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REPRODUCTIVE BIOLOGY OF HALIMIUM ATRIPLICIFOLIUM (LAM.) SPACH AND H. HALIMIFOLIUM (L.) WILLK. (CISTACEAE)

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Abstract

The reproductive biology of *Halimium atriplicifolium* and *H. halimifolium* was studied between 1993-1995 in South Spain. Both species showed the fenomenon of flowers lasting only a few hours in the day. Flower anthesis occurred very early in the morning: at 7 hours in *H. atriplicifolium* and at 9 h in *H. halimifolium*. The most important group of insect visitors to *H. atriplicifolium* flowers were *Hymenoptera* and to *H. halimifolium* were *Coleoptera*. *Halimium* spp. showed self-incompatibility, no fruit set was found after hand self-pollination. Fruit set was very different between species, and it was very low always in *H. atriplicifolium*. The results are discussed in relation to the exceptional flowering period of *H. halimifolium*.

Introduction

Cistaceae is one of the most important families of the Mediterranean flora. It has five genera [*Cistus, Fumana, Halimium, Helianthemun* and *Xolantha* (=*Tuberaria*)] which form an important part of the Mediterranean shrub. The genus *Halimium* comprises six species in the Iberian Peninsule [*H. calycinum* (L.) K. Koch (=*H. commutatum* Pau), *H. umbellatum* (L.) Spach, *H. halimifolium* (L.) Willk., *H. lasianthum* (Lam.) Spach, *H. ocymoides* (Lam.) Willk. and *H. atriplicifolium* (Lam.) Spach, NOGUEIRA & al., 1993]. The species studied here, *H. halimifolium* and *H. atriplicifolium* have big, pentamerous, yellow flowers (Fig. 1A, B). In *H. atriplicifolium* the flowers in lax cymes have 5 sepals and in *H. atriplicifolium* they are in numerous paniculate cymes with flowers with 3 sepals. Although there are some works on the reproductive biology of Cistaceae (BOSCH, 1992; TALAVERA & al., 1993; HERRERA, 1992; BRANDT & GOTTSBERGER, 1988), little is known about *Halimium* species (BRANDT & GOTTSBERGER, 1988; HERRERA, 1986, 1987). The aims of this work is to contribute to this knowledge.

Material and Methods

Study of *H. halimifolium* was carried out in an area of matorral near of the town of Hinojos (Huelva Province, S Spain) at 80-120 m altitude and at ca. 30 Km from the sea. The vegetation in this area consits of a mixed woodland pine (*Pinus pinea* L.) and cork oak (*Quercus suber* L.). The shrub layer is composed mainly by *Cistaceae*, *Leguminosae* and *Ericaceae*. *H. atriplicifolium* was studied in the Nature Reserve of

"Sierra de Grazalema" on dolomitic solis. This is a mountainous area located in Cádiz Province (S Spain) at 900-1000 m altitude. Vegetation in this zone consists of a dense scrub, with abundant scattered trees of *Quercus rotundifolia* Lam., *Ceratonia siliqua* L. and *Juniperus phoenicea* L. The shrub layer, much more diverse, comprises *Oleaceae, Anacardiaceae, Cistaceae, Leguminoseae* and *Labiatae*.

Field work was carried out in *H. atriplicifolium* from 1993 to 1995 and in *H. halimifolium* during 1995. During the flowering season the sequence of the following floral events were observed for both species. In 37 plants of *H. atriplicifolium* and in 32 of *H. halimifolium* the anthesis rhythm was recorded. These flowers were monitored in order to determine the anther dehiscence, stigma receptivity, corolla changes and corolla abscision. Observations of the activities of flower visitors were made from 6 to 19 h (local time).

The breeding system was assessed in the marked plants. Controlled self- and cross-pollinations were made. Most of the flowers were left to set fruit and them the number of fruits and number of seeds per fruit and seed set was determined for each treatment. The same procedure was followed in flowers left to natural pollinations in the field.

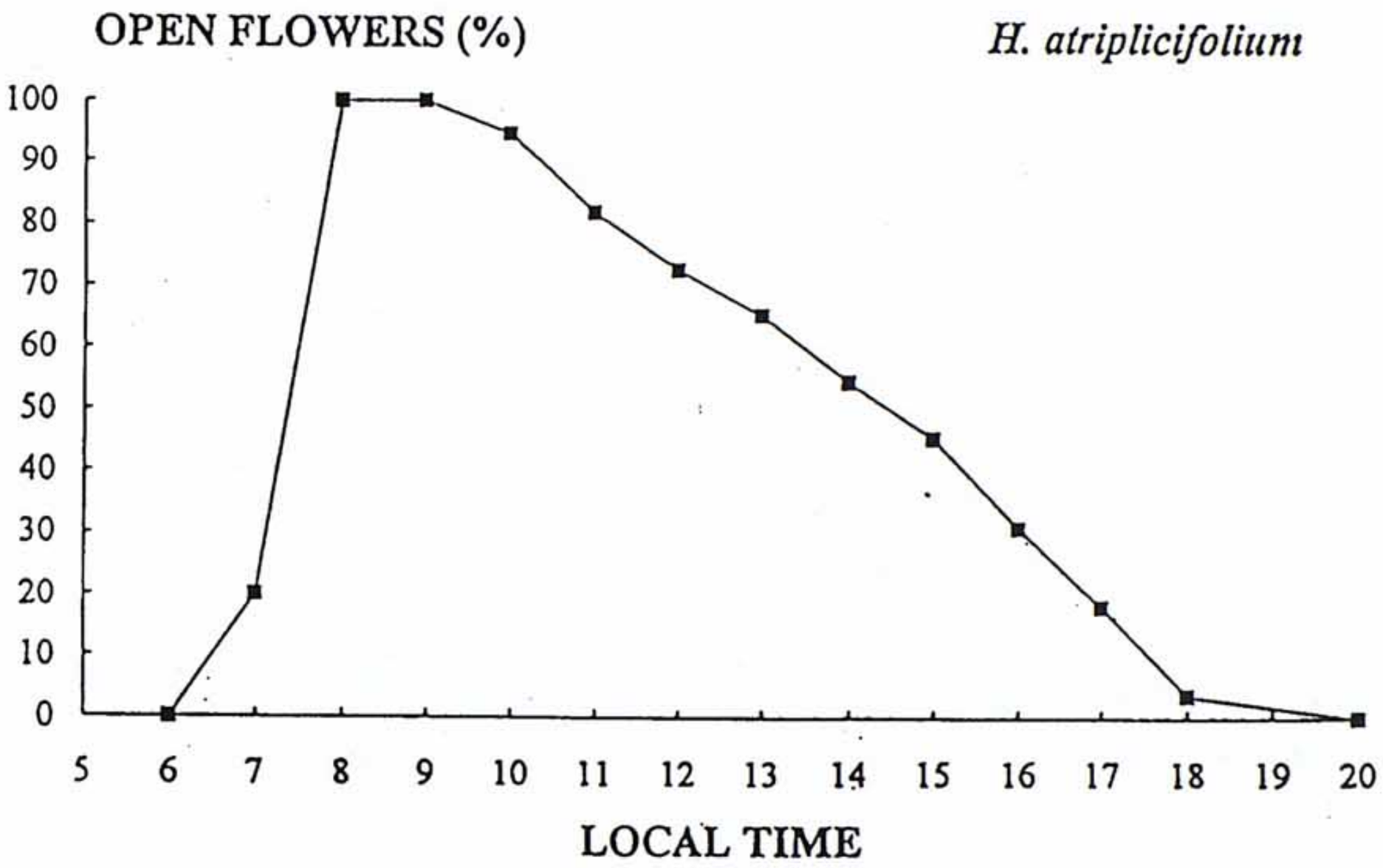
Subsamples of hand pollinated flowers (self and cross) were collected c. 5, and 24 h after pollination and fixed in FAA. Fixed flowers were subsequently used to check pollen grain germination and pollen tube growth by means of aniline blue staining under fluorescence microscopy (MARTIN, 1959).

Results

Flowering and pollination

H. halimifolium in the study area flowered in mid-April whereas *H. atriplicifolium* flowered at the beginning of June. Both species here studied show the phenomenon of flowers lasting only a few hours in early morning (Fig. 1). Flower opening occurred synchronously each day within the populations. *Halimium atriplicifolium* bud opening began at 6 h (local time) and at 7 h the flowers were completely open. *Halimium halimifolium* began to open its flowers at 8 h. In both cases, by the time any flower opens the majority of anthers have begun to dehisce and the pollen is readily taken up by the first insect visitors. In the studied *Halimium* the reward was only pollen, no nectar production was found. Between 11-12 h practically all the flowers of both taxa were pollinated by the insects showing pollen on the stigma and the petals began to drop. In *H. atriplicifolium* most of the 50% of the flowers had no petals at 14 h (n = 100 flowers).

Activity of insect visitors to *H. atriplicifolium* began ca. 7:45 h, but the peak of the visits occurs between 8-8:30 h (when most of the flowers were open) but continued sporadically until aproximately 13 h. About 47% of the visitors were *Hymenoptera*, 29% *Diptera* (mainly *Syrphidae*) and 24% *Coleoptera* (Fig. 2). The *Hymenoptera* attract to flower scent were the first visitors to arrive at the flowers and c. one hour before *Diptera* and *Coleoptera* began to arrive. In *H. halimifolium* the pattern of insect activity was very different to that of *H. atriplicifolium* (Fig. 2). About 77.7% of the flower



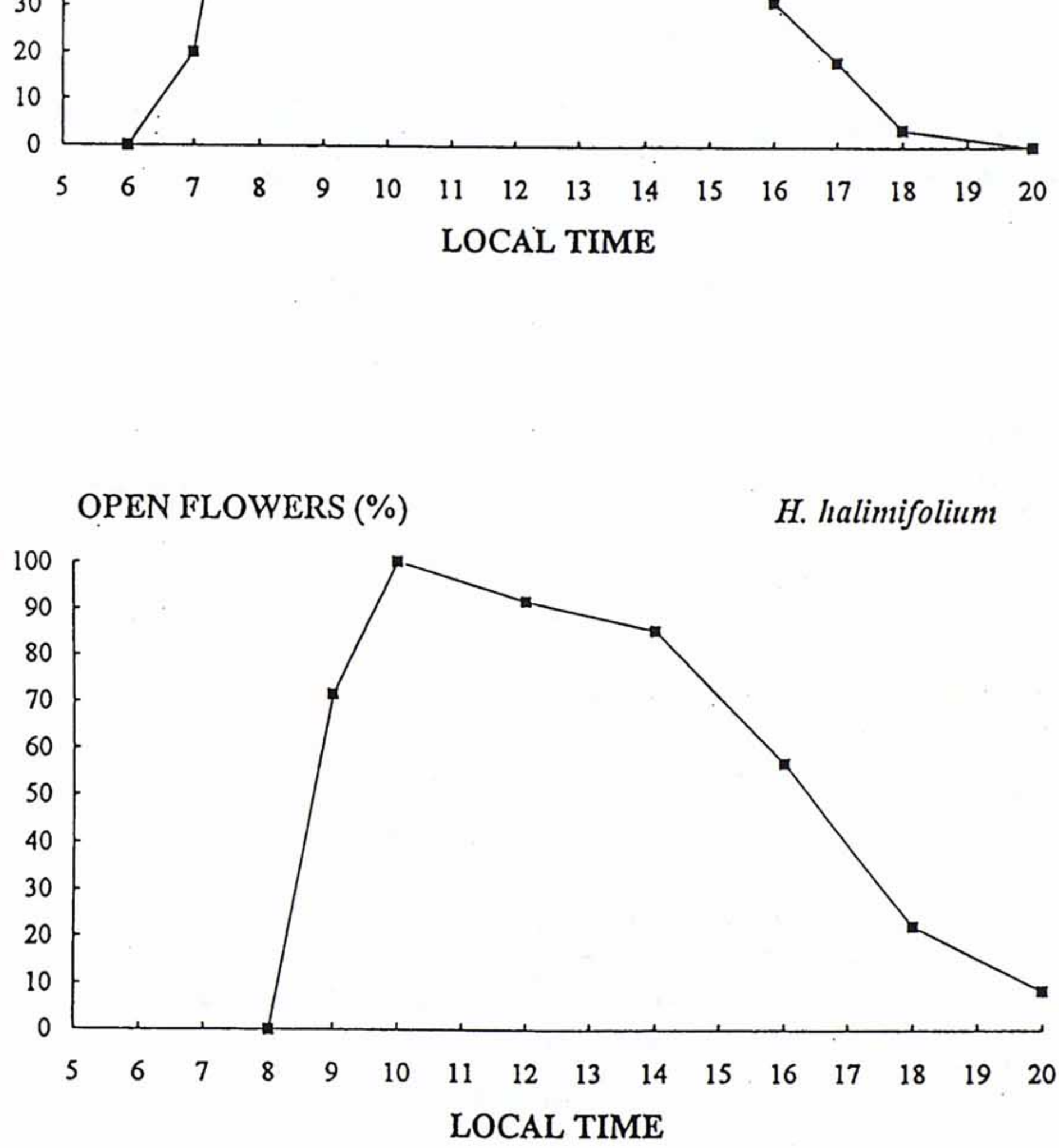


Fig. 1. Rhythm of anthesis of *H. atriplicifolium* and of *H. halimifolium* flowers.

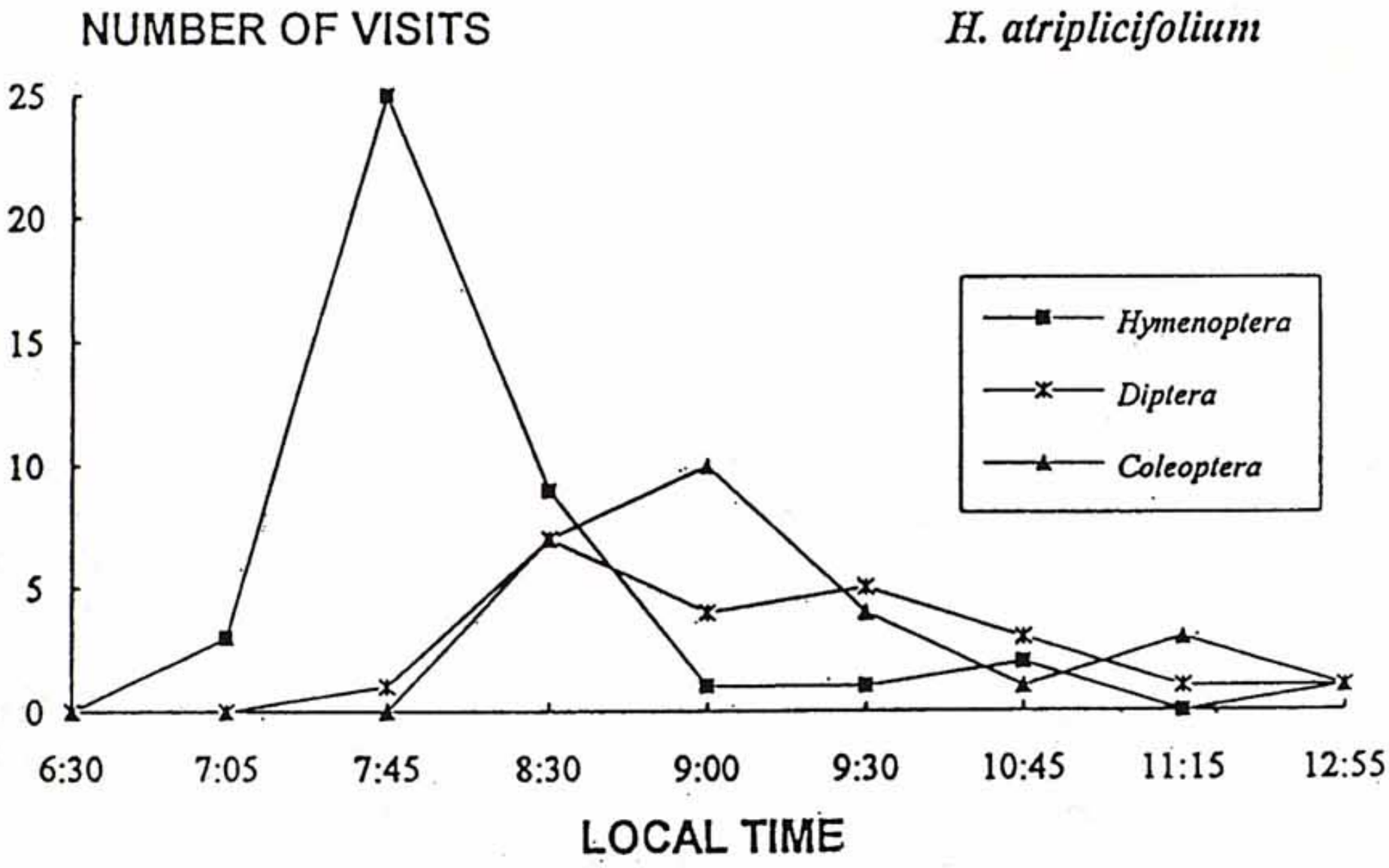


Fig. 2. Pattern of insect visits to *H. atriplicifolium* and to *H. halimifolium* flowers.

visitors were *Coleoptera,* 11.1% *Hymenoptera* and 11.1% *Diptera*. Peak of insect activity occurred at 12 h (in *H. atriplicifolium* there are no insects at that time) and finished between 18-20 h.

Breeding system

Results of the hand self- and cross-pollinations are given in Table 1. In both taxa, marked differences between fruit-set from cross- versus self-pollinated flowers were found. No flowers set fruits after self-pollination and thus *H. atriplicifolium* and *H. halimifolium* are strictly self-incompatible. In *H. atriplicifolium* pollen in both self- and cross-pollinated flowers germinated quickly (Fig. 3C). In only 5 h after pollination pollen tubes in crossed flowers reached the ovary and penetrated ovules were observed (Fig. 3D).

The fruit-set resulting after cross-pollination was significantly different in both species: 27% in *H. atriplicifolium* and 90% in *H. halimifolium*. The former accords well with that from natural fruit-set, whereas in *H. halimifolium*, hand cross-pollination produced significantly more fruits than nature pollination.

In naturally pollinated flowers the mean number of seeds per capsules was 52 in *H. atriplicifolium* and 24 in *H. halimifolium*. No significant differences in the numbers of seeds per capsule were found between crossed and naturally pollinated flowers either of *H. atriplicifolium* ($F = 1.326$, $p = 0.272$, 1df) or *H. halimifolium* ($F = 1.6$, $p = 0.38$, 1df). In *H. atriplicifolium* the seed-set in naturally pollinated flowers averaged 53% (mean ovules per ovary is 98.9 ± 4.2, n=227). In *H. halimifolium* ovaries have on average 49.9 ± 0.4 ovules (n =120) ovaries, and also the 53% of ovules develop into seeds (Table 1).

Species	Treatment	Number of flowers	Fruits (fruit-set)	Seeds/fruit x ± se	Seed-set (%)	Number of plants
Halimium	self-pollination	133	0 (0)	–	–	13
atriplicifolium	cross-pollination	92	25 (27.2%)	59.68 ± 4.6	60.3 ± 4.6	10
	control	135	47 (34.0%)	52.36 ± 3.0	52.9 ± 3.1	14
Halimium	self-pollination	60	0 (0)	-	-	9
halimifolium	cross-pollination	77	70 (90.0%)	23.80 ± 2.5	61.5 ± 4.1	7
	control	81	48 (59.2%)	23.71 ± 1.3	53.6 ± 2.3	16

Table 1. Numbers of flowers setting fruits, mean number of seeds per fruit and seed-set of *Halimium atriplicifolium* and *H. halimifolium* after hand pollination (self and cross) and with respect to the control (unmanipulled flowers).

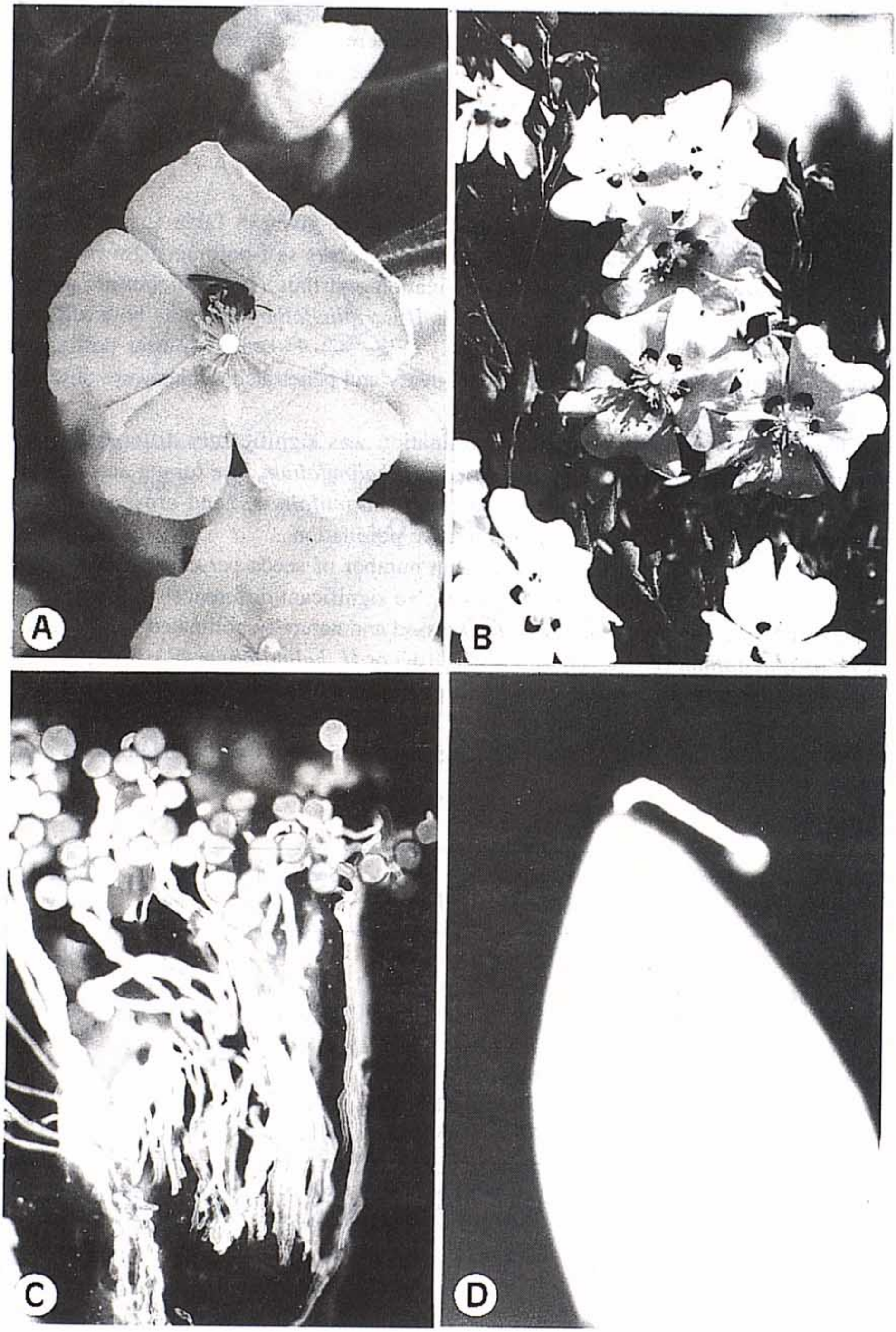


Fig. 3. A, Aspect of *Halimium atriplicifolium* flowers; B, Aspect of *H. halimifolium* flowers. C, Pollen germination in *H. atriplicifolium* 5 h after self-pollination. D, Penetrated ovule of *H. atriplicifolium* 5 h after cross-pollination.

Discussion

In the study year, 1995, *H. halimifolium* flowered 4-6 weeks earlier than its normal flowering period (HERRERA, 1986), due to unusually high temperatures which occurred during this spring season. As a consequence, difference in flower opening indicated in Fig. 2 probably reflects a difference in sunrise time (mid-April vs. early June). In a normal year, *H. halimifolium* flowers mid- to late May, and it is likely that both species exhibit a similar dawn anthesis. Similar situation could be happened with the pattern of insect visits. According with our observations in other flowering years the main group of insect visitors to *H. halimifolium* was *Hymenoptera* as in *H. atriplicifolium.*

Pollen germination ocurred in both self- and cross-pollinated flowers, but self-pollen tubes ceased growth in the style and not enter the ovary. This is similar to the situation described in *Cistus ladanifer* by TALAVERA & al. (1993) and suggest the presence of homomorphic, gametophytic self-incompatibility (GSI) in these species.

In general, natural fruit set in the species studied here was low compared with other *Cistaceae* species with similar breeding systems and flower morphology such as *Halimium commutatum* (92%, HERRERA, 1987), *Cistus ladanifer* (95% TALAVERA & al., 1993), *Cistus salvifolius* (83.3%, HERRERA, 1992; 93% HERRERA, 1987) or *Cistus libanotis* (80%, HERRERA, 1987). The low fruit-set of *Halimium atriplicifolium* in the field (only 34%) is not due to a lack of pollination because the manipulated flowers also show a low fruit-set. However, in *H. halimifolium* natural fruit-set is lower (52%) than that of the manipulated flowers (90%), and a lack of pollinator visitation could be the cause. Between species, fruit-set of *H. halimifolium* is practically twice as that of *H. atriplicifolium*. Moreover, although the number of seeds per fruit in *H. halimifolium* is lower (23) than that of *H. atriplicifolium* (52). But, since seed-set is similar in both species, the reproductive effort (fruit-set x seed-set) is higher in *H. halimifolium* (31.7%) than that of *H. atriplicifolium* (17.9%).

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