

Pollen transfer and diurnal versus nocturnal pollination in *Lonicera etrusca*.

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Abstract

We studied basic aspects of the reproduction and pollination ecology of *Lonicera etrusca* G. Santi (Caprifoliaceae) in the northwest Iberian peninsula. Our results indicate that the species is self-compatible and that its flowers are dichogamous. Fruit production requires insect pollination. Levels of fruit-set following xenogamous and geitonogamous pollen transfer (65.5% and 43.1% respectively) did not differ significantly from controls (53.8%). Fruit-set following autogamy was zero. The principal flower visitors were *Bombus terrestris* (51.4% of visits), *Xylocopa violacea* (18.6%) and *Macroglossum stellatarum* (8.2%). Fruit-set was 3.5 times higher when only diurnal pollination was allowed than when only nocturnal pollination was allowed.

Keywords: Pollen transfer, diurnal pollination, nocturnal pollination, *Lonicera etrusca*.

Résumé

Nous avons étudié les aspects fondamentaux de la reproduction et de la pollinisation de *Lonicera etrusca* G. Santi (Caprifoliaceae) dans le nord-ouest de la péninsule ibérique. Nos résultats indiquent que l'espèce est auto-compatible et que ses fleurs sont dichogames. La production de fruits nécessite la pollinisation par les insectes. Les niveaux d'apparition de fruits consécutifs au transfert de pollen xénogame et geitonogame (65.5 % et 43.1 % respectivement) ne diffèrent pas significativement des témoins (53.8 %). L'apparition de fruits consécutive à l'autogamie est nulle. Les principaux visiteurs des fleurs sont *Bombus terrestris* (51.4 % des visites), *Xylocopa violacea* (18.6%) et *Macroglossum stellatarum* (8.2 %). L'apparition de fruits est 3,5 fois plus élevée lorsque seule la pollinisation diurne est possible que lorsque seule l'est la pollinisation nocturne.

INTRODUCTION

The general association between the characteristics of flowers and those of their pollinators provides the basis for the hypothesis that interaction with pollinators is responsible for the diversity of floral types (CAMPBELL *et al.*, 1991). However, any study which sets out to investigate the effects of pollinators on floral morphology should take into account two questions. First, to what extent does the plant require pollinator visits in order to produce fruits and seeds? Second, how important are

the selective pressures resulting from illegitimate visitors that take nectar but, in most cases, do not transfer pollen.

As part of our general aim of investigating flowering and fruiting phenology and pollination of *Lonicera etrusca* in the northwest Iberian peninsula, we have attempted to answer these specific questions. First, we examined the effect on fruit-set of varying the pollen transfer system. Second, we evaluated the extent of diurnal variation in the relative importance of the various pollinator species, by excluding insects at various times throughout the day and monitoring eventual effects on fruit-set: since the diurnal activity patterns of the principal flower visitors are reasonably well-known, this allows us to draw conclusions about the importance of each species for pollination, and, indirectly, about the importance of nectar robbery. Species of the genus *Lonicera* (Caprifoliaceae) – especially those included in the section *Caprifolium* – are of particular value for the study of these questions, since their long and narrow corolla tubes greatly limit insect access. Previous studies have considered various aspects of the reproductive biology of the genus: flowering and fruiting phenology (SNOW & SNOW, 1988; GUITIAN & GUITIAN, 1990; JORDANO, 1990; FUENTES, 1991), reproductive system, pollination and nectar robbery (J. HERRERA, 1985; OTTOSEN, 1986; JORDANO, 1990), and fruit and seed characteristics and dispersal (DEBUSSCHE *et al.*, 1987; C. HERRERA, 1987; SNOW & SNOW, 1988; JORDANO, 1990; FUENTES, 1992). The information available about *Lonicera etrusca* G. Santi is still somewhat incomplete, although JORDANO (1990) has recently studied various aspects of the species' pollination in southern Spain. This information also allows us to consider, finally, the extent of geographical variation in the reproductive biology of this species.

STUDY AREA AND PLANT

The study was carried out in the El Bierzo region of León, NW Spain, on two thornscrub sites near the village of La Barosa, at altitudes of 500-550 m. The area has a Mediterranean climate with vegetation dominated by *Quercus rotundifolia* woodland (additional information can be found in GUITIAN & FUENTES, 1992). The study plots were cultivated until about 30 years ago, and now support a community dominated by *L. etrusca*, *Prunus mahaleb* and *Quercus rotundifolia*. *Lonicera etrusca* (Caprifoliaceae) is a climbing shrub which, in the study area, typically forms part of a thorny scrub fringe community around mature *Quercus rotundifolia* woodland. The flowers are initially white becoming yellowish with age. Pollination is largely by hawkmoths (Sphingidae). The fruit is a berry, bright red when mature, which is eaten by frugivorous birds, especially blackcaps *Sylvia atricapilla* (FUENTES, 1991).

METHODS

Studies of flowering and fruit phenology were carried out in 1991. Fifteen clearly heterogenetic adult plants were selected at random along a 60 m transect, and one branch per plant was marked with plastic labels; these plants were then inspected every three days during the flowering period, and every two weeks during the fruiting period. On the same plants, 10 flowers were marked and inspected every day, to determine floral duration and to monitor pistil receptivity and stamen dehiscence. Fruit-set (number of flowers producing mature fruit as a percentage of initial number of flowers) was calculated in open-pollinated flowers on the basis of data from branches marked in 1991 and 1992 in the same plants. Pollen transfer tests were carried out with flowers evenly distributed between 6 individuals, treated as follow:

1. Insect exclusion: bagging with mosquito netting (total number of flowers, $n = 119$).
2. Xenogamy: emasculation, pollination with pollen from another plant, then bagging ($n = 58$).
3. Geitonogamy: emasculation, pollination with pollen from the same plant, then bagging ($n = 51$).
4. Autogamy: pollination with pollen from the same flower, then bagging ($n = 26$).
5. Control: no treatment ($n = 91$).

In all cases, emasculation was carried out immediately prior to anthesis. Imminent anthesis is readily identifiable as the petals begin to detach distally from the corolla. Fruit-set was recorded after two months.

The amount of nectar available to insects (standing crop) was measured with capillary micropipettes in the early morning (0800 h GMT), before the onset of pollinator activity, in 20 "unrobbed" one-day-old flowers. Sugar concentration in the nectar was determined with a portable refractometer; the amount of sugar (mg per flower) was then calculated as per PRYS-JONES & CORBET (1978). Sugar composition was determined in the laboratory following dissolution of the nectar in distilled water. Reduced sugars were determined by colorimetry before and after acid hydrolysis (KÄPYLÄ, 1978). Insect visits were investigated by a series of 15 min surveillance sessions at each hour of the day, totalling 6 h. A total of 403 visits was observed; in each case the type of visit was recorded (nectar extraction, pollen collection, nectar robbery). We also examined 449 flowers at the end of their life (> 3 days old) for signs of nectar robbery: field determination of whether a flower has been robbed or not is straightforward, since the incision made by the insect in the tube of the corolla is clearly visible. Specimens of all insect taxa observed are in the authors' collection.

To evaluate the relative importance of pollination at different times of day we used 11 plants, bagging all flowers on four branches of each plant (one branch for each treatment) as follows:

1. Nocturnal exclusion: flowers exposed to visitors from dawn to dusk (0400 h GMT to 2000 h GMT), but bagged during the night ($n = 141$).
2. Diurnal exclusion: flowers exposed from dusk to dawn, but bagged during the day ($n = 146$).
3. "Midday" exclusion: flowers bagged between 1000 h GMT and 1500 h GMT ($n = 152$). Our observations suggest that *Xylocopa violacea* displays peak activity during this period, whilst the activity of *Bombus terrestris* drops considerably.
4. Control: no treatment ($n = 152$).

This experiment continued for three weeks, after which bagging was stopped: fruit-set for each treatment was recorded two months later. Three plants were slightly damaged during handling, and were therefore excluded from the analysis.

In all cases the chi-square test was used for comparison of data, with $p < 0.05$ being taken as significant. Spearman's rank correlation coefficient was calculated between duration of the flowering period on individual plants and the number of flowers produced by each plant. The possible effects of the factors "plant" and "treatment" on the probability of a flower setting fruit were examined using the procedure CATMOD (Categorical Data Modelling) in the statistical package SAS (SAS Institute, 1988).

RESULTS

Flowering phenology and fruit-set

The first floral buds appeared in the first week of April. The flowering period in the study area started on May 3 and finished on June 6 (55 days). The first fruits appeared on May 23. By July 28 all fruits had matured. The period of availability of mature fruit (between appearance of the first mature fruit and disappearance of the last mature fruit) started on June 14 and ended on August 8 (fig. 1). Mean length of the flowering period for individual plants was 21 ± 12 days, and was

positively correlated with the number of flowers produced by each plant ($r_s = 0.87$, $p < 0.05$). Mean fruit-set in plants not receiving either pollen transfer or pollinator exclusion treatments was 53.8% in 1991 and 58.5% in 1992; these values are not significantly different ($X^2 = 0.14$, d.f. = 1, n.s.).

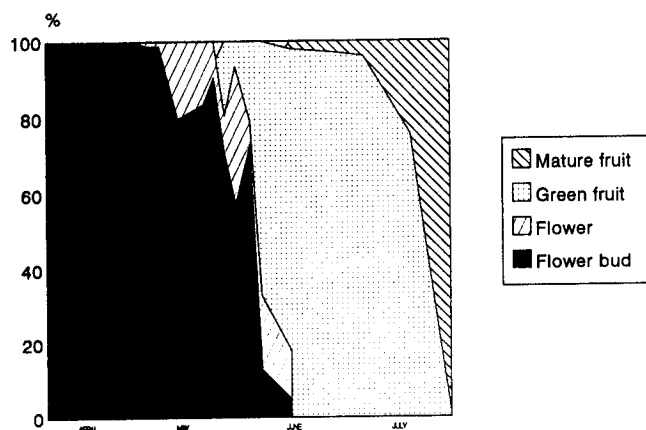


FIG. 1. – Flowering and fruiting phenology of *Lonicera etrusca*. The diagram shows the percentage of marked branches at each phenological stage over the period April–July.

Reproductive system

Floral duration was, in all cases, three days. The stigma is receptive when the flower opens, and anther dehiscence occurs from the second day onwards. Fruit-set obtained following xenogamous and geitonogamous pollen transfer (table I) do not differ significantly from those obtained in control plants (xenogamy: $X^2 = 0.51$, d.f. = 1, n.s.; geitonogamy: $X^2 = 0.51$, d.f. = 1, n.s.). Following insect exclusion and autogamous pollen transfer, fruit-set level was zero.

TABLE I. – Fruit-set from artificial pollen transfer and control pollinations.

	Control	Insect exclusion	Autogamy	Geitonogamy	Xenogamy
Flower No.	91	119	26	51	58
Fruit No.	49	0	0	22	38
Fruit-set (%)	53.8	0	0	43.1	65.5
Range (%)	25–94.4	–	–	20–80	33.3–90

Pollination

The mean volume of nectar available in the early morning, was $4.1 \pm 2.9 \mu\text{l}$ (range 1–9 μl) per flower; mean sugar concentration was $23 \pm 4.1\%$ (range 20–26%); mean sugar weight per flower was thus $0.8 \pm 0.6 \text{ mg}$. The mean saccharose-to-hexose ratio was 0.9. The principal flower visitors and types of visit are shown in table II. Of the 403 visits recorded, 15.1% were “normal” nectar extraction visits,

TABLE II. – Number of floral visits of the three foraging types (nectar extraction, pollen collection, and nectar robbing) by the various insect taxa. Figures in brackets show the percentage of total visits.

Taxon	Type of visit		
	Nectar extraction n (%)	Pollen collection n (%)	Nectar robbing n (%)
HYMENOPTERA			
Anthophoridae			
<i>Anthophora acervorum</i>	3 (0.7)		
<i>Anthophora hispanica</i>	11 (2.7)		25 (6.2)
Megachilidae			
<i>Megachile sp.</i>		5 (1.2)	
Vespidae		1 (0.2)	
Xylocopidae			
<i>Xylocopa violacea</i>			75 (18.6)
Apidae			
<i>Psithyrus vestalis</i>	2 (0.5)		
<i>Bombus terrestris</i>	12 (3.0)		195 (48.4)
LEPIDOPTERA			
Sphingidae			
<i>Macroglossum stellatarum</i>	33 (8.2)		
DIPTERA			
Syrphidae			
		19 (4.7)	
COLEOPTERA			
Carabidae			
		22 (5.5)	

11.7% were pollen collection visits and 73.2% were nectar robbery visits. In the first group the principal visitor was *Macroglossum stellatarum* (8.2% of all visits). In the second group the principal taxa were Carabidae (5.5%) and Syrphidae (4.7%). The principal nectar robbers were *Bombus terrestris* (48.4%) and *Xylocopa violacea* (18.6%). Our observations indicate that *M. stellatarum* (Sphingidae) is the principal "legitimate" pollinator, but that *B. terrestris* and *X. violacea*, the principal nectar robbers, may also enter into contact with the plant's sexual organs. *B. terrestris* tends to land on the basal part of the corolla tube, although we have occasionally observed it in the distal part contacting stamens and pistils (see also MACIÖR, 1966). *X. violacea* cuts through the basal part of the corolla, like *B. terrestris*, but (since it is larger) the ventral part of its abdomen often makes contact with the sexual organs of the flower at the same time. In our opinion both species may be accidental pollinators. Of the 449 flowers monitored for signs of nectar robbery throughout their lifespan, 99% were robbed: robbing occurred both before (especially when the robber was *X. violacea*) and after anthesis (J. GUITIAN, personal observation). The results of the nocturnal/diurnal exclusion experiments (fig. 2) indicate significant differences in fruit-set, both between control flowers and diurnally exposed flowers ($X^2 = 5.3$, d.f. = 1, $p < 0.05$) and between control flowers and nocturnally exposed flowers ($X^2 = 38.4$, d.f. = 1, $p < 0.05$); in both cases fruit-set was significantly higher in control flowers. Fruit-set was 3.5 times higher in diurnally exposed flowers than in nocturnally exposed flowers ($X^2 = 17.1$, d.f. = 1, $p < 0.05$). "Middy"

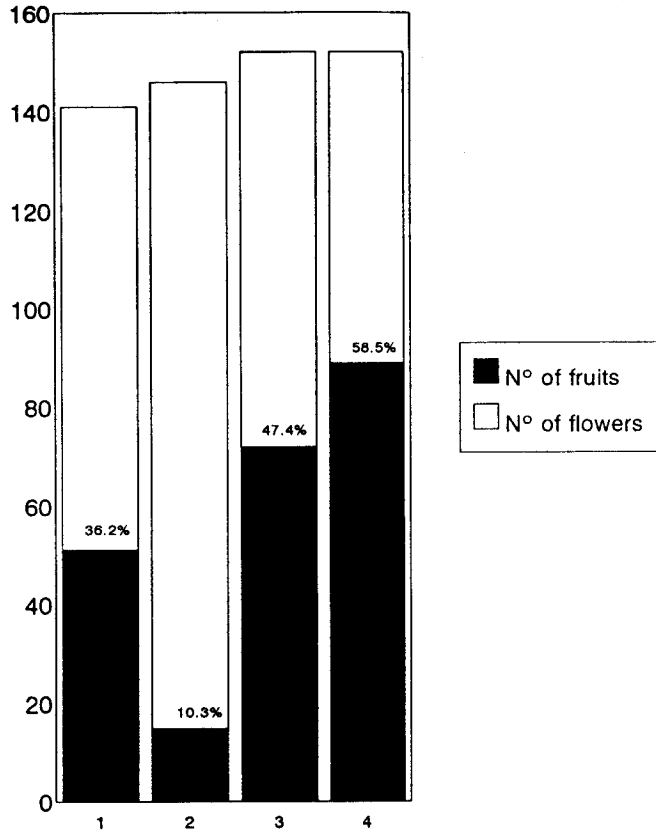


FIG. 2. – Fruit-set recorded following the different pollinator exclusion treatments (1: nocturnal exclusion; 2: diurnal exclusion; 3: midday exclusion; 4: control, *i.e.* no exclusion).

exclusion of insects had no significant effect on fruit-set either with respect to control flowers ($X^2 = 1.2$, d.f. = 1, n.s.) or with respect to diurnally exposed flowers ($X^2 = 1.5$, d.f. = 1, n.s.); fruit-set was, however, significantly higher than in nocturnally exposed flowers ($X^2 = 27.9$, d.f. = 1, $p < 0.05$).

The results of CATMOD analysis (table III) show that the factor “plant” was not a significant source of variability in the probability of a flower setting fruit,

TABLE III. – Analysis of variance table for the effects of plant and treatment on fruit-set of individual flowers (CATMOD procedure, SAS institute, 1988, using maximum likelihood method).

Source	D.F.	Chi-square	Prob.
Intercept	1	0.00	0.9620
Plant	7	10.16	0.1794
Treatment	3	10.77	0.0130
Plant × treatment	21	38.25	0.0120
Likelihood ratio		0.00	1.0000

whilst the factor "treatment" was significant. The interaction "plant x treatment" was also significant, indicating that the effect of the treatments on the probability of a flower setting fruit differed between plants, i.e. that there were individual differences in the magnitude of response.

DISCUSSION

Our results indicate that *L. etrusca* in the western Iberian peninsula is spring-flowering (May to June), and that its fruits mature in the summer (June to July), as is the case in other Mediterranean areas (DEBUSSCHE *et al.*, 1987). In the Sierra de Cazorla, a mountainous area in southern Spain, flowering occurs in the last third of spring, with variations of one or two weeks depending on altitude (JORDANO, 1990). Such altitude-related phenological delays are well-documented, and can be attributed to climatic restrictions in mountainous areas (see, for example, ARROYO, 1990). We found no significant between-year variation in fruit-set. Fruit-set in both 1991 and 1992 was higher than that reported for this species in Cazorla (JORDANO, 1990).

Since self-compatibility was indicated by the geitonogamy experiments (see also SCHÖEN & LLOYD, 1992), the absence of fruit-set following autogamy indicates that the flowers are dichogamous. Mean fruit-set was high with respect to controls following xenogamous pollen transfer and low with respect to controls following geitonogamous pollen transfer (as observed by JORDANO, 1990); the differences were not, however, statistically significant. Other authors (SEAVEY & BAWA, 1986; WIENS *et al.*, 1987) have suggested that fruit production in angiosperms is significantly limited when pollen transfer is geitonogamous: our results do not allow us to conclude that this is the case for *L. etrusca*.

Our results confirm the hypothesis that flowers with a tubular corolla tend to have saccharose-rich nectars (PERCIVAL, 1965; CORBET, 1978), and that they are largely pollinated by long-tongued bees or hawkmoths (BAKER & BAKER, 1983). *Lonicera* species have been previously reported to be largely pollinated by hawkmoths (PERCIVAL, 1965; JORDANO, 1990).

The results of the nocturnal/diurnal exclusion experiments indicate that fruit production when only diurnal pollination is allowed is 3.5 times greater than when only nocturnal pollination is allowed. This suggests that pollination of *L. etrusca* in our study area is largely diurnal. Likewise, in the Sierra de Cazorla, JORDANO (1990) found that *Macroglossum stellatarum* (whose activity peak – according to HERRERA, 1992 – occurs between 1800 and 2000 h GMT) was the principal pollinator of *L. etrusca*. In studies of other plant species considerable between-year variation in the relative importance of diurnal and nocturnal pollination has been reported (see for example JENNERSTEN, 1988). "Midday" bagging did not significantly reduce fruit-set with respect to controls: the level observed was in fact similar to that obtained by summing the levels observed following diurnal and nocturnal pollinator exclusion. This suggests that pollination during the central hours of the day is not important. Midday is a period of peak activity for *X. violacea* (J. GUITIAN, personal observation; HERRERA, 1990) and of reduced activity for *B. terrestris*. Again, this supports the view that *M. stellatarum* is the principal pollinator, and suggests that nectar robbery by *X. violacea* and *B. terrestris* does not have an important effect on fruit-set.

In conclusion, the reproductive biology of *L. etrusca* appears similar to that described for hawkmoth-pollinated plants in general (BAKER, 1961; BAKER & BAKER, 1983). Our data and those of JORDANO (1990) suggest that there are no important geographical differences in the plant's relationships with pollinators and nectar robbers.

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