

## Trade-offs between reproduction and growth influence time of reproduction in *Oenothera biennis*

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**Abstract:** Size at which reproduction occurs was examined in two populations of *Oenothera biennis* occupying contrasting habitats: (1) a gravel embankment without complete canopy closure, and (2) a second-year successional field with a closed canopy. One hundred plants were randomly harvested from each site and weighed. A greater proportion of plants reproduced in the closed versus open canopy site (83 versus 61%). There was no difference in mean size at reproduction between the two sites; however, variation in size at reproduction was greater in the closed site where both the smallest and largest plants were found. In the smallest size category, more plants reproduced in the closed than in the open site, indicating that the minimum size required for reproduction is smaller in the closed site. We suggest that plants reproduce at a smaller size in the closed site because of the marked stem elongation associated with reproduction in this species, which increases the ability of plants to compete for light. This question was addressed in a garden experiment in which seedlings were transplanted into either weeded plots (i.e., an open canopy) or a successional field (i.e., a closed canopy). Half the plants at each site were left as vegetative controls and the other half treated with gibberellic acid to induce bolting. Bolting had no effect on growth in the open canopy but increased growth in the closed canopy as predicted.

**Key words:** time of reproduction, *Oenothera biennis*, life history variation, stem elongation, bolting, size at reproduction, phenotypic plasticity.

**Résumé :** Les auteurs ont examiné l'effort de reproduction dans deux populations d'*Oenothera biennis* occupant des habitats contrastés : (i) un dépôt de gravier sans canopée complètement fermée et (ii) un champ en deuxième année de succession avec une canopée fermée. Cent plants ont été récoltés au hasard sur chaque site et ont été pesés. Une plus forte proportion des plants se reproduisent sous couvert fermé que sous la canopée ouverte (83 vs. 61%). Il n'y a pas de différence dans la dimension moyenne, à la reproduction, entre les deux sites, cependant la variation de la dimension à la reproduction est plus grande sous canopée fermée où on trouve à la fois les plantes les plus grandes et les plus petites. Dans la catégorie des plus petites, il y a plus de plantes qui se reproduisent sous canopée fermée par rapport à ouverte, ce qui indique que la grosseur minimum pour se reproduire est plus faible sous canopée fermée. Les auteurs suggèrent que les plantes se reproduisent avec une dimension plus petite sous canopée fermée parce qu'elles ont une tige beaucoup plus allongée, associée avec la reproduction de cette espèce. Ce qui augmente la capacité des plantes à compétitionner pour la lumière. Les auteurs ont examiné cette question en jardin expérimental où des plantes ont été transplantées dans des parcelles désherbées (canopée ouverte) ou un champ en succession (canopée fermée). Ils ont laissé la moitié des plants comme témoins végétatifs et ils ont traité les autres plants avec de l'acide gibbérellique, pour induire la floraison. La floraison est demeurée sans effets sur la croissance avec canopée ouverte mais la croissance a augmenté sous la canopée fermée, tel que prévu.

**Mots clés :** moment de la reproduction, *Oenothera biennis*, variation du cycle vital, elongation de la tige, floraison, dimension à la reproduction, plasticité phénotypique.

[Traduit par la rédaction]

### Introduction

There is marked intraspecific variation in time of reproduction in monocarpic perennials (Lee and Hamrick 1983; Reinartz 1984a, 1984b; Silvertown 1984; Lacey 1986a,

1986b; Klinkhamer et al. 1991). This variation can have a genetic basis, but most studies suggest that it is largely environmentally induced (Reinartz 1984a, 1984b; Lacey 1986a; Thomas and Bazzaz 1993). Resource-allocation studies show that monocarpic plants must reach a minimum critical size before reproduction can be initiated (Werner 1975; Gross 1981; Wesselingh et al. 1994). This means that time of reproduction in monocarpic perennials is more closely correlated with size than with age (Lacey 1986b). Therefore variation in light, nutrients, moisture, temperature, and other environmental factors which influence growth will also affect time of reproduction.

Although size is important in determining time of repro-

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duction, there is substantial variation in size at which flowering occurs. For example, in a given *Oenothera biennis* population, size at reproduction can differ by several factors, even among individuals in a particular year (Reekie 1997). One explanation for this is that plants may reach the minimum critical size at different points in the growing season. Because many monocarpic perennials require vernalization (i.e., a cold treatment to induce flowering), two plants which reach the minimum critical size at different points in the growing season could both postpone flowering until the following year. This would result in one being larger than the other at time of flowering (Baskin and Baskin 1979). This can explain variation in size at time of reproduction, but cannot explain vernalized plants that remain vegetative, even though they may be larger than plants that flower (Reekie 1997). It has been shown in a wide range of monocarpic plants that there is significant overlap in the size of flowering and nonflowering individuals (e.g., Kachi and Hirose 1985; Reinartz 1984a, 1984b; de Jong et al. 1986). This suggests that the minimum critical size required for reproduction varies among individuals, or that unknown factors may prevent reproduction, even though an individual has reached the minimum critical size (see also Lacey 1986a).

Differences in the extent to which growth occurs after reproduction is initiated could explain much of the intraspecific variation in size at flowering. Given that reproductive output will ultimately depend on the total amount of resources accumulated in growth, the extent to which growth continues after reproduction is initiated will have a major impact on reproductive output and, therefore, reproductive timing. Theoretical models suggest that reproduction should occur at an earlier age and smaller size when reproduction has little negative effect on growth, and at a larger size and later time when reproduction has more negative effects upon growth (e.g., see Cohen 1976).

The objective of the present study was to determine if variation in the effect of reproduction on growth influences time of reproduction in *O. biennis*. This species exists as an acaulescent rosette in the vegetative state but after reproduction is initiated, the stem elongates, carrying the leaves up into the canopy (Hall et al. 1988). *Oenothera biennis* requires a high red to far red light ratio for seed germination, and this limits germination to sites where bare ground is present (Gross 1985). This is important for the initial establishment and growth of this species, since the acaulescent vegetative plant is a poor competitor for light (Gross and Werner 1982). *Oenothera biennis* can be found in two very different habitats: (1) sites with low fertility, such as gravel pits where complete canopy closure never occurs due to nutrient shortage, and therefore this species forms a permanent population; and (2) successional fields in which *O. biennis* colonizes in the first year following a disturbance, completes its life cycle, and is finally eliminated from the site as canopy closure follows.

Given that stem elongation (i.e., bolting) is associated with reproduction, it can be predicted that reproduction in a closed canopy may actually enhance the growth of this species, as it would increase its ability to compete for light. On the other hand, in an open canopy, reproduction will either have no beneficial effect on growth or may decrease growth due to the diversion of resources from vegetative

activities to the production of seeds. Therefore, if the trade-off between reproduction and growth influences the size required for reproduction, we predict the minimum flowering size should be lower in a site with a closed canopy than in a site with an open canopy.

## Materials and methods

### Reproduction in closed versus open canopies

To test the prediction that plants reproduce at a smaller size in closed versus open canopies, size of vegetative versus reproductive plants at time of bolting was determined in two populations of *O. biennis* occupying contrasting sites. The first site was a previously cultivated field that was abandoned in mid-June of the previous year and subsequently left untilled. There was 100% ground cover and the 0.75-m canopy contained a variety of annuals and perennials including *Chenopodium album*, *Amaranthus retroflexus*, *Daucus carota*, *Poa pratensis*, and *Plantago major* in addition to *O. biennis*. The second site was a roadside gravel embankment. Approximately 30–50% of the surface was covered by vegetation, the principle species being *O. biennis*, *Verbascum thapsus*, and *D. carota*. Both sites were located in Wolfville, Nova Scotia, Canada (45°06'N, 62°24'W).

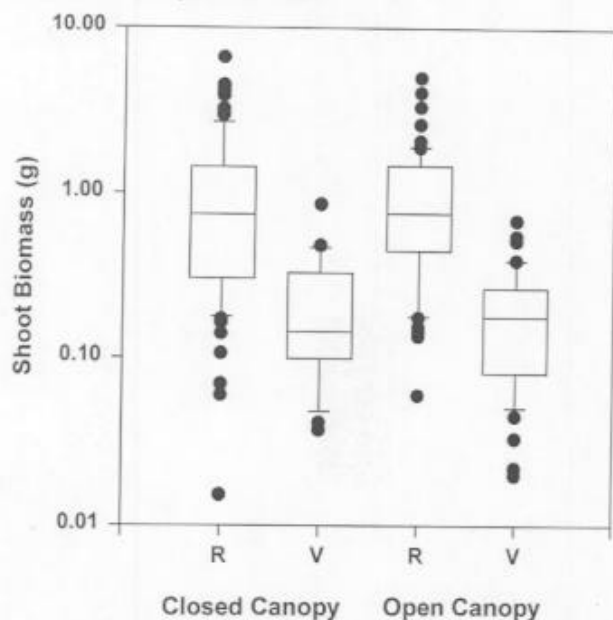
Between June 15 and 20, 1992, 100 adult plants were randomly selected at each site. Seedlings that germinated in 1992 were excluded from the sample. Therefore, all plants were at least 1 year old, had received a cold treatment the previous winter, and were potentially capable of reproducing in 1992. We examined each plant to determine if bolting had occurred. Since reproductive plants bolt in late May or early June (E. Reekie, unpublished data), plants that had not bolted by the time of the census were classified as vegetative. Two by two contingency tables were used to determine whether the two sites differed in the proportion of plants that reproduced. Tables were constructed for all individuals combined regardless of size, as well as for individuals in three size categories. Plants were divided into size categories by ordering individuals on the basis of size and dividing the resulting list into three groups of approximately equal numbers. The small size category consisted of 67 individuals ranging in size from 0.015 to 0.269 g, the medium category 67 individuals ranging from 0.270 to 0.826 g, and the large category 66 individuals ranging from 0.830 to 6.589 g.

After determining reproductive status, shoots were harvested and dried at 60°C for more than 48 h before weighing. The timing of the harvest allowed us to determine the size of both vegetative and reproductive plants at the time reproduction was initiated in these populations. Differences in the mean size of vegetative versus reproductive plants at the two sites were examined by a two-way factorial analysis of variance. To achieve a normal distribution, the dry weight data were transformed prior to analysis by taking the logarithm of the raw values. As mean size at reproduction will not necessarily reflect the minimum size necessary for reproduction (Baskin and Baskin 1979), data for reproductive plants were divided into quartiles. Since quartiles were determined separately for reproductive plants from the two sites, a comparison of plant size within the lower and upper quartiles provides a measure of whether the minimum and maximum size at reproduction differed between the two sites. Data were analyzed by a two-way analysis of variance, with site and quartile as the independent variables and the logarithm-transformed weight as the dependent variable. A significant quartile  $\times$  site interaction in this analysis indicates that the size distribution of the two populations differs.

### Effect of reproduction on competition for light

The question of whether or not the stem elongation associated with reproduction would increase the ability of plants to compete for light in a closed canopy and thereby increase growth was addressed

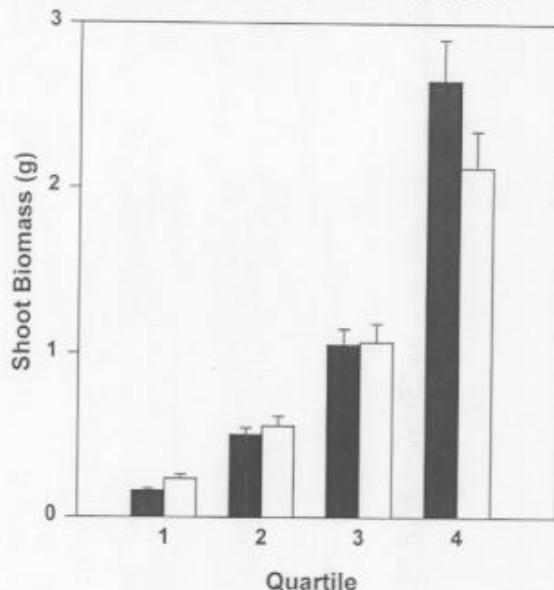
Fig. 1. Box plot depicting the size distribution of vegetative (V) and reproductive (R) individuals from sites with a closed vs. open canopy. The lower, middle, and upper lines of each box represent the 25th, 50th, and 75th percentiles, respectively. The lower and upper error bars represent the 10th and 90th percentiles, respectively, and the solid circles are the individual data points outside of the 10th and 90th percentiles. Note that shoot biomass is plotted on a logarithmic scale.



in a garden experiment. Seeds were planted in 7.6 cm diameter peat pots filled with vermiculite on May 24, 1989. Upon emergence, plants were thinned to one seedling per pot and placed in a greenhouse where they received ambient light and temperature conditions. Plants were fertilized by watering biweekly with a water-soluble fertilizer (Plant Products, Bramalea, Ont., General Use Fertilizer with micronutrients, 20-20-20 N-P-K) at a concentration of  $1 \text{ g} \cdot \text{L}^{-1}$ . On June 20, the seedlings were transplanted to one of two field plots: (1) a weeded experimental garden (open canopy), and (2) a successional field (closed canopy). The two plots were immediately adjacent to each other and located on the same soil type, a Wolfville clay loam. The successional field was a cultivated garden plot which was abandoned in June 1988. It was dominated by a variety of annual and perennial plants including *C. album*, *Ambrosia artemisiifolia*, *D. carota*, *O. biennis*, *Plantago major*, *Poa pratensis*, and *Taraxacum officinale*. The maximum height of the canopy in June 1989 was approximately 0.85 m.

A total of 118 seedlings were transplanted to the weeded garden plot and a total of 72 seedlings to the successional plot. Plants were placed in rows such that there was 0.5 m between adjacent plants. Seedlings were paired according to size (i.e., rosette diameter) prior to transplanting, and plants within a pair were planted adjacent to each other. One of these plants was randomly selected as a control, whereas the other was induced to bolt by spraying leaves with  $1000 \text{ mg} \cdot \text{L}^{-1}$  solution of gibberellic acid (at least 90% of total gibberellins in the form of gibberellin  $A_3$ ). Gibberellic acid is a naturally occurring plant hormone that induces many rosette-forming plants to undergo stem elongation and flower (Salisbury and Ross 1978). A preliminary experiment indicated that this procedure produced plants that were similar in morphology and seed output to naturally vernalized plants of the same size (see also Lang 1957). Gibberellic acid was applied from July 6 to July 10 on a daily

Fig. 2. Average shoot biomass of reproductive individuals from sites with a closed (solid bars) vs. open (open bars) canopy. Means were calculated separately for each of the four quartiles from the two sites. Error bars depict one standard error.



basis. Shoots in both plots were harvested on August 19 and dried at  $60^\circ\text{C}$  for at least 48 h, and their dry weight was determined.

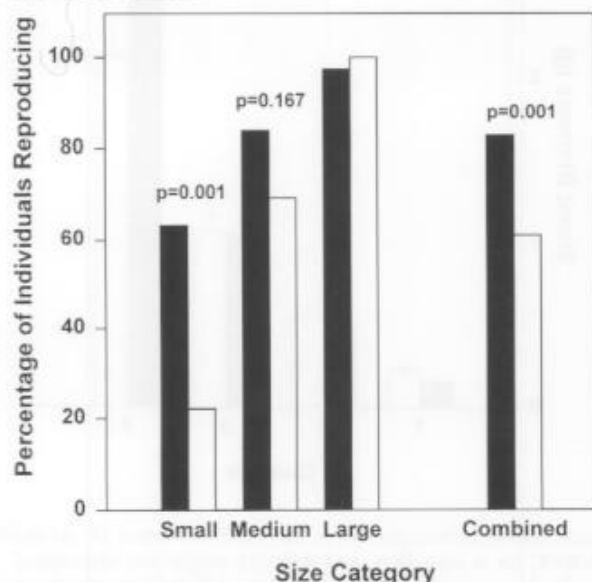
To determine if the effect of bolting differed between the two plots, the biomass of plants induced to bolt was divided by that of the corresponding vegetative rosette for each pair of individuals. The ratio was then used as the dependent variable in a one-way analysis of variance with plot as the independent variable.

## Results

There was substantial variation in the size of both vegetative and reproductive plants (Fig. 1). Vegetative plants ranged between 0.020 and 0.867 g, whereas reproductive plants ranged between 0.015 and 6.59 g. Despite the broad overlap in their distributions, reproductive plants were larger on average (0.710 g) than vegetative plants (0.158 g) ( $p = 0.0001$  for the main effect of reproductive state in a two-way ANOVA). There was no difference in mean size between the two sites ( $p = 0.6936$  for the main effect of site), nor did the mean size of vegetative versus reproductive plants differ between sites ( $p = 0.3935$  for the reproductive state  $\times$  site interaction). Although there was no difference in mean plant size between sites, the smallest reproductive plants in the closed site were smaller than those in the open site, and the largest ones were larger than those in the open site (Fig. 2,  $p = 0.0346$  for the quartile  $\times$  site interaction). This is also reflected in the wider range in size observed among reproductive plants in the closed site than in the open site (Fig. 1). The smallest reproductive plant in the open site (0.060 g) was four times larger than that in the closed site (0.015 g), whereas the largest reproductive plant in the open site was only 4.96 compared with 6.59 g in the closed site.

A greater proportion of plants reproduced in the closed site (83%) versus the open site (61%) ( $p < 0.0001$ ). As virtually all large plants reproduced regardless of site, the

**Fig. 3.** Percentage of individuals that reproduced in sites with a closed (solid bars) vs. open (open bars) canopy. Percentages were calculated separately for small, medium, and large size categories and for the combined data. The level of significance for the difference between sites is given for each category with one exception. It was not possible to calculate a probability in the case of the large size category due to the fact that virtually all plants reproduced.



difference between sites was due to differences in the proportion of small plants that reproduced (Fig. 3).

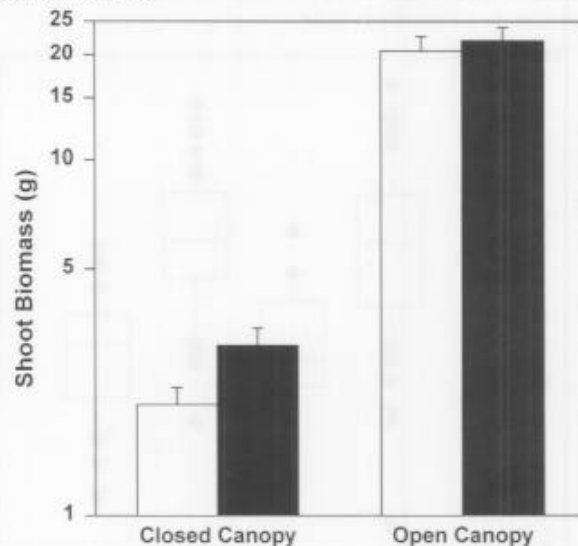
In the garden experiment there was greater growth in the weeded (open canopy) versus unweeded (closed canopy) plots (Fig. 4). Bolting had little effect on growth in the open canopy, whereas in the closed canopy there was a marked increase in growth as a result of the bolting induced by gibberellic acid application ( $p = 0.001$  for the difference between open and closed canopy in the biomass ratio of bolted versus vegetative plants).

## Discussion

Reproductive plants were larger on average than vegetative plants (Fig. 1). This is to be expected, given that plants must reach a minimum critical size before reproduction can occur. Although there was no difference in the mean size of reproductive plants between the two sites, the fact that in the smallest size category more plants reproduced in the site with the closed canopy (Fig. 3) clearly indicates that the minimum size required for reproduction is smaller in the closed canopy. This is supported by the observations that the smallest reproductive plant was found in the closed canopy (Fig. 1) and that plants in the lowest quartile were smaller in the closed-canopy site (Fig. 2). The only reason the mean size of reproductive plants did not differ between sites is that the low minimum size at reproduction in the closed-canopy site was counterbalanced by a large maximum size.

Although the present study does not address the question of why the maximum size at reproduction differs between

**Fig. 4.** Shoot biomass (g) of acaulescent vegetative individuals (open bars) vs. those that were induced to bolt through the application of gibberellic acid (solid bars). Plants were grown either in a second-year successional field (closed canopy site) or weeded garden plots (open canopy site). Error bars depict one standard error. Note that shoot biomass is plotted on a logarithmic scale.



sites, one can speculate that it is perhaps a reflection of the differences in soil fertility one would expect between a successional field and a gravel road embankment. On the other hand, this study does provide an explanation for why the minimum size at reproduction should differ between sites. The garden experiment found that bolting has the potential to substantially increase size when plants are competing for light (Fig. 4). Given that bolting is associated with reproduction in this species, leaves will be displayed higher in the canopy and will therefore be exposed to more light after reproduction has been induced, thereby increasing growth. If reproduction increases growth rate, plants may initiate reproduction at a small size and still accumulate sufficient resources to successfully mature seeds.

Another factor that could contribute to differences in size at reproduction between the two sites is the effect of nutrient availability on the trade-off between reproduction and growth. Saulnier and Reekie (1995) examined the influence of reproduction on growth of *O. biennis* under conditions of low versus high nutrient availability. In plants growing in high-nutrient environments, reproduction had no negative effects on growth, whereas in low-nutrient environments reproduction decreased growth. In the latter, plants resort to using component nutrients of the leaves (e.g., the nitrogen contained in leaf chlorophyll) to supply reproductive needs (Saulnier and Reekie 1995). This causes photosynthetic rate to decline with reproduction. In high-nutrient environments, nutrient uptake is sufficient to supply reproductive growth and nutrient content of leaves remains high. Therefore, photosynthesis does not necessarily decline with reproduction, allowing growth to continue. Assuming the successional field is likely to supply more nutrients than the gravel road



embankment, growth may continue after reproduction to a greater extent in the successional field, facilitating reproduction at a smaller size.

It might be argued that growth after reproduction has been initiated is irrelevant in so far as the timing of reproduction is concerned, i.e., the "decision" to reproduce must be made before the growth occurs. However, there are indicators of postreproductive growth potential such as variation in light quality which can be detected by a plant before reproduction occurs. Through changes in the ratio of red to far red light, the plant pigment phytochrome is used to detect the extent of canopy closure (Smith 1995). These changes in light quality, and the effect these changes elicit on phytochrome, are known to regulate various aspects of development, including stem elongation, seed germination, branching patterns, and leaf morphology. Through the dark decomposition of phytochrome, this pigment is also used to measure day length and in doing so plays a role in controlling when reproduction is initiated in plants sensitive to day length (Smith 1995). Our results suggest that phytochrome may be playing a role in regulating time of reproduction via alterations in light quality as well. If a plant "senses" that it is being shaded by a neighboring plant through changes in the red to far red ratio, it may reproduce at a smaller than normal size to increase its ability to compete for light.

Attempts to explain variation in timing of reproduction have largely focussed on the demography of the populations involved, i.e., variation in survivorship schedules and how this may select for different life histories (see literature cited in Willson 1983). Such studies clearly illustrate the importance of survivorship patterns in explaining genetic differentiation in time of reproduction and other life history characteristics. Law (1979), for example, has shown that populations of *Poa annua* where adults have a short life expectancy due to frequent disturbance reproduce at a younger age than populations where disturbance is less frequent and adults have a longer life expectancy. However, the majority of life history variation observed in the field has an environmental rather than a genetic basis (Willson 1983; Lotz and Blom 1986; Sultan 1987). Environmental variation is not addressed by traditional life history theory, which was developed to explain genetic differentiation among populations (Reznick 1985). Survivorship patterns can only explain phenotypic plasticity in life history characteristics if individuals can foresee the likelihood of their future survival, and the possible success of their offspring in the environments to which they are likely to be dispersed (Willson 1983). Although it is possible that survivorship may be correlated with environmental cues that can be sensed by an individual, it is more parsimonious to explain variation in terms of direct environmental effects. The present study provides one example of such direct effects. Simple physiological mechanisms such as those used to explain the varying trade-off between reproduction and growth in *O. biennis* could explain much of the phenotypic variation in plant life history characteristics.

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