a plant in the within-stand size hierarchy is the main determinant of RA,
- (ii) apart from the effect in (i), plant size is not an important determinant of RA and
- (iii) RA is negatively affected by CO₂.

MATERIALS AND METHODS

Species, growth conditions and measurements

Sinapis arvensis L. (formerly *Brassica kaber* var. *pinnaftida* (Stokes) L. C. Wheeler) (field mustard, charlock, Brassicaceae) is native to Eurasia (Fogg 1950) and is an important

agricultural weed in the mid-western regions of North America (Gleason and Cronquist 1991; Warwick *et al.* 2000). *Sinapis arvensis* flowers in April to July in IL (Mohlenbrock 1980). Plants germinate from seed in early spring, and because of a well-developed and persistent soil seed bank, populations can represent recruitment of seed from several past years (Mulligan and Bailey 1975). At densities of 10–80 plants m^{-2} , *S. arvensis* can produce 5 700–30 100 seeds m^{-2} and significantly reduce yield of spring sown crops by 19–77% (Blackshaw *et al.* 1987). Seed of *S. arvensis* collected from a population in Woodstock, IL (F & J Seed Service, Woodstock, IL, USA), were used in this greenhouse experiment. The experiment included two experimental factors: density and CO₂. On 23 December 1996, seed were directly sown into 84 round 5.5 l, 25 cm diameter pots (stands) filled with a 2:1 mixture of Promix BX (Premier Horticultural Inc., Redhill, PA, USA) and horticultural washed sand. Each pot used a combination of one of six densities and one of two CO₂ regimes. The six densities were 1, 2, 4, 8, 16 and 32 plants per pot corresponding to densities of ~20, 41, 81, 163, 326 and 652 plants m^{-2} . The two CO₂ regimes were concentrations of 350 and 700 $\mu l l^{-1}$. Seeds within all pots were equally spaced and at the two highest densities were sown in a regular hexagonal design. To eliminate the need for transplanting and to minimize initial variation in seedling emergence time (and thus seedling size), numerous seeds were sown at each desired seedling location. Five days after cotyledons began to emerge, seedlings were thinned to one seedling per location, choosing seedlings of similar size within and between pots within each CO₂ treatment. To minimize edge effects that might have affected all but the lowest density stands, a collar of neutral density shade cloth was placed around all pots. The height of the shade cloth was regularly adjusted to match average canopy height within a pot. Location within the pot was not recorded for each individual as it was believed that any advantage to plants located at the edge of a group during the experiment was considerably reduced through the shading device. In addition to light, there may be other resources that can contribute to size hierarchies, such as nutrient and water availability; however, data were not recorded here to address such questions. Conditions in the greenhouse were 70% full sunlight supplemented with metal halide lamps and $26 \pm 2^{\circ}C$ day/ $21 \pm 2^{\circ}C$ night temperatures. These conditions were similar to those used in previous studies of IL annual plants (Garbutt *et al.* 1990; Gedroc *et al.* 1996; Mabry and Wayne 1997; Mc Connaughay and Coleman 1999; Wayne *et al.* 2002).

The experiment was laid out as a split plot design with three blocks (greenhouses), each containing two main plots, one for each of the two CO₂ concentrations. Each main plot contained 14 stands (subplots), with the lowest density (1 plant per pot) replicated four times and the other five densities each replicated twice. Within main plots, pot locations were randomized approximately every 10 days. The purpose of having a wide range of densities was to create differences in mean plant sizes across stands and to produce within-stand-size hierarchies to

assess the effects of CO₂ on RA over a wide range of competitive conditions. Intra-specific competitive effects have been observed in field populations of *S. arvensis* at densities >20 plants m^{-2} (Edwards 1980). In common with other crucifers, *S. arvensis* is insect pollinated (Fogg 1950; Warwick *et al.* 2000) and has a single locus, multiallelic sporophytic incompatibility system (Ford and Kay 1985).

The 84 stands were harvested on 17 February 1997, when a large number of flowers had matured into fruits but before many leaves had senesced (leaf area index = 2.9 and 2.1 for ambient and elevated CO₂, respectively). After separating leaves and support structures (stems and petioles), above-ground biomass for all available individual plants was oven dried at 70°C for 1 week and weighed giving a total of 704 responses. The biomass of all reproductive structures (flowers and fruits) of each of these individuals was also measured. Further experimental details are in Wayne *et al.* (1999).

Statistical analysis and modelling

To analyse these data, we followed the modelling approach detailed in Brophy *et al.* (2007). We use the following notation, where the prefix L indicates the natural logarithm (log) of the variable. For the *i*th plant in the *j*th stand M_{ij} (LM_{*ij*} for logarithm) is aboveground biomass, R_{ij} is biomass of all reproductive structures and RA, $RA_{ij} = R_{ij}/M_{ij}$, is the ratio of the biomass of all reproductive structures to the biomass of all aboveground structures. LRA_{*ij*} is the log of RA_{*ij*} except when RA_{*ij*} = 0, where we define LRA_{*ij*} to be the log of the minimum non-zero *R* minus LM_{*ij*}. The value LR takes when *R* = 0 can be included in the model (described in detail later in this section) as a parameter and tested using maximum likelihood (lambda in Brophy *et al.* 2007). The log of the smallest non-zero *R* (=0.005g) is the maximum-likelihood estimate of this parameter agreeing with our definition of LRA when LR = 0. The average aboveground biomass of individual plants in a stand is \bar{M}_j . The size of an individual relative to the average size of its neighbours within its stand is LRatio_{*ij*} = log(M_{ij}/\bar{M}_j) on the logarithmic scale. Ratio = 0.5, 1 and 2 indicates an individual plant is half, equal to and double the stand average plant size, respectively. Stands 1–42 (43–84) were grown at ambient (elevated) CO₂. The index variable CO₂ takes values 0 and 1 for ambient and elevated levels of CO₂, respectively.

Initial exploratory analyses showed no evidence of block or main plot variation, so the split plot nature of the design was ignored in subsequent analysis. Similar findings were reported on the same experimental data in Wayne *et al.* (1999). A preliminary view of the data (Fig. 1) suggests a strong relationship between LR and LM among reproducing plants at both ambient and elevated CO₂. However, there were many plants that did not reproduce (~40% of all individuals) and some of these (circled in each panel of Fig. 1) were large plants that could not be part of the LR/LM relationship. These data suggest two groups of plants in the population. The first group follows the LR/LM relationship; this group includes all reproducing individuals and some small individuals that did not reproduce.

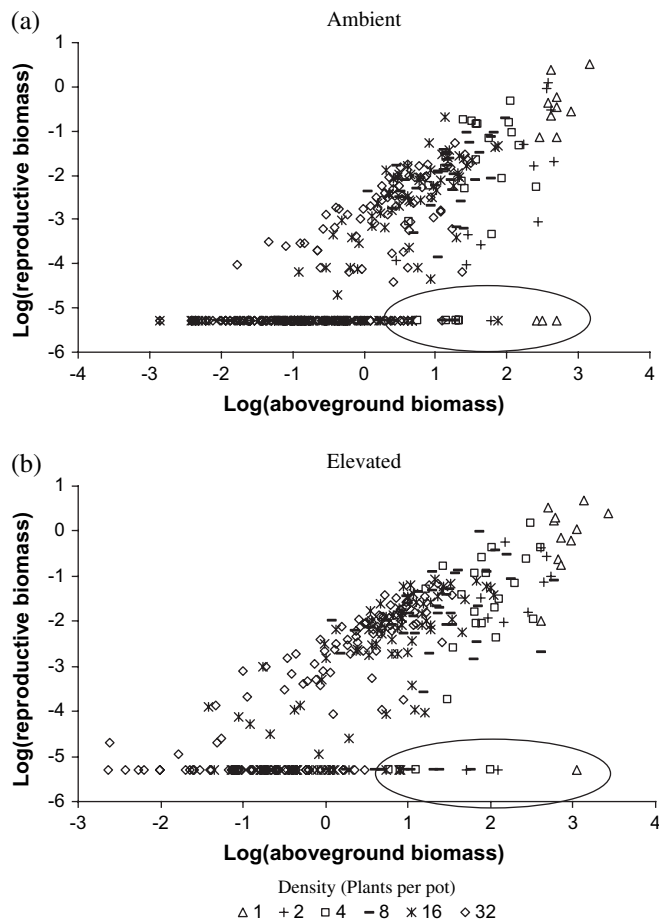


Figure 1: log(reproductive biomass) versus log(aboveground biomass) for plants of *Sinapis arvensis* grown at different densities (# plants per stand) under ambient and elevated CO₂. Note: The log of non-reproducing plants is defined as -5.299 , the log of the smallest non-zero reproductive biomass. Circled values highlight the number of large individuals that did not reproduce. Panel (a) is a more detailed version of Fig. 1 in Brophy *et al.* (2007).

The second group does not follow this relationship and this group consists solely of the remaining non-reproducing individuals. We modelled LRA assuming these two groups (Brophy *et al.* 2007). (Note that since $LRA = LR - LM$, a linear regression of LR on LM is directly related to a linear regression of LRA on LM, the intercept is the same and the slope is changed by -1 .) Within the first group (Group 1), we assumed a truncated linear allometric relationship between LRA and LM (Amemiya 1985; Gelfand *et al.* 1997; Schmid *et al.* 1994) and tested the effect of additional variables on this relationship. Non-reproducing individuals can arise in either group but cannot be unambiguously assigned to one or other group. We say that a non-reproducing individual is in Group 2 with probability P , and in Group 1 with probability $1 - P$. This probability has to be estimated from the data and may depend on a number of factors, including size and CO₂ level. Including a range of densities in the experiment was primarily to produce a range of

plant sizes but its effect as an explanatory variable instead of and in addition to plant size in both components of the model was also tested. We jointly modelled the allometric relationship and P using the two-component mixture model framework of Brophy *et al.* (2007). The algebraic forms of the two components are given in equations (1) and (2) below. Note that the explanatory variables included in these equations were those that appeared in the final models after testing the inclusion of explanatory variables and their interactions using likelihood ratio tests (Pawitan 2001). The allometric model is of the form

$$LRA_{ij} = \beta_0 + \beta_1 LM_{ij} + \beta_2 CO_2 + u_j + \varepsilon_{ij}, \quad (1)$$

where β_0 is the intercept, β_1 and β_2 are regression coefficients of the terms LM and CO₂, respectively, u_j is a random effect for the j th stand that induces correlation between all responses within the stand (Verbeke and Molenberghs 2000) and ε_{ij} is the residual term. The random terms ε_{ij} and u_j are assumed to be independently normally distributed with mean zero and variances σ_1^2 and σ_2^2 , respectively. The model for P is

$$\log\left(\frac{P_{ij}}{1 - P_{ij}}\right) = \alpha_0 + \alpha_1 LM_{ij} + \alpha_2 CO_2 + \alpha_3 LRatio_{ij} + \alpha_4 LM_{ij} \times LRatio_{ij} + w_j \quad (2)$$

where α_0 is the intercept, α_1 , α_2 , α_3 and α_4 are regression coefficients of the terms LM, CO₂, LRatio and the interaction between LM and LRatio, respectively, and w_j is a normally distributed random effect for the j th stand assumed to have mean zero and variance σ_3^2 and its covariance with u_j is γ . The logit transformation is used to ensure that estimates of P lie between 0 and 1 (Collett 1993). Inclusion of the random terms for stand (σ_2^2 , σ_3^2 and γ) was tested using likelihood ratio tests (Pawitan 2001). The Bayesian Information Criterion (BIC) statistic (Schwartz 1978) was used to test for the presence of the second group of non-reproducing plants (Mc Lachlann and Peel 2000). The model was fitted using the NLMIXED procedure in the SAS/STAT software, Version 9.1 (SAS Institute Inc., Cary, NC, USA).

The fitted mixture model can be interpreted in several different ways which we discuss here to avoid confusion in the Results section. RA can be predicted from the model in two ways; from equation (1) and therefore predicted RA is conditional on being in Group 1 or from the combination of equations (1) and (2) when predicted RA is for the experimental population for specific values of covariates (i.e. for the two groups combined). We will differentiate between the two types by referring to RA predicted from equation (1) as 'RA conditional on being in Group 1' and referring to RA predicted from the two components of the mixture model as 'RA'. Using equation (2), we can predict P , the probability of being in Group 2. However, biologically it is of more interest to predict what the probability of flowering is for *S. arvensis*. This can be done in two ways: using equation (1) (note that Group 1 also contains zero RA values) and hence the probability is conditional on being in Group 1 or by combining equations (1) and (2)

and predicting flowering probability for the experimental population for specific values of covariates. Here when we refer to flowering probability, we mean the latter. Thus, our overall interpretations and conclusions from this paper are based on the assessment of both RA and flowering probability from the combination of the two components of the model.

We predicted the probability of flowering (producing reproductive biomass) and RA from the model for a range of values of the explanatory variables in equations (1) and (2) and used bootstrapping (Efron and Tibshirani 1993) to calculate standard errors for the predictions and to test for specified differences between these predictions. A thousand bootstrap data sets were constructed by re-sampling with replacement at the stand level within each density by CO₂ combination and again at the plant level within stand. The model was fitted to each of these data sets. The standard error for any prediction from the original model was calculated as the standard deviation of the predictions obtained from these 1000 models. A bootstrap interval using the BC_a method (Efron and Tibshirani 1993) was calculated for the difference between particular predictions of interest and the significance of any difference was determined by whether or not this interval contained zero.

RESULTS

Manipulating density successfully generated a large range of average stand plant sizes (\bar{M}_j = average of individuals within a stand) across the 84 stands. The smallest (0.76 g) and largest (30.69 g) average plant sizes were found in stands at the highest density (32 individual plants per pot) and the lowest density (1 individual plant per pot), respectively. The variable Ratio (= M_{ij}/\bar{M}_j) measured an individual plant's position within its size hierarchy and this variable ranged from 0.07 to 5.03 (i.e. there was an individual plant with size 7% of its stand average plant size and another individual plant with size five times larger than its stand average plant size).

The large individuals with no reproductive biomass (circled values in Fig. 1) suggested two groups within the population. Using the BIC statistic, we found strong evidence for a second group of non-reproducing plants that did not follow the allometric relationship between RA and M (smaller BIC value indicates better model). We observed BIC = 678 and 1143 for the models with and without the second group, respectively, i.e. a much smaller BIC for the model including the second group. For the allometric regression model for Group 1, LRA conditional on being in Group 1 decreased as biomass (LM) increased and it increased under elevated CO₂ ($P = 0.069$) (Table 1). The probability, P , of being in Group 2 was related to LM, CO₂, LRatio and the interaction between LM and LRatio (Table 1); P decreased as biomass, CO₂ and Ratio increased but the effect of Ratio was more pronounced in smaller plants. Using density instead of size in the models did not provide a better fit. Density also did not have an effect in addition to that of size in the model of Group 1 but there was some evidence ($P =$

Table 1: parameter estimates, standard errors and significance levels for the allometric regression model for LRA conditional on being in Group 1 (equation (1)) and the model for P , the proportion in Group 2 (equation (2)).

	Parameter*	Estimate	Standard error	P value**
Equation (1)	Constant	-3.14	0.074	-
	LM	-0.11	0.041	0.011
	CO ₂	0.15	0.080	0.069
Equation (2)	Constant	-0.20	0.267	-
	LM	-1.00	0.230	-
	CO ₂	-0.68	0.309	0.034
	LRatio	-1.28	0.292	-
	LM*LRatio	-0.39	0.170	0.030
Variance components	σ_1^2	0.468		
	σ_2^2	0.021		
	σ_3^2	0.410		
	γ	-0.059		

*LM = log(aboveground biomass), CO₂ is coded 0 and 1 for ambient and elevated CO₂ respectively, LRatio = log(individual size relative to the average size of plants in its stand), σ_1^2 is the variance within stand, σ_2^2 is the variance of u_j , the random stand term in equation 1, σ_3^2 is the variance of w_j , the random stand term in equation 2, γ is the covariance between u_j and w_j .

**Calculated using likelihood ratio tests. In equation (2), likelihood ratio tests were not carried out for the terms LM and LRatio since they are involved in a higher order interaction and therefore cannot be excluded from the model. Likelihood ratio tests were also not carried out for the constant in either equation as there is no biological justification for excluding these terms from the model.

0.046) for including it in addition to size in the model for P . However, the effect of density was very small relative to the effect of plant size and so it was excluded from both components of the model.

RA and flowering probability were predicted by combining predictions from the models of Groups 1 and 2 and are shown for various combinations of Ratio, CO₂ and size in Fig. 2a and b, respectively. The model was not used to predict RA or flowering probability at Ratio = 0.5 >4 g or at Ratio = 2 <2 g as these values were outside the range of the data. Standard error bars for predicted RA and predicted flowering probabilities for plants of size 2 g are in Fig. 2 (calculated as the standard error over the 1000 predictions from the bootstrap samples).

The P values in this paragraph and the next two are based on the 95% bootstrap BC_a interval for each effect on RA and probability of flowering as predicted by combining the two components of the mixture model. (Note: If zero is not in the BC_a interval, the effect is significant at $\alpha = 0.05$ and otherwise the effect is non-significant.) The higher a plant's position in the size hierarchy (measured by Ratio), the higher its predicted RA (Fig. 2a). A plant of size 2 g grown at ambient CO₂ allocated on average 2.3% of aboveground biomass (size) to

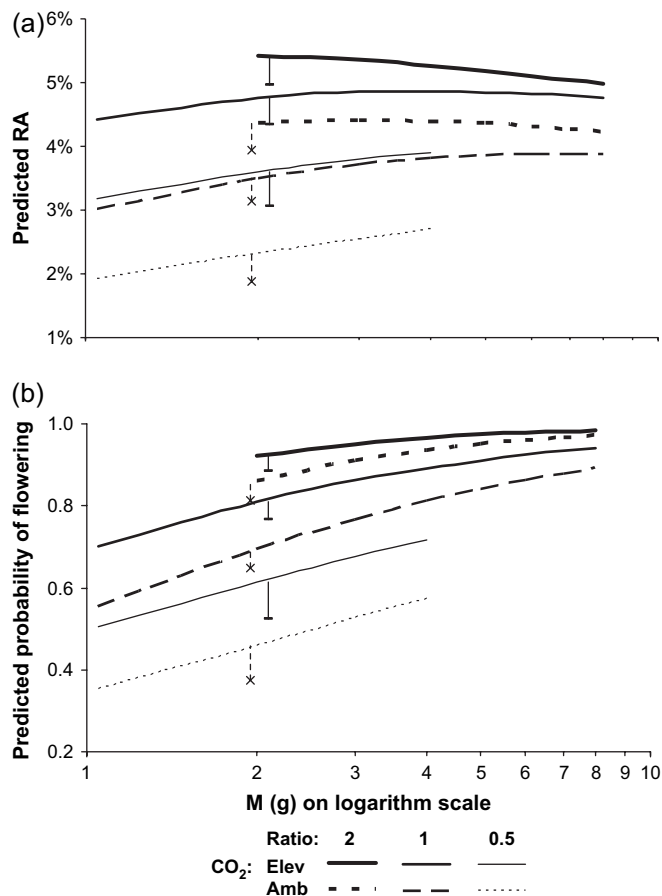


Figure 2: (a) Predicted RA (%) and (b) predicted probability of flowering versus aboveground biomass, M (g), on the log scale for various combinations of Ratio and CO₂. Predictions in (a) and (b) are combined predictions from the estimated equations (1) and (2) presented in Table 1. Predictions for each CO₂ × Ratio combination are restricted to the range of M values observed in the experiment. Standard error bars are presented for predictions at 2 g and are staggered for illustration; error bars from the lines representing ambient (elevated) CO₂ are to the left (right) and are dashed (solid) with the symbol × (-) at the end.

reproduction if it was half the average plant size in the stand (Ratio = 0.5) but this increased to 3.5 and 4.4% if the plant was equal to (Ratio = 1) or double (Ratio = 2) the average stand plant size, respectively. This is close to a 2-fold increase in RA. The effect of Ratio was significant at 2 g as each of the pairwise comparisons between these three predictions were significant ($P < 0.05$ for each comparison). By comparison, a plant of size 8 g grown at ambient CO₂ allocated 3.9 and 4.2% to reproduction if it was equal to or double the stand average plant size, respectively, and these predictions differed ($P < 0.05$). There was no evidence of a difference between the comparison of Ratio = 1 and 2 at 8 g to the same comparison for a plant of size 2 g.

Despite this strong relative size effect, there was no evidence for an effect of plant size on RA (Fig. 2a). We found no evidence of any interaction between CO₂ and either Ratio or

M . The effect of CO₂ was always positive and almost always significant, e.g., at Ratio = 1 the effect of CO₂ was significant at $M = 2$ g ($P < 0.05$) and at $M = 8$ g ($P < 0.05$).

The higher a plant's position in the size hierarchy (measured by Ratio), the higher its probability of flowering (Fig. 2b); at ambient CO₂, a plant of size 2 g has a probability of flowering of 0.86 if it is double the average stand size of plants within its stand compared to 0.69 if it is equal to or 0.46 if it is half the average stand size. Flowering probability increased with increasing size; this effect was measured by comparing the predictions for M values at the beginning and end of each of the six lines in Fig. 2b, representing each Ratio by CO₂ combination ($P < 0.05$ for four of the six tests). The effect of CO₂ on flowering probability was always positive but this effect was not usually significant.

Inclusion of the random terms for stand (σ_2^2 , σ_3^2 and γ) was a necessary feature of the model ($P = 0.003$, 3 degrees of freedom (df)). The covariance between the two random stand effects was not strong ($P = 0.237$, 1 df); there was strong evidence for a random stand effect in the model for P ($P = 0.002$, 1 df) but not in the allometric regression model ($P = 0.129$, 1 df).

DISCUSSION

The primary result from this experiment is the importance of position within stand size hierarchy on the RA of plants, with substantially reduced RA of plants of a given size when with larger as opposed to smaller neighbours (supporting Hypothesis 1). There was no evidence of an additional effect of plant size on RA (supporting Hypothesis 2). This was a trade-off between a negative effect of size on RA conditional on being in Group 1 (equation (1) Table 1) and a positive effect of size on flowering probability (Fig. 2b). There was a positive effect of CO₂ on RA (contradicting Hypothesis 3). The new analytical framework described in Brophy *et al.* (2007) proved useful in testing the relative importance of a variety of mechanisms influencing RA, while simultaneously accounting for others. Such a joint assessment of multiple factors allows a comparison of their relative importance within a single study and a single analysis.

Our results show that growing plants in a competitive environment can dramatically modify their RA. A plant of a given size (e.g. 2 g) low down in the stand size hierarchy with Ratio = 0.5 would allocate much less to reproduction than another plant of the same size but from a stand where it was high in the hierarchy with Ratio = 2 (Fig. 2a). This asymmetric effect was largely driven by a strong positive effect of Ratio on flowering probability (Fig. 2b), which could be due to below average sized plants in a stand being shaded by their larger neighbours and so not receiving as much light, perhaps delaying their flowering (Schwinning and Weiner 1998; Steinger *et al.* 2003). Smaller plants can suffer a double penalty in contributing to stand total R (biomass of reproductive structures). On average, RA was almost constant at the stand level across

all plant sizes and so their contribution to R is automatically lower than that of larger plants. In addition, when they are the smaller plants in a stand size hierarchy (Ratio < 1), their RA is reduced and hence their R is further reduced. This observation may have implications for the fitness of slow developing plants in a competitive setting, if this reduction in RA reflects a consistent downward shift in the allocation trajectories of such individuals. Asymmetric responses in plant biomass to competition have been observed in individuals in monoculture populations (Damgaard and Weiner 2008; Weiner and Thomas 1986) and between species in mixed communities (Connolly and Wayne 1996). Our study extends this result to RA.

We found very little effect of average plant size on RA, agreeing with Cheplick (2005) for annual plants. The negligible effect of size on RA (Fig. 2a) is caused by a trade-off (Weiner 2004) of opposite effects: the negative effect of size on RA in equation (1) (Table 1) and the positive effect of plant size on flowering probability (Fig. 2b). Our results show that size tightly controls the probability of/ability to flower, agreeing with the findings of Méndez and Karlsson (2004). Our study supports the finding that density is one of the main components in determining plant size but has little direct effect on RA (Samson and Werk 1986; Sugiyama and Bazzaz 1998). Equivocal results of previous studies investigating the relationship between RA and density (Karlsson and Méndez 2005) may be partly caused by ignoring the effect of size relative to neighbours and the random stand effect.

The positive (but not significant) effect of CO₂ on RA conditional on being in Group 1 (Table 1) and on flowering probability (Fig. 2b) combined to give a positive effect of elevated CO₂ on RA (Fig. 2a) (e.g. average percentage increase in RA of 39% at 2 g). This is in contrast with the average negative effect reported for wild species in the meta-analysis of Jablonski *et al.* (2002). However, of the 48 species used in that analysis, the maximum response of RA to elevated CO₂ was 64% and six species showed an increase greater than 39% (P. Curtis, personal communication). Our results for *S. arvensis* emphasize that the effect of CO₂ on RA is species specific and varies greatly among species.

The benefits of temporal sampling of populations and individuals within competitive stands over the static approach inherent in studies based on a single harvest have long been argued (Connolly *et al.* 1990; Damgaard *et al.* 2002; Mc Connaughay and Coleman 1999). Nevertheless, most experiments on plant competition use only a single harvest (Gibson *et al.* 1999). In a population that is only harvested at a particular time, plants may be at different ontogenetic stages and much of the observed variation in RA may be due to ontogenetic drift (Mc Connaughay and Coleman 1999), abiotic environment, density or neighbour relationships, or position on non-linear allocation trajectories. Nevertheless, it is quite appropriate to model RA across various populations at a given time to describe how it is affected by plant size and other factors at that time. These relationships should be interpreted with caution as a description of the dynamic nature of reproduction–size rela-

tionships and of the ultimate implications for the fitness of individuals. Alternative models that allow the trajectories of individuals to be non-linear or to approach some boundary trajectory at a certain stage in their development (e.g. Aigner *et al.* 1977) may be more appropriate to an overall study of developmental trajectories but they require multiple time point data which are rarely available. Were additional temporal data available our modelling framework could be adapted to deal with these issues.

Regression of RA on M has been criticized because M is the denominator of RA (Samson and Werk 1986). However, our model regressing the log of the ratio of reproductive to total mass as a function of log of total mass is a standard example of compositional data analysis (Aitchison 1986).

In this paper, we provide the first application of the general framework of Brophy *et al.* (2007) which can handle zero values and allows for more than one cause for zero reproductive output. Instead of viewing a lack of reproductive output as a nuisance or irrelevant, our search to explain it shows that the patterns of the zero reproductive output values contain valuable biological information. It has identified the large effect of position within the stand size hierarchy on flowering probability and on RA (Fig. 2). In this experiment, ~40% zero RA values were observed. Such high numbers of zero values have been reported in the literature previously (e.g. Gibson *et al.* 2002); however, it is difficult to estimate exactly how common zero RA values are due to the equivocal ways in which they have been dealt with (Brophy *et al.* 2007). It is possible that zero reproduction is underreported in the literature; this would be particularly likely in studies with high numbers of zero reproductive values.

Failure to reproduce can be due to a number of causes such as small plant size (Schmid *et al.* 1994; Sugiyama and Bazzaz 1998), day length, temperature, red/far red ratio or genetic make-up (Franklin and Whitelam 2004; He *et al.* 2005). While we may expect many small plants not to reproduce (Schmid *et al.* 1994; Weiner 2004), the larger plants with zero RA (circled in Fig. 1a and b) are more unusual. One explanation is the possible occurrence within our original field collection of *S. arvensis* seed of genetically distinct populations that differ in their ability to reproduce under our range of experimental conditions. Populations of *S. arvensis* show high within-population genetic variability (Warwick *et al.* 2000) including light- and dark-coloured seed morphs with different germination capacities (Fogg 1950; Luzuriaga *et al.* 2006) that may be adapted to different flowering thresholds. If the only reason for these larger plants not reproducing was genetic make-up or size-independent physiological mechanisms, the only term in equation (2) would be the intercept but clearly this is not the case here.

Overall, the analysis from our experiment confirms the existence of two distinct groups in the data, the major importance of position in the competitive within stand size hierarchy in determining flowering probability and RA and positive effects of CO₂ on both of these responses.

FUNDING

Environmental Protection Agency (Ireland) Doctoral Scholarship to C.B.; Enterprise Ireland International Collaboration grant to J.C. and US Department of Agriculture to D.J.G.

ACKNOWLEDGEMENTS

We acknowledge and thank Peter Curtis and Ed Reekie for useful discussions and advice. We acknowledge the helpful comments of Adrian Dunne, John Finn, Laura Kirwan and Kristina Stinson.

Conflict of interest statement: None declared.

REFERENCES

- Ackerly DD, Bazzaz FA (1995) Plant growth and reproduction along CO₂ gradients—nonlinear responses and implications for community change. *Global Change Biol* **1**:199–207.
- Aigner D, Lovell CAK, Schmidt P (1977) Formulation and estimation of stochastic frontier production function models. *J Econometrics* **6**:21–37.
- Aitchison J (1986) *The Statistical Analysis of Compositional Data*. London: Chapman and Hall. Reprinted in 2003 by The Blackburn Press.
- Amemiya T (1985) *Advanced Econometrics*. Oxford: Basil Blackwell Ltd.
- Blackshaw R, Anderson G, Dekker J (1987) Interference of *Sinapis arvensis* L. and *Chenopodium* L. in spring rapeseed (*Brassica napus* L. *Weed Res* **27**:207–13.
- Brophy C, Gibson DJ, Wayne PM, *et al.* (2007) A modelling framework for analysing the reproductive output of individual plants grown in monoculture. *Ecol Modell* **207**:99–108.
- Cheplick GP (2005) The allometry of reproductive allocation. In: Reekie EG, Bazzaz FA (eds). *Reproductive Allocation in Plants*. San Diego, CA: Elsevier Academic Press, 94–125.
- Collett D (1993) *Modelling Binary Data*, 2nd edn. London: Chapman & Hall/CRC.
- Connolly J, Wayne P (1996) Asymmetric competition between plant species. *Oecologia* **108**:311–20.
- Connolly J, Wayne P, Murray R (1990) Time course of plant-plant interactions in experimental mixtures of annuals—density, frequency and nutrient effects. *Oecologia* **82**:513–26.
- Damgaard C, Weiner J (2008) Modeling the growth of individuals in crowded plant populations. *J Plant Ecol* **1**:111–6.
- Damgaard C, Weiner J, Nagashima H (2002) Modelling individual growth and competition in plant populations: growth curves of *Chenopodium album* at two densities. *J Ecol* **90**:666–71.
- Edwards M (1980) Aspects of the population ecology of charlock. *J Appl Ecol* **17**:151–71.
- Efron B, Tibshirani RJ (1993) *An Introduction to the Bootstrap*. London: Chapman & Hall.
- Fogg GE (1950) *Sinapis arvensis* L. *J Ecol* **38**:415–29.
- Ford MA, Kay QON (1985) The genetics of incompatibility in *Sinapis arvensis* L. *Heredity* **54**:99–102.
- Franklin KA, Whitelam GC (2004) Light signals, phytochromes and cross-talk with other environmental cues. *J Exp Bot* **55**:271–6.
- Garbutt K, Williams WE, Bazzaz FA (1990) Analysis of the differential response of five annuals to elevated CO₂ during growth. *Ecology* **71**:1185–94.
- Gedroc JJ, McConnaughay KDM, Coleman JS (1996) Plasticity in root shoot partitioning: Optimal, ontogenetic, or both? *Funct Ecol* **10**:44–50.
- Gelfand AE, Mallick BK, Polasek W (1997) Broken biological size relationships: a truncated semiparametric regression approach with measurement error. *J Am Stat Assoc* **92**:836–45.
- Gibson DJ (2002) *Methods in Comparative Plant Population Ecology*. Oxford: Oxford University Press.
- Gibson DJ, Connolly J, Hartnett DC, *et al.* (1999) Essay review: designs for greenhouse studies of interactions between plants. *J Ecol* **87**:1–16.
- Gibson DJ, Spyreas G, Benedict J (2002) Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. *J Torrey Bot Soc* **129**:207–19.
- Gleason HA, Cronquist A (1991) *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2nd edn. Bronx, NY: New York Botanical Garden, 910.
- Harper JL (1977) *Population Biology of Plants*. London: Academic Press.
- He JS, Bazzaz FA (2003) Density-dependent responses of reproductive allocation to elevated atmospheric CO₂ in *Phytolacca americana*. *New Phytol* **157**:229–39.
- He JS, Wolfe-Bellin KS, Bazzaz FA (2005) Leaf-level physiology, biomass, and reproduction of *Phytolacca americana* under conditions of elevated CO₂ and altered temperature regimes. *Int J Plant Sci* **166**:615–22.
- Jablonski LM, Wang XZ, Curtis PS (2002) Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytol* **156**:9–26.
- Karlsson PS, Méndez M (2005) The resource economy of plant reproduction. In: Reekie EG, Bazzaz FA (eds). *Reproductive Allocation in Plants*. San Diego, CA: Elsevier Academic Press, 1–49.
- Luzuriaga AL, Escudero A, Perez-García F (2006) Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Res* **46**:163–74.
- Mabry CM, Wayne PW (1997) Defoliation of the annual herb *Abutilon theophrasti*: mechanisms underlying reproductive compensation. *Oecologia* **111**:225–32.
- McConnaughay KDM, Coleman JS (1999) Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. *Ecology* **80**:2581–93.
- McLachlan P, Peel D (2000) *Finite Mixture Models*. New York: John Wiley & Sons Inc.
- Méndez M, Karlsson PS (2004) Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates. *Oikos* **104**:59–70.
- Mohlenbrock R (1980) *The Illustrated Flora of Illinois: Flowering Plants Willows to Mustards*. Carbondale, IL: Southern Illinois University Press.
- Mulligan G, Bailey L (1975) The biology of Canadian weeds. 8. *Sinapis arvensis* L. *Can J Plant Sci* **55**:171–83.
- Pawitan Y (2001) *In All Likelihood: Statistical Modelling and Inference Using Likelihood*. Oxford: Clarendon Press.
- Reekie E, Bazzaz F (2005) *Reproductive Allocation in Plants*. San Diego, CA: Elsevier Academic Press.
- Samson DA, Werk KS (1986) Size-dependent effects in the analysis of reproductive effort in plants. *Am Nat* **127**:667–80.

- Schmid B, Polasek W, Weiner J, et al. (1994) Modeling of discontinuous relationships in biology with censored regression. *Am Nat* **143**:494–507.
- Schwartz G (1978) Estimating the dimension of a model. *Ann Stat* **6**:461–4.
- Schwinning S, Weiner J (1998) Mechanisms determining the degree of size asymmetry in competition among plants. *Oecologia* **113**:447–55.
- Sletvold N (2002) Effects of plant size on reproductive output and offspring performance in the facultative biennial *Digitalis purpurea*. *J Ecol* **90**:958–66.
- Steinger T, Roy BA, Stanton ML (2003) Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *J Evol Biol* **16**:313–23.
- Stinson KA, Bazzaz FA (2006) CO₂ enrichment reduces reproductive dominance in competing stands of *Ambrosia artemisiifolia* (common ragweed). *Oecologia* **147**:155–63.
- Sugiyama S, Bazzaz FA (1998) Size dependence of reproductive allocation: the influence of resource availability, competition and genetic identity. *Funct Ecol* **12**:280–8.
- Thompson BK, Weiner J, Warwick SI (1991) Size-dependent reproductive output in agricultural weeds. *Can J Bot* **69**:442–6.
- van Kleunen M, Stephan MA, Schmid B (2006) CO₂ and density-dependent competition between grassland species. *Glob Change Biol* **12**:2175–86.
- Verbeke G, Molenberghs G (2000) *Linear Mixed Models for Longitudinal Data*. New York: Springer.
- Warwick SI, Beckie HJ, Thomas AG, et al. (2000) The biology of Canadian weeds. 8. *Sinapis arvensis* L. (updated). *Can J Plant Sci* **80**:939–61.
- Wayne P, Foster S, Connolly J, et al. (2002) Production of allergenic pollen by ragweed (*Ambrosia artemisiifolia* L.) is increased in CO₂-enriched atmospheres. *Ann Allergy Asthma Immunol* **88**:279–82.
- Wayne PM, Carnelli AL, Connolly J, et al. (1999) The density dependence of plant responses to elevated CO₂. *J Ecol* **87**:183–92.
- Weiner J (2004) Allocation, plasticity and allometry in plants. *Perspect Plant Ecol* **6**:207–15.
- Weiner J, Thomas SC (1986) Size variability and competition in plant monocultures. *Oikos* **47**:211–22.