

Variations in ageing and meristematic activity in relation to flower-bud and fruit excision in the *Beta* species complex

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Summary

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- Removal of developing fruits delays ageing in many annual plant species. This has long been seen as evidence that ageing is caused by resource depletion due to fruit development. Excision experiments can be interpreted from both evolutionary-ecology and resource-allocation viewpoints. Iteroparous (reproducing repeatedly) and annual plants may show different responses to excision. Furthermore, under the resource-depletion hypothesis, the more precocious the excision of reproductive organs, the greater the effect on allocation patterns.
- A controlled-conditions experiment was set up involving six life cycles (from long-lived iteroparous to annual taxa of wild beets, *Beta* spp.) under three treatments of excision of reproductive-parts (buds, fruits and control).
- Treatment effect was similar in semelparous and iteroparous beets, although effect on reproduction was more obvious in iteroparous ones. Flower-bud removal tended to induce resource allocation in new reproductive meristems, whatever the life cycle, without any effect on ageing in semelparous beets and probably none on survival in most iteroparous plants. Fruit removal had no effect.
- These results and the occurrence of between-accessions variation are discussed in terms of the ecological significance of optimal allocation strategies.

Key words: senescence, ageing, reproductive-parts excision, meristematic activity, *Beta* species complex (wild beets), resource-depletion hypothesis, life-cycle gradient, resource allocation.

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Introduction

Semelparity, or monocarpy, is the life history shared by annual and some long-lived perennial plants such as Bamboos or Agaves. It is characterised by a single massive reproductive episode, followed by the death of the individual (Gadgil & Bossert, 1970; Kirkendall & Stenseth, 1985). Economically this life history is of major importance, since it is selected in crops (Noodén, 1988b) as well as in ruderals (Grime, 1979). Its alternative is iteroparity or polycarpy, that is repeated reproduction, which may be accompanied by progressive senescence, or ageing, defined here as the persistent decline in the age-specific fitness components (survival and reproduction) of an organism due to internal physiological deterioration (Rose, 1991). A distinction between whole-plant senescence and somatic tissue senescence has to be

made: leaves of deciduous trees fall each year due to somatic tissue senescence without any resulting negative consequences on fitness (for a discussion on the relationship between life history of the whole plant and senescence of its constituent parts) (Thomas *et al.*, 2000). In this paper we will therefore follow the above-cited evolutionary definition of senescence and use the word 'ageing' for 'whole-plant senescence' and 'end of the reproductive cycle' for the 'somatic tissue senescence' occurring each year after the reproductive season. The difference between iteroparity and semelparity relates not only to the number of reproductive episodes but also to the pattern of ageing. Semelparous organisms undergo a very abrupt ageing whereas iteroparous organisms undergo a progressive or apparently nonexistent ageing (Noodén, 1988c; Rose, 1991). All functions of an organism, such as reproduction, growth and survival, depend on the same

limiting resources and are consequently related to each other by negative correlations, the so-called trade-offs (review in Stearns, 1992). Trade-offs may be expressed as, for example, increased mortality rate with fruiting (Sohn & Policansky, 1977) or reduced probability of subsequent reproduction and reduced growth with fruit production (Snow & Whigham, 1989; Worley & Harder, 1996; Primack & Stacy, 1998). Trade-offs between reproduction and survival are central to the models of evolution of semelparity and ageing. Since the late 19th century (Wallace and Weismann) ideas that potential somatic immortality is sacrificed for the sake of reproduction have been developed (Rose, 1991). Gardeners and scientists observed that excision of developing fruits might delay the onset of ageing or restore vegetative growth in some semelparous plant species (Noodén, 1988c). This provided preliminary evidence on the occurrence of some kind of physiological 'communication' between fruits and other tissues of a plant, which was termed correlative control (Hensel *et al.*, 1994). It has been hypothesised that whole-plant senescence results from the depletion of nutrients from somatic tissues due to their diversion to reproductive organs (Rose, 1991). This hypothesis which is based on a very simple concept (reproduction and survival compete for limiting resources) is very attractive. Such excision experiments have therefore been carried out until the present day (with various results, see Discussion) in the hope of a better knowledge of ageing mechanisms. This has generally been done in annual plants. Information in perennials is rare.

Removal of flower buds, or of developing fruits, implies that some amount of resources will not be committed to the development of these fruits, as it normally should. The fate of these released resources will depend on the allocation strategy of the plant and its ability to modify this strategy after reproductive structures are lost. The allocation strategy is the way individuals deal with the obligation to allocate their resources, which are limited, between several compartments. In plants, an individual (that cannot reproduce vegetatively) can invest in three major compartments: reproductive structures, survival structures (i.e. reserves necessary for survival until the following reproductive season) and growth and maintenance of the vegetative structures that are necessary for other compartments. The strategy is tightly related to the life cycle, that is iteroparous plants invest in all compartments, in proportions depending on their life history: long-lived iteroparous plants will invest more resources in survival than short-lived ones, and annual plants obviously do not invest in survival. Under one specific strategy, relative investment in each part will also vary throughout the season. For example, at the beginning of the reproductive season an annual plant will invest in growth, maintenance and reproduction, whereas at the end of the season theoretically it will invest all available resources into reproduction. Allocation patterns are probably established very early in the season: in low-nutrient conditions some iteroparous plants may not initiate flowering at

all (Noodén, 1980; Hautekèete *et al.*, 2001). Reversibility of allocation patterns remains an unsolved question, however. If the strategy is irreversible, resources released by the excision of flower buds or developing fruits should be allocated to each compartment in proportions corresponding to the normal strategy of the plant. A part of the released resources should therefore be allocated to the production of new flower buds, whereas investment in vegetative growth and maintenance should not differ from control. However, these new flowers will require delayed plant ageing to give seeds, that is prolonged maintenance of existing vegetative structures or production of some new vegetative structures. An irreversible allocation strategy is consequently not optimal. Therefore only two optimal patterns of reversion of the allocation pattern are possible.

First, reproduction continues, but is sustained by prolonged vegetative growth and maintenance, which allows seed maturation. In some species investment in survival for the following year can also be increased. Ageing is delayed and reproduction continues after reproductive parts are excised.

Second, reproduction stops to the benefit of survival until the following year, through increased reserves and/or increased vegetative growth and maintenance. No new reproductive structure is produced, while survival until the following year is enhanced.

Phylogenetically related iteroparous and semelparous species are likely to share common characteristics due to their partly joint evolution. Families or genera with taxa differing in life histories are therefore ideal for the comparative study of life-history traits. Iteroparous taxa can invest both in reproduction and survival, and their investment to reproduction is known to be plastic (Noodén, 1980; Hautekèete *et al.*, 2001) contrary to semelparous taxa which invest at a constant maximum rate in reproduction. In other words, reversible reproductive effort should be more expected in iteroparous plants. However ageing in iteroparous taxa is not easily observed in short-term experiments, whereas semelparous taxa might provide more clear-cut patterns of ageing (Noodén, 1988c).

Finally if the relationship between reproduction and ageing is based on a trade-off due to the limitation of resources, the effect of bud removal should be more important than the effect of fruit removal, since fewer resources are already committed to reproduction.

The *Beta* species complex is an ideal plant model for comparative studies. It includes many phylogenetically closely related taxa or populations with contrasting life cycles. It includes the cultivated beet *Beta vulgaris* ssp. *vulgaris* L. and four wild relatives: its closest wild relative *B. v. maritima* (L.) W. D. J. Koch, and *B. v. adanensis* Pamuk., *B. patula* Aiton. and *B. macrocarpa* Guss. (Letschert, 1993). Within the *Beta* species-complex, we observed a gradient from pronounced iteroparity (*B. v. maritima*) to pronounced semelparity (*B. macrocarpa*). Among populations of the iteroparous taxa *B. v. maritima*, the range of life span is very large: mean life spans range from two to 10 yr under controlled conditions.

B. patula is mostly semelparous, although some individuals may survive and reproduce for at least two years (Letschert, 1993; Hautekèete *et al.*, 2001). The subspecies *B. v. adanensis* is semelparous, *B. macrocarpa* dies immediately after seed maturity and may therefore be termed strictly semelparous (Hautekèete *et al.*, 2001). All taxa of the *Beta* species-complex are able to interbreed (although such crosses are unlikely in the wild). They are not able to reproduce repeatedly. In a controlled-conditions experiment, involving six different life cycles of the *Beta* species complex and three excision treatments (excision of flower buds, of fruits and control), we addressed the following questions:

1 What is the effect of the excision of reproductive organs (flower buds or developing fruits) on the pattern of allocation for reproduction and survival in semelparous and iteroparous taxa of the *Beta* species-complex: does excision induce the production of new reproductive structures in semelparous taxa and does it delay ageing? do iteroparous taxa produce new reproductive structures or stop investing in reproduction and instead reallocate to survival?

2 Is the effect of bud excision stronger than the effect of excising a fruit?

Materials and Methods

Experimental design

We selected six different life cycles to represent the gradient of life history: three iteroparous life cycles of *B. v. maritima* with different life expectancies and three more or less strictly semelparous species, *B. macrocarpa*, *B. v. adanensis* and *B. patula*. To separate life-cycle effects from population effects, two accessions were chosen per life cycle, except for *B. patula* which is endemic to Madeira and for which only one population is known (Table 1).

Seeds of *B. macrocarpa* (accessions VS1 and VS2, Table 1), *B. v. adanensis* (accessions LS1 and LS2 from two different

Greek localities), and *B. patula* (SI) were provided by L. Frese (BAZ Gene Bank at Braunschweig, Germany, each accession being collected as a bulk). These taxa are essentially autogamous, unlike *B. v. maritima* which is self-incompatible. The *B. v. maritima* accessions were part of our laboratory collections (Van Dijk *et al.*, 1997). Plants were sown during April 2000 and grown in a glasshouse under long-day photoperiod (16 h, 400 W high pressure sodium lamps, PAR: 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ over the waveband 400–700 nm). Plants were grown in enriched compost with 1 kg m^{-3} of 14 : 16 : 18 NPK and microelements fertiliser (3 dm³ pots). Fertiliser (8/6/16S with Mg) was supplied each fortnight starting at age 28 d. They were also randomised each fortnight throughout the experiment.

Individuals of each accession were randomly distributed between the three treatments (bud excision, fruit excision and control).

In all taxa of the *Beta* species-complex, flowers are clustered as inflorescences of two to eight flowers called glomerules. Hundreds of glomerules are produced per individual, especially in *B. v. maritima*. Seeds of one glomerule mature as a seedball in which perianths form a corky pericarp firmly attached to the seeds (Letschert, 1993). Each day for almost one year (330 d, beginning from sowing date) we proceeded to flower-bud excision before bloom (bud-excision treatment) and to the surgical excision of ovules in fruits before the formation of the corky pericarp (fruit-excision treatment).

This experiment therefore involved 11 accessions and three treatments per accession. To obtain around five individuals per treatment, 200 plants were potted at the beginning of the experiment. After elimination of the plants that required vernalization or that had been accidentally damaged, 150 individuals remained for further analysis.

Data collection

At the end of the reproductive season, iteroparous beets produce vegetative rosettes (overwintering stage). All aerial

Table 1 Taxa, life cycles, codes and number of individuals of the various accessions used in the experiment. Labelling was chosen to obviate future necessity of cross-referencing to this table: long-lived iteroparous life-cycles or accessions are referred to as VI for 'very' and 'iteroparous', M means 'medium', L 'less' and S alone denotes 'semelparous'

Taxon	Accession	Life cycle	Code	Number of individuals
<i>B. v. maritima</i>	French Atlantic coast 1	Iteroparous, 4.7 years \pm 1.9	VI1	9
<i>B. v. maritima</i>	French Atlantic coast 2	Iteroparous, 5.5 years \pm 2.0	VI2	10
<i>B. v. maritima</i>	Mediterranean 1	Iteroparous, 4.1 years \pm 1.5	M11	14
<i>B. v. maritima</i>	Mediterranean 2	Iteroparous, 2.8 years \pm 1.2	M12	15
<i>B. v. maritima</i>	Agen area 1	Iteroparous, 2.0 years \pm 0.9	LI1	12
<i>B. v. maritima</i>	Agen area 2	Iteroparous, 2.0 years \pm 1.0	LI2	11
<i>B. patula</i>	Madeira	Semelparous, or rarely iteroparous	SI	17
<i>B. v. adanensis</i>	Greece 1	Semelparous	LS1	17
<i>B. v. adanensis</i>	Greece 2	Semelparous	LS2	15
<i>B. macrocarpa</i>	Cyprus	Strictly semelparous	VS1	15
<i>B. macrocarpa</i>	Tunisia	Strictly semelparous	VS2	15

parts (stems, leaves, flowers and seeds with the exception of embryos) degenerate and dry out. This is obviously the fate of all organs of semelparous plants. At this unambiguous physiological stage, which therefore corresponds to the end of the reproductive cycle in iteroparous plants and to ageing in semelparous plants, we recorded the age of individuals as well as the number of reproductive meristems estimated from the number of inflorescences.

Statistical analyses

A partially nested mixed-model ANOVA (generalised linear model (GLM) procedure (SAS Institute, 1988)) was conducted to compare age at the end of reproduction and the number of reproductive meristems between life cycles, between accessions within life cycles and between treatments. Life cycle and treatment were fixed effects and all others were random. This model was as follows:

$$Y_{ijkn} = \mu + \alpha_i + B_{ij} + \delta_k + (\delta\alpha)_{ik} + (\delta B)_{ijk} + e_{ijkn} \quad \text{Eqn 1}$$

(Y_{ijkn} , the reproductive effort of the n th individual of the j th accession nested in the i th life cycle and undergoing the k th treatment; μ , the mean; α_i , the fixed effect of the i th life cycle; d_k , the fixed effect of the k th treatment; B_{ij} , the random contribution of the j th accession of the i th life cycle.) $(\delta\alpha)_{ik}$ and $(\delta B)_{ijk}$ are, respectively, the life cycle \times treatment interaction effect and the accession \times treatment interaction effect, and e_{ijkn} is the error term. Test denominators were mean squares of accession (life cycle) for life cycle effect, of treatment \times accession (life cycle) for treatment and treatment \times life cycle effects, and of the error term for other effects. F -tests used type III sums of squares. Data were ln-transformed to meet the normality and homoscedasticity requirements of ANOVA for treatments (Sokal & Rohlf, 1995).

When the accession (life cycle) effect or the interaction treatment \times accession (life cycle) were significant, we also conducted one-way ANOVAs per accession with treatment as a factor. Significance levels were adjusted to multiple inferences by a sequential Bonferroni test (Rice, 1988), with $\alpha = 0.05$ and 11 tests. Multiple comparisons among means were conducted following a Student-Neumann-Keuls procedure (Sokal & Rohlf, 1995).

We finally tested the effect of treatment following the Bernoulli's law (probability test). The null hypothesis is that there is no effect of treatment. Under this null hypothesis, the probability of having a greater value (i.e. the number of meristems) under bud excision than in control group is 0.5. The Bernoulli's law gives the probability that x accessions of 11 have a greater value under bud excision than in the control group. With 11 accessions and $\alpha = 0.05$, the null hypothesis is rejected if the number of successes is equal to or higher than nine (i.e. nine accessions of 11 have a higher value under bud excision than in control group). Since the number

of reproductive meristems may depend on the age at the end of the reproductive cycle, we also conducted these analyses for a third parameter, namely the number of reproductive meristems weighted by the age at the end of reproductive cycle.

Results

Effect of excision on the number of reproductive meristems

The mixed model analysis of variance reveals a significant effect of the life cycle (Table 2), as well as highly significant effects of accession and treatment on the number of flowering meristems. The life cycle \times treatment interaction and the accession \times treatment interaction are not significant (Table 2).

The per-accession average number of reproductive meristems produced is always higher in bud excision treatment than in control (Figs 1 and 3). Numbers do not appear to be different in fruit excision treatment from control (all values are near to the diagonal compensation line, see Fig. 1).

The one-way ANOVAs conducted per accession give significant or highly significant effect of treatment on the number of reproductive meristems in VI1, VI2 and MI1 ($P = 0.0008$, $P = 0.0262$, $P = 0.0011$). Treatment effect is still significant after correction for multiple inferences (Bonferroni test) in VI1 and MI1. In these accessions, fruit-excision treatment was clustered with control and was significantly different from bud-excision treatment under a Student-Neumann-Keuls procedure.

Nevertheless, in all of the 11 accessions the number of reproductive meristems is higher in the bud-excision treatment than in control; therefore Bernoulli's law allows us to reject the null hypothesis that there is no effect of treatment at $\alpha = 0.0005$. This strongly supports the occurrence of a general effect of treatment on the number of reproductive meristems.

Results are strictly similar when the number of reproductive meristems is weighted by the age at the end of the reproductive cycle and are therefore not shown.

Table 2 Mixed model analysis of variance (GLM) on ln-transformed number of flowering meristems. Significant effects at $\alpha = 0.05$ are in boldface

Source	df	Type III MS	F Value
Life cycle	5	27.7473	6.87*
accession(life cycle)	5	4.0414	15.61***
Treatment	2	7.5151	26.02***
Life cycle \times treatment	10	0.5982	2.07
Treatment \times accession(life cycle)	10	0.2888	1.12
Error	117	0.2588	

***, significant at $P < 0.001$; *, $P < 0.05$. P -values are similar when the number of reproductive meristems is weighted by the age at the end of the reproductive cycle and are therefore not shown.

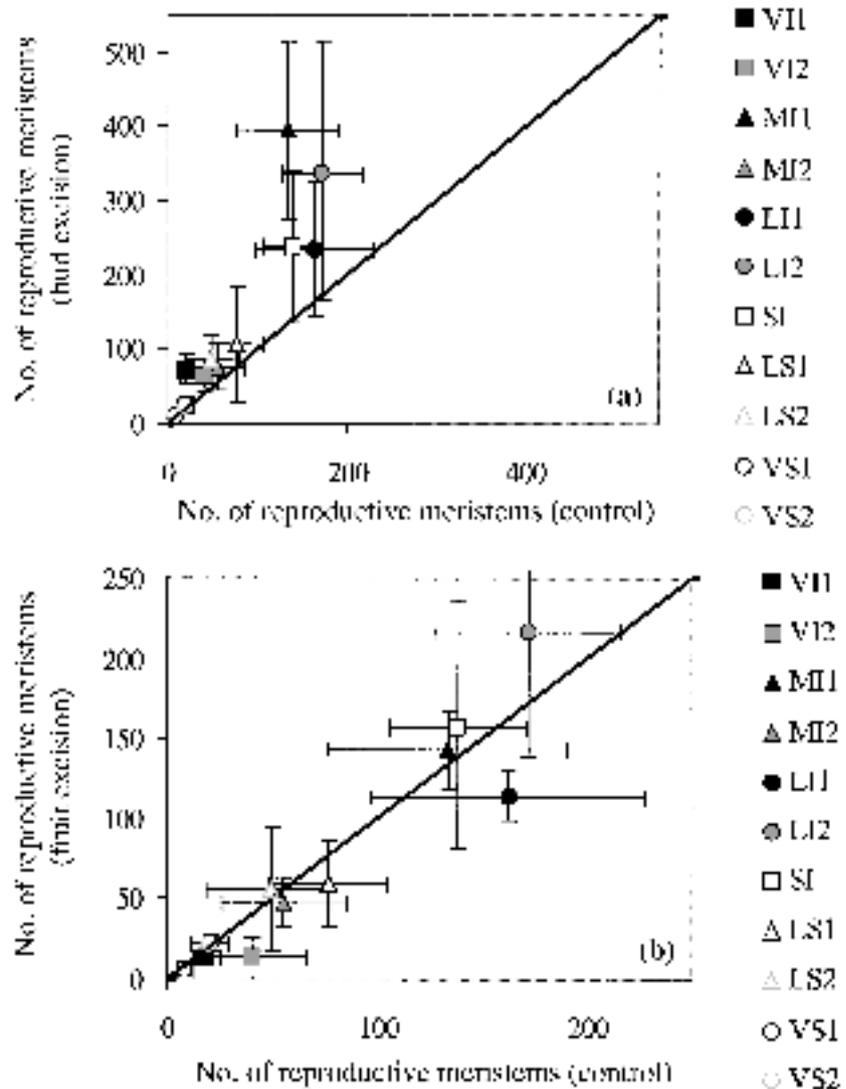


Fig. 1 Average number of reproductive meristems produced (a) in bud excision and in control treatments and (b) in fruit excision and in control treatments. Horizontal bars: SD in control treatment; vertical bars: SD in excision treatment. At the diagonal compensation line, plants in both treatments produce the same number of reproductive meristems.

Effect of excision on the end of the reproductive cycle (i.e. death in semelparous plants)

We stopped the experiment at day 330 (day 0 is the sowing date) and at that moment all MI1 individuals were still producing new reproductive meristems. We therefore decided to record their age at the end of the reproductive cycle as 320 (the average value, since on average seeds germinated 10 days after sowing date).

The mixed model analysis of variance (GLM) reveals highly significant effects of life cycle (Table 3), and of accession on age at end of the reproductive cycle (i.e. death in semelparous plants). Treatment effect, life cycle \times treatment and accession \times treatment interactions are insignificant (Table 2).

The per-accession averages of ages at the end of the reproductive cycle do not appear to be different in bud-excision treatment from control, except in the MI1 accession (Figs 2

Table 3 Mixed model ANOVA (GLM) on \log_e -transformed age at death (semelparous taxa) or age at end of reproductive cycle (iteroparous taxa)

Source	df	Type III MS	F Value
Life cycle	5	0.2999	48.10**
Accession (life cycle)	5	0.0204	3.27**
Treatment	2	0.0246	2.04
Life cycle \times treatment	10	0.0081	0.68
Treatment \times accession (life cycle)	10	0.0119	1.91
Error	117	0.0062	

Significant effects at $\alpha = 0.05$ are in boldface. **, significant at $P < 0.01$.

and 3). They also do not appear to be different in fruit-excision treatment from control (Figs 2 and 3).

The one-way ANOVAs conducted per accession give significant or highly significant effects of treatment on the age at

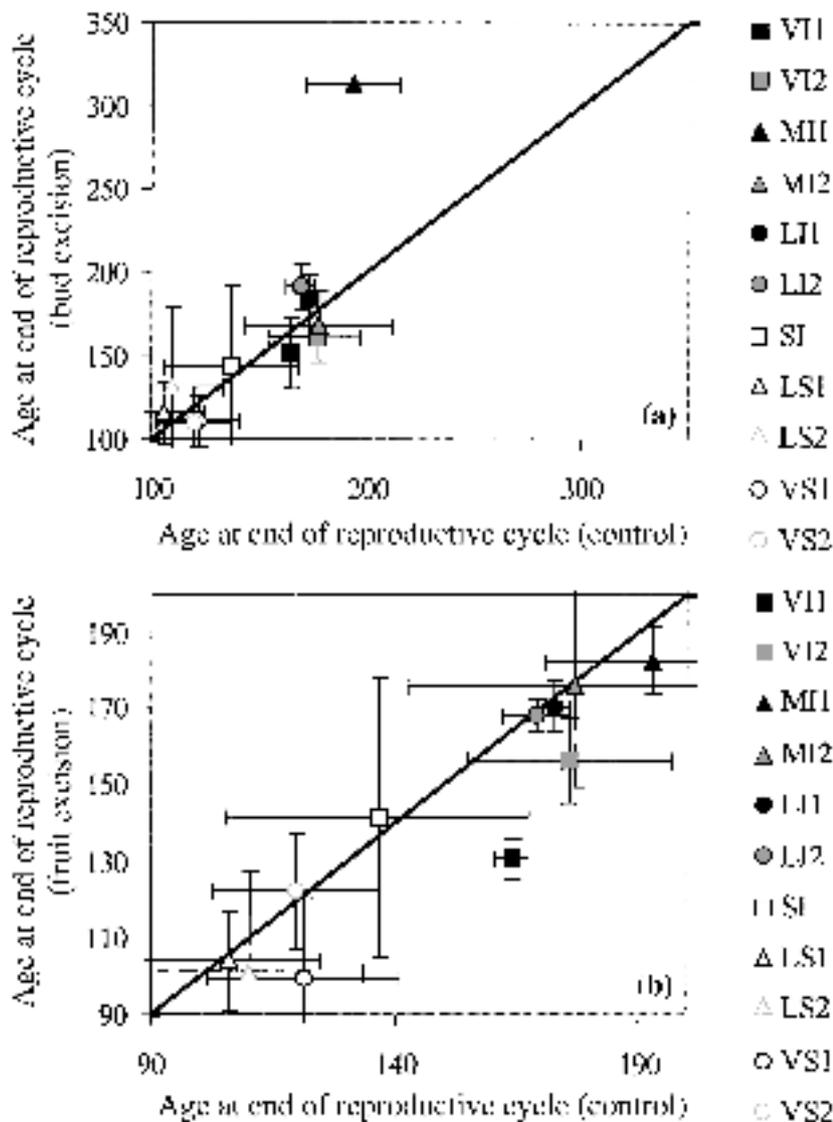


Fig. 2 Average age at the end of the reproductive cycle (a) in bud excision and in control treatments and (b) in fruit excision and in control treatments. Horizontal bars: SD in control treatment; vertical bars: SD in excision treatment. At the diagonal compensation line, plants in both treatments end their reproductive cycle at the same age. End of the reproductive cycle means senescence and death in semelparous plants (accessions VS1, VS2, LS1, LS2, SI) and the entering into a vegetative stage (rosette) in iteroparous plants (accessions VI1, VI2, MI1, MI2, LI1, LI2).

the end of the reproductive cycle in VI1 and MI1 ($P = 0.0472$ and $P < 0.0001$, respectively). Treatment effect is still significant after correction for multiple inferences (Bonferroni test) in MI1. In these accessions fruit-excision treatment was clustered with control and was significantly different from bud-excision treatment under a Student-Neumann-Keuls procedure.

In only 6 accessions, out of 11, the age at the end of the reproductive cycle is higher in the bud excision treatment than in control; therefore Bernoulli's law does not allow us to reject the null hypothesis that there is no effect of treatment (at $\alpha = 0.05$).

Discussion

Effect of excision on reproduction

Floral-bud excision induces the production of new reproductive meristems (i.e. originally quiescent meristems at

leaf axils which are activated and produce a new inflorescence in indeterminate flowering plants such as wild beets). This is particularly clear in iteroparous plants. However this is a general tendency in the *Beta* species-complex (as shown by the absence of life cycle \times treatment and accession \times treatment interactions, as well as by the probability test). This production of new reproductive meristems is not significantly affected by the length of the reproductive period.

Iteroparous taxa Floral buds or fruits act as sinks for nutrients and for products of photosynthesis. Flower-bud and developing-fruit excision releases some amount of resources which then becomes available for reproduction or survival. Theoretically, iteroparous plants might be able to reallocate those resources to survival. It was expected therefore that reproductive allocation would be arrested in iteroparous plants and consequently the release of resources would turn out to be advantageous for

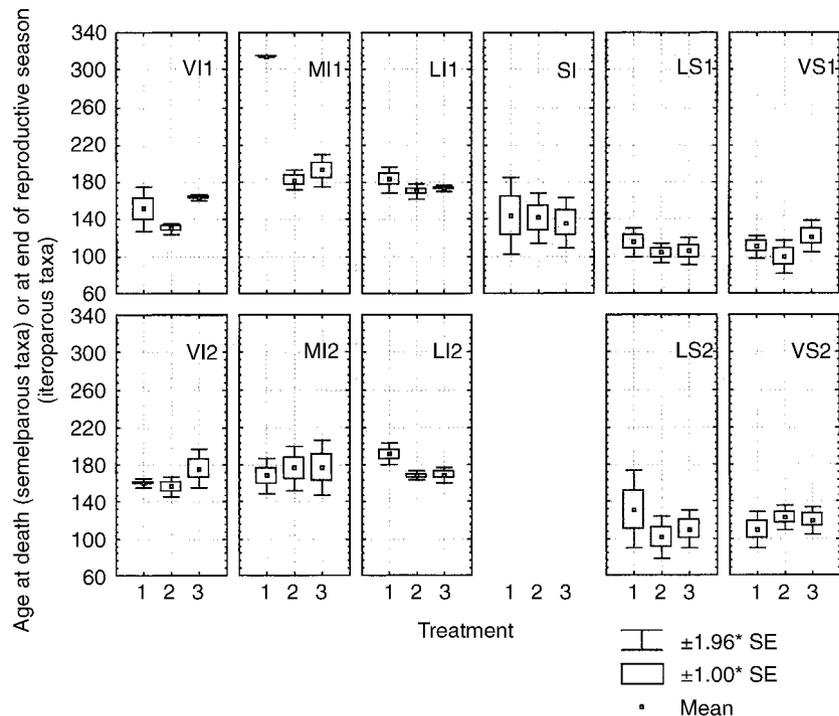


Fig. 3 Box-wiskers plots for age at end of reproductive cycle in the three treatments: 1, bud excision; 2, in fruit excision; and 3, control. End of the reproductive cycle means senescence and death in semelparous plants (accessions VS1, VS2, LS1, LS2, SI) and the entering in a vegetative stage (rosette) in iteroparous plants (accessions VI1, VI2, MI1, MI2, LI1, LI2).

survival and/or reserve organs. However the observed strategy is completely different, since iteroparous beets produce new floral meristems continually when sinks are removed. Allocation patterns appear to be fixed in the *Beta* species complex: released resources are probably not reallocated to survival but instead keep being allocated to reproduction.

Two nonexclusive explanations may be relevant. They are both based on the statement that bud excision probably mimics damages caused by some common herbivores or parasites of wild beets or even damages caused by some abiotic factors (though to a higher extent). Herbivores and abiotic factors may damage floral buds meristems. Some pathogens are known to castrate plants (Clay, 1990; Clay, 1991). Sugar beet, *B. v. vulgaris*, is a very close relative of the studied taxa and, as a crop, its enemies are obviously very well documented. Some herbivores and pathogens are known to cause the destruction of reproductive parts in sugar beet: among them *Botrytis cinerea* dries seeds up and *Aphis fabae* damages inflorescences (Lejealle *et al.*, 1982). Moreover wild beets grow along seashores and their inflorescences are often highly damaged by the spray (N.-C. Hautekèete & Y. Piquot, unpublished). Since flower-bud or fruit excision (although representing an extreme situation) has an ecological meaning, excision experiments may be interpreted in an ecologically orientated view.

The first of these explanations is based on the annual probability of destruction of reproductive organs. If flower-bud destruction is sporadic and does not happen each year, herbivory or parasitism may constitute a good clue for a bad season, and therefore it may be advantageous to skip reproduction

and wait for a better year to flower (Stearns, 1976). This would furthermore allow a higher investment in survival, since resources are released by sink removal. Alternatively, if bud destruction is recurrent in wild beets and happens almost each year, investing in survival and future reproduction should not represent a more appropriate strategy. Plants would therefore keep allocating the released resources to reproduction, following the reproductive effort that is optimal in this environment. This is the most probable situation here.

The second possible explanation is based on the hypothesis that bud destruction is not continuous throughout the reproductive season, and that herbivory gives information about risks of future damage. If early herbivory is a good clue that risk of future damage is high, induced resistance should be selected for (Karban *et al.*, 1999). Alternatively, if early herbivory is a good clue that risk of future damage is low, for example in case of migratory herbivores (Karban *et al.*, 1999), overcompensation should be selected for, which means a higher reproduction with, than without, grazing (Belsky, 1986; Paige & Whitham, 1987; Gronemeyer *et al.*, 1997; Lennartsson *et al.*, 1997). In intermediate situations, tolerance might be selected for and plants would simply compensate for damages. Overcompensation and tolerance both fit the pattern observed in wild beets (for a discussion of relationships between overcompensation or tolerance and resistance see Mauricio (2000).

The effect of bud removal on reproduction is highly accession-dependent. MI1 shows the most significant effects of treatment on the number of reproductive meristems and it is the only accession in which treatment effect on the age at the

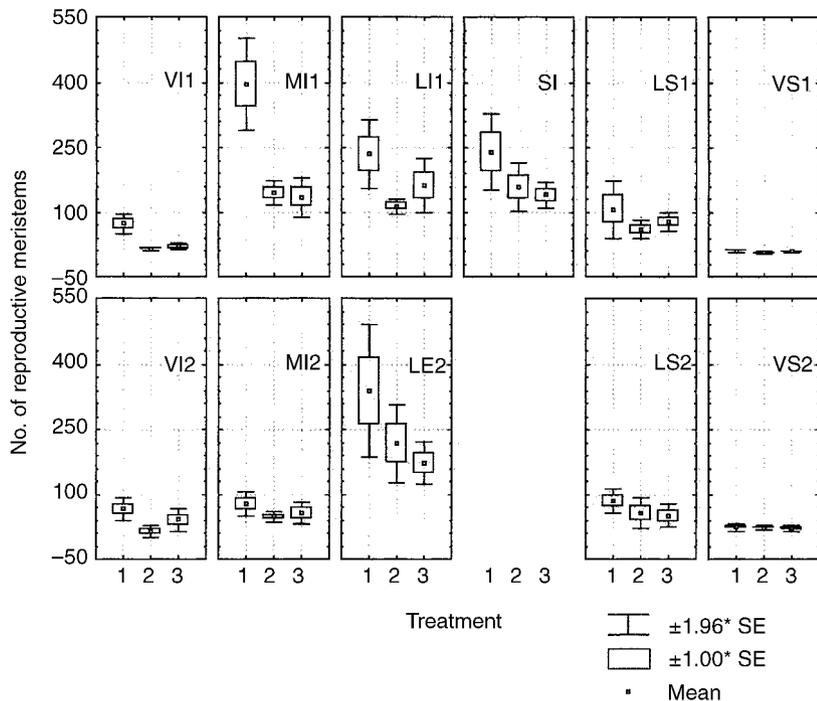


Fig. 4 Box-wiskers plots for number of reproductive meristems produced in the three treatments: 1, bud excision; 2, fruit excision; and 3, control.

end of the reproductive cycle is still significant after correction for multiple inferences. Under bud excision, individuals of the MI1 accession produce new reproductive meristems and end their reproductive cycle later through prolonged maintenance of the vegetative parts. This is the most coherent strategy, since seed maturation is delayed in the new inflorescences. The observed variation might depend on local ecological context. For example, in *Gentianella campestris* overcompensation is observed in the most grazed and late-flowering populations (Lennartsson *et al.*, 1997). Another explanation based on ecological disparity would be that in some regions (e.g. in the Mediterranean or in south-western France), too much delay in the production of flowers may be inefficient because of summer drought. Therefore selection for the production of new inflorescences would only be possible for a slight delay which would not be detectable. However ecological studies to explain that pattern in MI1 are lacking. It is also noticeable that, in our experiment, the longest-lived iteroparous beets (VI1 and VI2) are not the most treatment-dependent. These plants are very long-lived (*c.* 5 yr) and might therefore have more chance of experiencing good years (and thus of producing many seeds) than shorter-lived iteroparous ones. Since in our experiment no information is available on the following years, long-term experiments would be of interest, giving information about future allocation to survival vs reproduction (Haukioja & Koricheva, 2000).

Semelparous taxa Although treatment effect on the number of reproductive meristems is not significant in semelparous accessions, a general tendency to a higher number of floral

meristems under bud excision treatment than in control is observed (Figs 1 and 4). Resources released by the flower-bud removal are still allocated to reproduction. Similarly, the activity of inflorescence meristems is dependent on the number of developing fruits in *Arabidopsis thaliana* (L.) Heynh. (Hensel *et al.*, 1994) and in *Glycine max* (L.) Merr. (Proebsting *et al.*, 1976).

Ageing

Removal of reproductive organs does not delay whole-plant senescence in the *Beta* species-complex. This is obvious in semelparous plants, for which the end of the reproductive cycle coincides with whole-plant senescence. Fruit removal does not systematically result in delayed ageing in other plant species. The range of responses to such treatments that has been observed in previous experiments (Leopold, 1961; Carr & Pate, 1967; Thrower, 1967; Wareing & Seth, 1967; Leopold, 1980; Noodén, 1980; Crafts-Brandner *et al.*, 1984; McKersie *et al.*, 1988; Noodén, 1988a; Noodén, 1988b; Noodén, 1988c; Van Staden *et al.*, 1988; Wilson, 1997) is not surprising, since, due to the multiple origins of semelparity from iteroparous ancestors (Young & Augspurger, 1991; Watkinson, 1992; Bena *et al.*, 1998), semelparous ageing may imply various mechanisms and/or environmental stimuli. Furthermore, genotype effect on the delay of ageing induced by sink removal was demonstrated in maize by Crafts-Brandner *et al.* (1984), which suggests that ecological factors might have evolutionary consequences on ageing mechanisms.

Ageing does not appear to be delayed at all in semelparous beets. However they produce new reproductive structures. Production of new reproductive structures without any increased vegetative growth or maintenance is probably not optimal: seed maturation cannot be complete. It is therefore probable that, in their natural habitats, irreversible allocation strategies are not highly counter-selected, for example flower-bud herbivores and pathogens are rare or do not have extensive impact throughout the season. Physiological constraints are also possible.

It is plausible that survival does not increase in iteroparous beets either. They do not stop investing in reproduction and as a consequence they cannot invest surplus resources in survival. However extreme iteroparous beets do not invest much in reproduction and this might reflect a higher probability of survival to the following year.

Bud vs fruit excision

The stage at which floral organs are excised proved to be crucial in our experiment. Production of new reproductive meristems cannot be induced by sink removal when fruits begin to develop, whereas it is still possible at the floral bud stage. In the strawberry, compensation of flower removal is limited compared to compensation of bud removal (English-Loeb *et al.*, 1999). This might be interpreted in terms of resources, since, contrary to later stages, few resources are already committed in reproduction at the flower bud stage, and many resources are released by bud excision. Contrary to wild beets, developing-fruit removal allows the development of new inflorescences in *A. thaliana* (Hensel *et al.*, 1994; Bleecker & Patterson, 1997) and soybean (Proebsting *et al.*, 1976). At early fruit stage, pods and siliques are not very developed, and their excision might release some resources, whereas excision of ovules in glomerules just before the formation of their corky pericarp might not release enough resources: they might not be important sinks at this stage. However, release of hormones or growth regulators (e.g. abscisic acid, ethylene, cytokinin) at a particular stage (precocious in beets, late in soybean and *A. thaliana*) is not incompatible with this hypothesis.

A non-significant decrease of age at death is observed in all beet taxa under fruit-excision treatment, which might be interpreted as a slight effect of stress due to excision.

Semelparous vs iteroparous beets

Tendencies in semelparous and iteroparous taxa of wild beets are similar despite different scales of response to treatment. Since a rather large within-accession genetic variability might be expected from seeds collected in bulks, future experiments should involve a higher number of individuals per treatment to increase chances of observing differences. However in both life histories bud removal induces allocation of resources to

reproduction and has a null or undetectable effect on life span. Fruit removal does not induce any allocation to reproduction nor survival. Since reproduction and survival are negatively correlated in wild beets the optimal resource allocation might differ from an ecological context to the other (in terms of disturbance, richness or climate), leading to semelparity (100% reproduction) or iteroparity (reproduction and survival in various proportions). However this experiment suggests once more that semelparity and long-lived iteroparity are extreme strategies of a continuum (for a discussion about quantitative aspects of annuality/perenniality see Thomas *et al.* (2000)). Many architectural, physiological and, what is more surprising, life-history characteristics, such as control of meristematic activity by reproductive organs, are shared by these phylogenetically related taxa.

Conclusion

No radical change of strategy is observed in iteroparous beets: under flower-bud excision treatment they keep on investing in reproduction. This is also the case in the semelparous taxa of wild beets, and it is remarkable that they do not show delayed ageing. It is therefore probable that the strategy (investment in reproduction vs investment in survival) is stated at a precocious stage (see Introduction) and is not reversible. Only one iteroparous population shows prolonged vegetative maintenance enabling complete seed maturation.

The effect of sink removal on ageing in semelparous plants should not be considered universal. Specific, populational or genotypic variability does exist and should be kept in mind. To help in understanding this variability, complementary information may be given by related iteroparous species in which the choice between reproduction and survival is clearer. Investment in reproduction is related to life span by trade-offs and, as a consequence, both ageing and reproductive meristematic activity should be jointly studied as related parts of the same strategy. Separated studies of iteroparous or semelparous species, life span or reproduction, might focus the attention on particular strategies resulting from phylogeny or from differential selective pressures in local habitats in relation to ecology. When too many discrepancies between studies are found, future experiments might require more evolutionary and ecological approaches.

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