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Effects of defoliation on reproductive success in two orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*

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Herbivory is a very common phenomenon in nature, which may considerably alter subsequent survival and reproduction of affected plants. We examined the effects of three levels of experimental defoliation (0%, 50% or 100% of the leaves removed) on reproductive behaviour, leaf production, survival and flowering of two nectarless orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*. Neither orchid species showed differences between the non-defoliated and defoliated plants in total pollinia mass, but seed production was significantly lower in defoliated plants. The male reproductive traits appear to be more buffered against the effect of defoliation than female traits. Defoliation also decreased leaf area and the probability of flowering in the year following the treatments. In this paper, we show that natural levels of herbivory can severely affect both current and future reproduction in these two orchid species.

Key words: fruit, herbivory, nectarless orchid, pollinia

Introduction

In the Orchidaceae, the sexual reproduction is predominantly pollinator or automatic self-pollination dependent even if, sometimes, seeds may be successfully produced asexually by agamospermy. Seed production of flowering plants is determined by pollinator limitation and/or resource limitation (Bierzychudek 1981, Stephenson 1981). Under natural conditions, it is difficult to determine whether low seed production is due to the absence of effective pollinators or to resource limitation. Life history of plants may be affected both by biotic factors, such as herbivores (Reichman & Smith 1991) and by abiotic ones (nutrient and water availability), which may considerably alter subsequent survival and future reproduction, since resources used by individuals for current reproduction are not available for future use (Charlesworth 1980, Reznick 1985). Leaf herbivory removes photosynthetically active tissue, which decreases the amount of energy available for growth, flower and fruit production. A decrease in photosynthetic area has been found to be limiting for male and female reproductive success, by decreasing directly plant growth (Nunez-Farfan & Dirzo 1991), pollen production (Lehtilä & Strauss

1999), flower and seed production (Stephenson 1984, Meyer 1998), and fruit set (Obeso 1993, Koptur *et al*. 1996), or indirectly by decreasing attractiveness to pollinators (Strauss *et al*. 1996). Moreover, defoliation may cause a reduction in the probability of flowering in subsequent years (Primack & Hall 1990, Whigham 1990, Vallius & Salonen 2000). The consequences of herbivory for male and female reproductive success have been assessed in only a few species (McKone 1989, Quesada *et al*. 1995, Gronemeyer *et al*. 1997, Aizen & Raffaele 1998, Lehtilä & Strauss 1999, Vallius & Salonen 2000). The most effective and unambiguous way to determine if there is a cost of herbivory is to conduct a manipulative experiment in the field.

This paper presents the results of a defoliation experiment conducted to evaluate the effect of leaf removal on male and female reproductive success, growth and survivorship of two perennial terrestrial orchids. In natural populations of two nectarless orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*, we investigate if defoliation affects male and female reproductive traits, and if it affects subsequent growth and probability of flowering.

Methods

Study species and site

Serapias vomeracea has a single viscidium and two guiding swellings at the base of the labellum. Sepals, petals and lateral lobes of the hypochile together form a small tube. The epichile, narrow distal part of the labellum, points downwards. Pollen is packed into two pollinaria and the entire pollinarium is removed when visited by a single successful pollinator. The insects use this "floral tube" to rest or sleep or as a draught-free hiding place in bad or rainy weather conditions (Gumprecht 1977, Dafni *et al*. 1981). Interestingly, although the main pollinators of *S. vomeracea* are *Eucera* bees, small bees (i.e. *Prosopis* sp. and *Ceratina* sp.) have also been observed to shelter without taking pollinia (Dafni *et al*. 1981). Studies on populations of *S. vomeracea* showed that occasional pollinators were bees of the genera *Andrena*, *Osmia* and *Tetralonia* (Vöth 1980, Felicioli *et al*. 1998, Pellegrino *et al*. 2005a) and Coleoptera (Oedemeridae and Lymexylidae) (Pellegrino *et al*. 2005a). The observations were conducted during the flowering period of *Serapias vomeracea* in May–June 2002 and May–June 2003 in the National Park of Pollino (Calabria, southern Italy) in a relatively dense population consisting of about 150–200 flowering plants.

Dactylorhiza sambucina is a widespread non-rewarding orchid, growing disjunctly all over Europe (Tutin *et al*. 1980). Flowers of *D. sambucina* do not produce nectar, and pollination occurs mainly by non-mimetic food deception of naïve bees (van der Cingel 1995). Each flower has a pollinarium with pollen packed into two pollinia, as it is in all the Orchidinae (Dressler 1981). The entire pollinarium can be removed by a single successful visit by a pollinator. The main pollinators of *D. sambucina* are bumblebees (*Bombus* sp.), principally queens of *B. lapidarius*, *B. terrestris* and *B. lucorum*, cuckoo bumblebees (*Psithyrus* app.) and bees of other genera, i.e. *Apis* sp. and *Osmia* sp. (Nilsson 1980, Vöth 1980). The experiments were conducted in a population of 400–500 plants in the National Park of Calabria (Sila, southern Italy) during spring 2002 and 2003 (April–June).

Field experiment

In the early spring of 2002, before blooming, 75 plants in each population, selected for study based on their uniformity of size and healthy appearance, were bagged with a fine mesh cloth to exclude pollinators. Plants were permanently marked with plastic wire. Each plant was randomly assigned to one of three levels of leaf removal treatments: non-defoliated plants (control), partially defoliated plants (50% of leaf area was removed by clipping away the top half of each leaf), and completely defoliated plants (all leaves were cut off from the base). These levels of leaf damage are comparable to natural damage commonly seen in the field as a result of herbivory by cows, sheep and insects. To determine the leaf area, we measured plants for leaf length and width just before the treatment. Because leaves of both orchid species are rhomb-shaped,

leaf area was calculated using the empirically derived formula: leaf area = 0.5(leaf length)(leaf width). During the flower period, the pollinaria were collected using a toothpick and successively weighed on a microscale balance. Immediately after removal of pollinia, each flower was cross-pollinated with pollinaria taken from plants growing outside the experimental area.

In June, after capsule maturation, we counted the capsules and quantified the proportion of treated flowers producing seeds for each experiment. Capsules were dried at 80 °C for 24 hours and weighed. In the year following the treatments (2003), each experimental plant was examined to assess its stage (dead or underground, vegetative or flowering) and the leaf area of each vegetative and flowering plant was calculated from the length and width of leaves.

Statistical analysis

The effects of defoliation (0%, 50% or 100% of leaf area removed) on male (weight of pollinia) and female function (number of produced capsules) were evaluated with the analysis of variance (ANOVA) followed by Tukey's test (SPSS statistic software package) (Norusis 1992). Data were log-transformed prior to the analysis. Normal distribution was analyzed using Shapiro-Wilk's *W*-test (Royston 1992). The effect of treatments on probability of flowering, and on probability of a plant being vegetative in the following year, and the change in leaf area between years were tested with the χ^2-test at the 0.05 level of statistical significance.

Results

Defoliation did not significantly affect the mass of pollinia produced in *S. vomeracea* and in *D. sambucina* (Table 1). Defoliation showed no significant effect on the relative capsule production (ranging from 19.3% to 21.2% in *D. sambucina*, and from 15.8% to 17.4% in *S. vomeracea*), but capsules produced by the completely defoliated plants were lighter than those produced by non-defoliated plants (Table 1). In both orchid species examined, capsules produced by completely defoliated plants were significantly lighter than those produced by non-defoliated plants (P = 0.001 and P = 0.003 in *D. sambucina* and *S. vomeracea*, respectively). There were no differences in dry weight of capsules produced by partially defoliated and non-defoliated plants (Table 1).

The completely defoliated plants had a lower probability of flowering in the year following treatment than the non-defoliated or partially defoliated plants (Table 2). There was no difference between the treatments in number of plants being vegetative in 2003, while defoliation seemed to increase significantly the probability of plants to stay subterranean or die, and to decrease significantly the number of flowering plants (Table 2).

Mean (± S.E.) leaf areas measured before and in the year following the treatment in the non-defoliated, partially defoliated and completely defoliated plants are reported in Table 3. Complete defoliation significantly decreased the leaf area of vegetative and flowering plants in the following year. Non-defoliated plants produced

Table 1. Mean (± S.E.) pollen mass per flower, mean (± S.E.) capsule production and mean (± S.E.) capsule weight and results of ANOVA in the non-defoliated (0%), partially defoliated (50%) and completely defoliated (100%) plants of *Dactylorhiza sambucina* and *Serapias vomeracea*. n = 25 for each treatment.

	0%	50%	100%	MS	*F*	*P*
Dactylorhiza sambucina						
Weight of pollinia (μg)	202.0 (± 3.17)	197.0 (± 2.98)	195.3 (± 3.02)	441.4	0.391	0.587
Capsule production (%)	21.2 (± 0.57)	20.2 (± 0.67)	19.3 (± 0.65)	7.1	0.671	0.421
Weight of capsules (mg)	14.9 (± 0.21)	13.9 (± 0.18)	8.9 (± 0.10)	30.5	9.243	0.001
Serapias vomeracea						
Weight of pollinia (μg)	188.0 (± 3.89)	178.2 (± 2.97)	175.5 (± 4.02)	452.1	0.361	0.689
Capsule production (%)	16.0 (± 0.41)	15.8 (± 0.15)	14.4 (± 0.51)	8.4	0.881	0.418
Weight of capsules (mg)	18.0 (± 0.65)	17.6 (± 0.32)	9.1 (± 0.15)	28.1	7.121	0.003

more leaf area in 2003 than completely defoliated plants (Table 3).

Discussion

We evaluated the effects of artificial defoliation treatments on male and female reproductive traits, and on probability of flowering in the season following the experiments in two orchid species with different pollination strategies. The levels of leaf damage are comparable to natural damage commonly seen in our populations in the two National Parks. Defoliation had no effect on pollinia mass per flower in *S. vomeracea* and in *D. sambucina* (Table 1). From this evidence we can conclude that, during the season of damage, pollen quantity is not affected by defoliation in nectarless orchids. A similar response has been found in another orchid (*Dactylorhiza maculata*, Vallius & Salonen 2000), while in other cases defoliation decreased pollen production (*Bromus inermis*, McKone 1989) or the quality of pollen (*Lobelia siphilitica*, Mutikainen & Delph 1996). The fact that no significant differences were found in male reproductive traits among plants indicates that, to compensate for loss of tissue, an efficient translocation of resources from an underground corm has occurred (Primack & Hall 1990, Robertson & Wyatt 1990, Primack *et al*. 1994) to fully satisfy the requirement of pollen production in the completely defoliated plants. In contrast to annuals, it may be advantageous for a long-lived perennial to maintain the male function in case of poor resource availability, because one orchid pollinaria may fertilize more than just one flower (Inoue 1986), and if pollination success is good, a plant suffering from defoliation may gain higher fitness through pollen donation than through the production of costly seed capsules.

Because it has been demonstrated that the dry weight of a capsule and the number of seeds it contains are strongly positively correlated in *Dactylorhiza maculata* (Vallius 2001), it can be

Table 2. The number of *Dactylorhiza sambucina* and *Serapias vomeracea* plants flowering, non-flowering or remaining cryptic (subterranean or dead) in the year following treatments (0%, 50% and 100% of the leaves removed) and results of χ^2-test.

	0%	50%	100%	χ^2	*P*
Dactylorhiza sambucina					
Flowering	15	11	1	10.81	0.005
Vegetative	10	13	17	2.01	0.422
Underground	0	1	7	9.81	0.005
Serapias vomeracea					
Flowering	14	9	0	11.02	0.005
Vegetative	10	14	16	2.65	0.421
Underground	1	2	9	10.01	0.005

Table 3. Mean (± S.E.) leaf area of *Dactylorhiza sambucina* and *Serapias vomeracea* plants measured before to treatment (before) and of flowering and vegetative plants in the year following the treatment (after) (0%, 50% and 100% of the leaves removed) and results of χ^2-test.

	0%	50%	100%
Dactylorhiza sambucina			
Leaf area (cm^2) before	11.7 (± 1.89)	11.8 (± 1.39)	11.5 (± 0.93)
Leaf area (cm^2) after	12.6 (± 1.91)	9.4 (± 1.01)	6.9 (± 0.89)
χ^2	1.34	2.01	10.98
P	0.544	0.121	0.001
Serapias vomeracea			
Leaf area (cm^2) before	8.7 (± 1.09)	8.7 (± 0.84)	9.0 (± 1.19)
Leaf area (cm^2) after	8.6 (± 0.79)	7.3 (± 0.66)	5.9 (± 0.49)
χ^2	0.88	1.81	11.01
P	0.782	0.562	0.001

concluded that in the *S. vomeracea* and *D. sambucina* populations completely defoliated plants produced fewer seeds than non-defoliated plants as a consequence of a negative influence of defoliation on resource availability. Defoliation has also been found to reduce female reproductive success in other perennial orchids (Vallius & Salonen 2000). Insufficient availability of photosynthates is probably a major reason for the observed reduction in capsule number among the defoliated plants.

Orchids show a mutualistic association with mycorrhizal fungi. If the flow of carbohydrates to the fungi decreases due to the defoliation, the supply of mineral nutrients by the fungi could decrease (Alexander *et al.* 1984, Allen 1991). The allocation to male reproduction seems to suffer less from the effects of defoliation than the female function. A possible explanation for this lack of response may be that pollen production always precedes fruit production; therefore the male function can have a temporal advantage. Both carbon and mineral nutrients can be sufficiently available for pollen production, but can become insufficient before the seed production has been completed.

In addition to the decrease in seed production, the ability of plants to store sufficient resources for next season also diminished due to defoliation. The non-defoliated plants had a greater probability of flowering in the year following the treatment than the completely defoliated ones (Table 3). A similar effect of defoliation on flowering has also been found in other orchid species (*Tipularia discolor*, Whigham 1990, *Cypripedium acaule*, Primack *et al.* 1994, *Dactylorhiza maculata*, Vallius 2001). In these defoliated plants, leaf area was also reduced in the next season (Table 3). It is probable that the reproduction of *S. vomeracea* and *D. sambucina* will be further delayed, as has been observed in another orchid species, *Tipularia discolor* (Whigham 1990).

Both orchids studied in this paper showed a similar response to defoliation. Capsule production may compete with pollen production especially for nutrients (Mutikainen & Delph 1996), indeed nutrient and water availability (Mattila & Kuitunen 2000) has also been found to limit seed production in orchids.

Previous studies have shown that reproduction success of such deceptive orchids is often limited by pollinator visitation due to the lack of rewards (Janzen *et al.* 1980, Firmage & Cole 1988, Zimmerman & Aide 1989, Proctor & Harder 1995, Johnson & Nilsson 1999, Matsui *et al.* 2001). In previous studies (Pellegrino *et al.* 2005a, 2005b) it has been shown that reproductive success of two investigated rewardless orchids (*D. sambucina* and *S. vomeracea*) is generally pollinator-limited within a season, while this study shows that seed production is limited by reduction of photosynthetic products due to decrease of leaf surface.

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