

SHORT COMMUNICATION

Marcescent corollas as functional structures: effects on the fecundity of two insect-pollinated plants

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- **Background and aims** Persistence of withered corollas after anthesis ('corolla marcescence') is widespread in angiosperms, yet its functional significance does not seem to have been explored for any species. This note reports the results of experiments assessing the fecundity effects of marcescent corollas in two southern Spanish insect-pollinated plants, *Lavandula latifolia* (Lamiaceae) and *Viola cazorlensis* (Violaceae).
- **Methods** The effect of marcescent corollas on seed production was evaluated experimentally on wild-growing plants. Newly open flowers were randomly assigned to either control or treatment groups in experimental plants. After anthesis, withered corollas of treatment flowers were removed and those in control flowers were left in place. Fruits produced by treatment and control flowers were collected shortly before dehiscence and the number of seeds counted.
- **Key Results** In *V. cazorlensis*, removal of withered corollas had no effect on percentage of fruit set, but mean seeds per fruit increased from 9.5 to 11.4. In *L. latifolia*, corolla removal had no effect on the number of seeds per fruit, but reduced the proportion of flowers ripening fruit from 60 % to 40 %. The detrimental effect of corolla removal on *L. latifolia* fecundity resulted from the drastic increase in fruit infestation by seed-predatory cecidomyiid larvae, which occurred in 4 % and 34 % of control and treatment fruits, respectively.
- **Conclusions** Because of their potential effects on plant fecundity, marcescent corollas should not be dismissed *a priori* as biologically irrelevant leftovers from past floral functions. The simplicity of the experimental layout required to test for short-term fecundity effects of corolla marcescence should help to achieve a better understanding of the ecological and evolutionary correlates of this widespread but poorly understood trait.

Key words: Corolla marcescence, fecundity, *Lavandula latifolia* (Lamiaceae), seed predation, *Viola cazorlensis* (Violaceae).

INTRODUCTION

In the wake of a tradition initiated by Sprengel (1793) and consolidated by Darwin (1862), virtually every discernible trait of angiosperm flowers has been subjected to careful scrutiny and meticulous investigation over more than two centuries. Despite this vast research effort, however, the biological significance of certain floral features still remains poorly understood. In many flowering plants, parts of the perianth persist beyond the completion of anthesis, which suggests that they are co-opted for some new function(s) unrelated to pollination. In some instances, such post-anthesis functionality is quite apparent, as in species where spiny or plumose calices play decisive roles in seed dispersal (van der Pijl, 1982). Functionality is far less obvious in other cases, as exemplified by the withered corollas that remain attached to the developing fruits of many plants ('marcescent corollas'; Little and Jones, 1980). The post-anthesis functionality of persistent perianth parts has been rarely investigated, and the few studies available have focused exclusively on the function of the calyx (Yonemori *et al.*, 1995; Nakano *et al.*, 1997; Sisterson and Gould, 1999; Salopek-Sondi *et al.*, 2000, 2002; Aschan *et al.*, 2005; Herrera, 2005b). Investigations addressing the post-anthesis functionality of the corolla are needed to assess

the significance and possible adaptive value of the various modalities of perianth persistence in angiosperms, a widespread but poorly understood floral feature. An improved understanding of the post-anthesis functionality of the perianth would also contribute to the more general issue of whether, because of possible contrasting selective pressures operating at the anthesis and post-anthesis stages, multifunctionality of a particular floral structure might favour the evolution of compromise floral attributes, an aspect that remains essentially unexplored in the floral biology literature (Herrera, 2005b). This note reports the results of an experimental study designed to assess the biological significance of corolla marcescence in two southern Spanish insect-pollinated plants, *Lavandula latifolia* (Lamiaceae) and *Viola cazorlensis* (Violaceae). Specifically tested was whether the marcescent corollas that remain attached to the developing fruits of these species enhanced plant fitness through effects on seed production, a finding that would support an adaptive role for the trait.

MATERIALS AND METHODS

Study area and species

This study was carried out at two localities of Parque Natural Sierras de Cazorla-Segura-Las Villas, Jaén province,

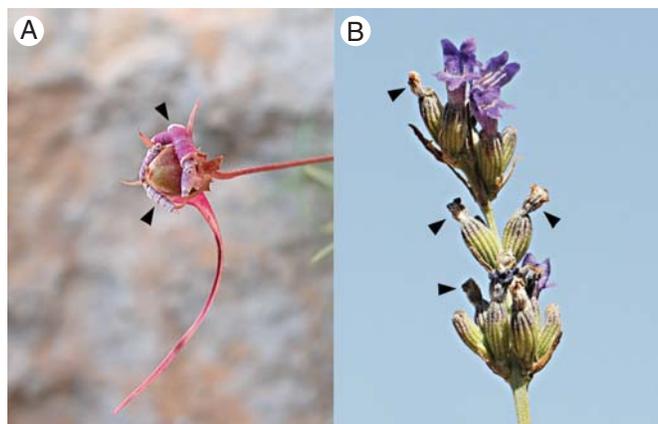


FIG. 1. Developing fruits of *Viola cazorlensis* (A) and *Lavandula latifolia* (B) showing the attached marcescent corollas characteristic of these species (black arrows). In the hawk-moth-pollinated *V. cazorlensis*, the long floral spur also persists attached to the fruit until the latter's dehiscence.

south-eastern Spain. Experiments on *Viola cazorlensis* were carried out during June–July 2005 on a population located in Cerrada del Escalón (1520 m a.s.l.), and those on *Lavandula latifolia* during July–September 2000 on plants growing at Arroyo Aguaderillos (1180 m a.s.l.). These two species were chosen for study because their reproductive biology is known in considerable detail and they are representative of genera where corolla marcescence occurs frequently.

Viola cazorlensis is a perennial, suffruticose violet endemic to a few contiguous limestone mountain ranges in south-eastern Spain (Morales Torres, 2009). The flowers have pinkish-purple corollas, are characterized by a long and thin spur, and are pollinated by a single species of long-tongued day-flying hawk moth (*Macroglossum stellatarum*, Sphingidae). The dialypetalous corolla is not shed after anthesis, with the petals rolling up after withering and persisting closely associated to, and thoroughly enclosing, the basal portion of the developing capsule until fruit dehiscence and seed shedding (Fig. 1A). Additional information on *V. cazorlensis* can be found in Herrera (1993), Herrera and Bazaga (2008a, 2009), and references therein.

Lavandula latifolia is a summer-flowering, insect-pollinated shrub of open woodlands on limestone substrates that is widely distributed in southern France and the eastern Iberian Peninsula (Upson and Andrews, 2004). Flowers are hermaphroditic, self-compatible, and are pollinated by a diverse assemblage of bees, flies and butterflies. The sympetalous corolla persists after anthesis, forming a dry, plug-like structure that blocks the entrance of the slightly inflated calyx during the whole period of fruit development (Fig. 1B). Prior to fruit maturation, the calyx aperture widens and the dry corolla gets detached and is shed before seeds are dispersed. Further information on *L. latifolia* in the study area can be found in Herrera (2000, 2005a), Herrera and Bazaga (2008b), and references therein.

Corolla removal experiments

A manipulative experiment adopting a randomized block design was designed to evaluate the possible effect of

corolla marcescence on fecundity. Individual *V. cazorlensis* and *L. latifolia* plants ($n = 21$ and 10 plants, respectively) were treated as blocks, and within each plant newly open flowers were marked individually and randomly assigned to either control or treatment groups. Marked flowers were exposed to natural pollinator visitation. Within each *L. latifolia* plant, control and treatment flowers were distributed equally among ten different inflorescences. Immediately after anthesis, the withered corollas of flowers in the treatment group were carefully removed using fine forceps ($n = 104$ and 388 flowers, for *V. cazorlensis* and *L. latifolia*, respectively), while those of flowers in the control group were left in place ($n = 102$ and 218 flowers, for *V. cazorlensis* and *L. latifolia*, respectively). Touching experimental flowers while removing their corollas was inevitable. To avoid possible artefacts arising from differential manipulation (e.g. Cahill *et al.*, 2002), control flowers were also touched in similar ways. Fruits produced by control and treatment flowers developed naturally on plants until collected shortly before dehiscence, and the number of seeds in each fruit was counted. In the case of *L. latifolia*, presence in fruits of the seed-eating larvae of an unidentified cecidomyiid fly was also recorded.

Statistical analyses

Statistical analyses were conducted using the SAS statistical package. The effects of corolla removal on fruit set (= proportion of flowers setting sound fruits, i.e. fruits bearing at least one seed) and number of seeds per sound fruit were tested by fitting generalized linear mixed models to the data using SAS procedure GLIMMIX, residual pseudolikelihood estimation, and the containment method for computing denominator degrees of freedom (SAS Institute, 2006). For fruit set, the response variable (fruits/flowers, i.e. the probability of a flower producing a fruit) was modelled as a binomial response, using logits as the link function. In *L. latifolia*, the probability of fruits being infested by Cecydomiid larvae was likewise modelled as a binomial response. In analyses of seed number per fruit, seed counts were modelled as a normal response. The two-level (corolla removed vs. marcescent) experimental factor (corolla persistence) was treated as a fixed effect, and individual plants (i.e. blocks) were included as a random effect in models. Means and associated standard errors reported below are model-corrected means obtained with the LSMEANS statement.

RESULTS

The protracted persistence of withered corollas attached to the developing fruits of *V. cazorlensis* and *L. latifolia* (Fig. 1) was consequential for seed production in both species, although the sign, magnitude and nature of effects were different. In *V. cazorlensis*, manual removal of the corolla immediately after withering had no effect on fruit set, but did increase the mean number of seeds ripened per fruit by about 20% (from 9.5 to 11.4 seeds/fruit), the effect being marginally significant (Table 1). In *L. latifolia*, in contrast, removal of the withered corolla had no significant effect on number of seeds per fruit, but did induce a highly significant reduction in the proportion of flowers that produced fruits, which fell

TABLE 1. Summary of results of generalized linear mixed models testing for the effect of post-anthesis corolla removal on fruit set (proportion of flowers producing sound fruits, i.e. bearing at least one seed) and fruit seediness (number of seeds per sound fruit) in *Viola cazorlensis* and *Lavandula latifolia* (for the latter species, the effect of corolla removal on the proportion of fruits infected by seed-predatory cecidomyiid larvae was also tested)

	Post-anthesis corolla		Treatment effect		
	Present	Removed	F	d.f.	P
<i>Viola cazorlensis</i>					
Fruit set	0.73 ± 0.05	0.77 ± 0.05	0.38	1,20	0.54
Fruit seediness	9.5 ± 0.8	11.4 ± 0.8	3.62	1,60	0.062
<i>Lavandula latifolia</i>					
Fruit set	0.62 ± 0.42	0.40 ± 0.47	18.71	1595	<0.0001
Fruit seediness	1.50 ± 0.11	1.52 ± 0.11	0.08	1279	0.77
Proportion of infected fruits	0.04 ± 0.06	0.34 ± 0.06	54.9	1365	<0.0001

Model-adjusted means are shown ± s.e.

from 60 % to 40 % (Table 1). The detrimental effect of corolla removal on *L. latifolia* fecundity was largely the consequence of a drastic increase in fruit infestation by cecidomyiid larvae. The latter destroyed most or all the seeds in those fruits where they occurred (mean ± s.e. = 1.42 ± 0.11 and 0.36 ± 0.13 seeds per fruit for uninfested and infested fruits, respectively; $F_{1,365} = 153.0$, $P < 0.0001$). Cecidomyiid larvae occurred in only 4 % of developing fruit bearing the natural marcescent corollas, and their incidence rose to 34 % in fruits whose withered corollas were removed after anthesis, the difference being highly significant (Table 1).

DISCUSSION

The few investigations that have examined the post-anthesis role of perianth parts have focused on the functionality of the calyx. These studies have shown that persistent calices often contribute photosynthates to developing fruits and seeds. In cultivated persimmon fruits (*Diospyros kaki*), photosynthesis by the persistent calyx lobes contributes to fruit development (Yonemori *et al.*, 1995), and in species of *Helleborus* the expanded green sepals are a source of assimilates for the developing seeds (Salopek-Sondi *et al.*, 2000, 2002; Herrera, 2005b). In *Helleborus viridis*, photosynthesis by the persistent sepals may contribute up to 60 % of whole-plant CO₂ gain during certain periods (Aschan *et al.*, 2005). In contrast, there seems to be no equivalent investigations explicitly addressing the biological significance of marcescent corollas, and the experiments reported here apparently represent the first attempt at exploring the subject. Although limited in scope, the results for *V. cazorlensis* and *L. latifolia* show that marcescent corollas can have nontrivial effects on plant fecundity, and that the nature of such effects and the mechanisms involved can be species- or locality-specific, as discussed below.

In *L. latifolia*, marcescent corollas played a protective function of the developing seeds by reducing the susceptibility of fruits to infestation by cecidomyiid larvae. After withering, corollas quickly dry up, harden, and are tightly held by the embracing calyx, becoming plug-like structures which presumably hinder the access of ovipositing female flies to the calyx interior and the enclosed achenes. Although the experiments

reported here were conducted on a single season, observations conducted at the same *L. latifolia* population in the course of other studies likewise support a seed-protective function of marcescent corollas in this species. In 1997, cecidomyiid flies occurred in 24.9 % of developing *L. latifolia* fruits with the corollas removed ($n = 181$ fruits), while their occurrence in unmanipulated fruits was always <10 % in most years (C. M. Herrera, unpubl. res.).

In *V. cazorlensis*, the effects of corolla removal on fecundity ran opposite to those for *L. latifolia*, since seed production tended to be enhanced by corolla removal, although the effect was only marginally significant. The beneficial effect on fecundity of corolla removal could perhaps be accounted for by enhanced photosynthetic contribution from capsule walls to seed development due to greater exposure to solar radiation. This result would suggest that corolla marcescence is a maladaptive trait in *V. cazorlensis*, but this interpretation must remain speculative at present. The developing capsules of *V. cazorlensis* are often severely preyed upon by a species of microlepidopteran larvae (*Heliothella wulfeniana*; Herrera, 1993), but evidence of fruit predation by this species was not found at the study site during the experiment reported here. Marcescent corollas could limit microlepidopteran infestation in years or sites where these seed predators are present, which would enhance *V. cazorlensis* fecundity by playing a protective function similar to that documented here for *L. latifolia*. This raises the possibility that the functionality and possible adaptive value of marcescent corollas are contingent on features of the ecological context, a suggestion which remains to be investigated by future studies.

The adaptive value of marcescence, the retention of dead plant organs that normally are shed, is insufficiently understood. Leaf marcescence occurs frequently in temperate deciduous trees and alpine plants, and experimental evidence suggests that it can reduce the impact on plants of large mammalian herbivores, and water and temperature stress (Smith 1979; Svendsen, 2001). Marcescent corollas occur in many flowering plant families, and corolla marcescence has been used as a diagnostic character to discriminate among taxonomic entities at the species level (e.g. Albert and Struwe, 1997) and above (e.g. Watson, 1964; Panero *et al.*, 1999; Gillespie and Kron, 2010). Results presented here show that

marcescent corollas, like marcescent leaves, are sometimes functional structures with direct effects on plant fitness, and should therefore not be dismissed *a priori* as biologically irrelevant leftovers from past functions. Retaining dead, physiologically inert structures may under some circumstances be an effective means for plants to perform certain functions without incurring additional energy and nutrient costs, which may explain the commonness of the phenomenon. The simplicity of the experimental layout required to test for the short-term fecundity effects of marcescence should help to achieve a better understanding of the ecological and evolutionary correlates of this widespread trait.

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LITERATURE CITED

- Albert V, Struwe L. 1997. Phylogeny and classification of *Voyria* (saprophytic Gentianaceae). *Brittonia* **49**: 466–479.
- Aschan G, Pfanz H, Vodnik D, Batic F. 2005. Photosynthetic performance of vegetative and reproductive structures of green hellebore (*Helleborus viridis* L. agg.). *Photosynthetica* **43**: 55–64.
- Cahill JF, Castelli JP, Casper BB. 2002. Separate effects of human visitation and touch on plant growth and herbivory in an old-field community. *American Journal of Botany* **89**: 1401–1409.
- Darwin C. 1862. *The various contrivances by which British and foreign orchids are fertilised by insects*. London: Murray.
- Gillespie E, Kron K. 2010. Molecular phylogenetic relationships and a revised classification of the subfamily Ericoideae (Ericaceae). *Molecular Phylogenetics and Evolution* **56**: 343–354.
- Herrera CM. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecological Monographs* **63**: 251–275.
- Herrera CM. 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. *Ecology* **81**: 15–29.
- Herrera CM. 2005a. Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany* **92**: 13–20.
- Herrera CM. 2005b. Post-floral perianth functionality: contribution of persistent sepals to seed development in *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* **92**: 1486–1491.
- Herrera CM, Bazaga P. 2008a. Population-genomic approach reveals adaptive floral divergence in discrete populations of a hawk moth-pollinated violet. *Molecular Ecology* **17**: 5378–5390.
- Herrera CM, Bazaga P. 2008b. Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. *Heredity* **100**: 275–285.
- Herrera CM, Bazaga P. 2009. Quantifying the genetic component of phenotypic variation in unpedigreed wild plants: tailoring genomic scan for within-population use. *Molecular Ecology* **18**: 2602–2614.
- Little RJ, Jones CE. 1980. *A dictionary of botany*. New York, NY: Van Nostrand Reinhold.
- Morales Torres C. 2009. Violaceae. In: Blanca G, Cabezudo B, Cueto M, Fernández López C, Morales Torres C, eds. *Flora vascular de Andalucía Oriental*, Vol. 2. Sevilla: Consejería de Medio Ambiente, 291–297.
- Nakano R, Yonemori K, Sugiura A. 1997. Photosynthesis by calyx lobes has no contribution to early fruit development in persimmon. *Acta Horticulturae* **436**: 345–353.
- Panero JL, Jansen RK, Clevinger JA. 1999. Phylogenetic relationships of subtribe Ecliptinae (Asteraceae: Heliantheae) based on chloroplast DNA restriction site data. *American Journal of Botany* **86**: 413–427.
- van der Pijl L. 1982. *Principles of dispersal in higher plants*, 3rd edn. Berlin: Springer.
- Salopek-Sondi B, Kovac M, Ljubesic N, Magnus V. 2000. Fruit initiation in *Helleborus niger* L. triggers chloroplast formation and photosynthesis in the perianth. *Journal of Plant Physiology* **157**: 357–364.
- Salopek-Sondi B, Kovac M, Prebeg T, Magnus V. 2002. Developing fruit direct post-floral morphogenesis in *Helleborus niger* L. *Journal of Experimental Botany* **53**: 1949–1957.
- SAS Institute. 2006. *The GLIMMIX procedure*, June 2006. Cary, NC: SAS Institute. Available over the internet at <http://support.sas.com/rnd/app/papers/glimmix.pdf>
- Sisterson MS, Gould FL. 1999. The inflated calyx of *Physalis angulata*: a refuge from parasitism for *Heliothis subflexa*. *Ecology* **80**: 1071–1075.
- Smith AP. 1979. Function of dead leaves in *Espeletia schultzii* (Compositae), an Andean caulescent rosette species. *Biotropica* **11**: 43–47.
- Sprengel CK. 1793. *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen*. English translation by Haase P. In: Lloyd DG, Barrett SCH, eds. 1996. *Floral biology*. New York, NY: Chapman and Hall, 3–43.
- Svendsen CR. 2001. Effects of marcescent leaves on winter browsing by large herbivores in northern temperate deciduous forests. *Alces* **37**: 475–482.
- Upson T, Andrews S. 2004. *The genus Lavandula*. Portland, OR: Timber Press.
- Watson L. 1964. The taxonomic significance of certain anatomical observations on Ericaceae: the Ericoideae, *Calluna* and *Cassiope*. *New Phytologist* **63**: 274–280.
- Yonemori K, Hirano K, Sugiura A. 1995. Growth inhibition of persimmon fruit caused by calyx lobe removal and possible involvement of endogenous hormones. *Scientia Horticulturae* **61**: 37–45.