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POLLINATION
BIOLOGICAL INVASION
POLLEN CARRIAGE
LINEPITHEMA HUMILE
EUPHORBIA CHARACIAS
EUPHORBIA BIUMBELLATA

ABSTRACT. – We have studied the influence of Argentine ant invasion on the level of pollen grain carriage by ants in *Euphorbia characias* and *E. biumbellata*, two deciduous shrubs visited by ants. The observations were made in two contiguous areas, invaded and non-invaded by the Argentine ant *Linepithema humile*, in a Mediterranean cork-oak forest. In the invaded area *L. humile* displaced all native ants that climb to the cyathia of the inflorescences, except *Plagiolepis pygmaea*, a tiny ant species. Eight native ant species were detected in non-invaded areas. *Camponotus cruentatus* (52.06 ± 5.57 pollen grains carried per ant worker of *E. characias* and 38.84 ± 6.82 pollen grains of *E. biumbellata*, mean ± se) and *Camponotus piceus* (42.80 ± 21.57 pollen grains of *E. biumbellata*) carried much more pollen than *L. humile* worker ants (0.37 ± 0.06 pollen grains of *E. characias* and 0.44 ± 0.21 pollen grains of *E. biumbellata*). The Argentine ant and the native ants collected nectar, but *C. cruentatus*, the visiting ant species most abundant in the non-invaded area, touched the anthers or the stigma of the flowers three times more frequently than the Argentine ant. These results suggest the Argentine ant displace the native ants and that the invasion could interfere with natural visitors or potential pollinators in several plants.

INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), an invasive species native of Brazil and Argentina, has invaded all Mediterranean ecosystems (Suarez *et al*. 2001). In invaded areas this ant displaces native ant species (Human & Gordon 1997) and disrupts ecological processes like seed dispersal by ants (Bond & Slingsby 1984, Christian 2001, Gómez & Oliveras 2003, Gómez *et al*. 2003) or pollination. For the South African *Protea nitida* Mill, a negative effect has been verified in the invaded areas: the Argentine ant foraging in the flowers had a repellent effect on the pollinator insects. There was also a collapse in insect diversity and an abundance of infested inflorescences, with a resulting delay in pollination and a negative effect on the plant reproductive capacity (Visser *et al*. 1996).

In the Mediterranean Basin the Argentine ant basically inhabits the coastal strip (Giraud *et al*. 2002, Espadaler & Gómez 2003), and many species of ants are usual flower visitors in this area (Herrera *et al*. 1984, Bosch *et al*. 1997). Ants are usually considered poor or ineffective pollinators: their small size means they pass by flowers without touching the anthers or the stigma; the pollen doesn't adhere to their bodies, they clean their bodies often (Faegri & Van der Pijl 1979) and their metapleural gland secretions alter or disrupt the function of pollen (Beattie *et al*. 1984). In many cases ants opportunistically exploit the floral nectar (Schaffer *et al*. 1983, Herrera *et al*. 1984) but in several plants they are effective pollinators (see review in Peakall *et al*. 1991, Gómez & Zamora 1992, Garcia *et al*. 1995, Gómez 2000). Even though the role of ants as pollinators seems to depend heavily on their relative abundance with respect to other species of the pollinator assemblage, ant pollination becomes evident when ants outnumber other floral visitors (Gómez *et al*. 1996).

Euphorbia characias L. and *Euphorbia biumbellata* Poiret cyathia of the inflorescences were visited in the studied areas: in the non-invaded area by several native ant species, and in the invaded area only by the Argentine ant and *Plagiolepis pygmaea* (Latreille), a tiny ant species. The main objective of this work is to determine the level of pollen load due to ants, and to determine if the Argentine ant can replace native ants in pollen load and eventual pollen transfer within a given plant or between nearby plants.

MATERIAL AND METHODS

Study area: This study was carried out during April-May 2003 in the Serra Llonga (Gavarres Massif) near the village of Castell d'Aro (NE Spain) (41º 49' N, 3º 00' E). In this area there are contiguous invaded and non-invaded zones situated at an elevation of 250 m and having a vegetation of open cork-oak secondary forests dominated by *Quercus suber* (L.). The study area was 4 km from the Mediterranean coast. The climate in this region is Mediterranean subhumid, with 627 mm of annual rainfall, a minimum monthly average temperature in January (7.2ºC) and a maximum in July (22.6ºC).

Euphorbia characias is a deciduous shrub and *E. biumbellata* is a biannual herb. They grow to 100 cm tall in scrub and holm-oak forests (Bolòs *et al.* 1990). The genus *Euphorbia* has simple flowers gathered in a singular structure, the cyathium, that has a unique female flower surrounded by a variable number of male flowers with only one stamen; this combination of unisexual flowers in bisexual inflorescences, surrounded by coloured petaloid appendages and nectaries, acts functionally as a single flower (Strasburger *et al.* 1994).

Ant pollen load: In a border area between the invaded and non-invaded zones, we collected all ant species on the cyathia of the inflorescences of *E. characias* and *E. biumbellata* in a 100 meter-long transect, at the hours of greatest ant activity (3 days in April for *E. characias*, and 2 days in May for *E. biumbellata*). In the field, and with binocular glasses, we counted the pollen grains adhering to their bodies. The pollen of those plants is characteristic yellow, and the flowering periods was not the same. For each plant species the number of pollen grains carried by each species of ant was compared using a one-way-ANOVA.

Ant activity on the inflorescences: During the flowering season of *E. characias*, and in a zone with invaded and non-invaded areas next to each other, we chose 5 plants in each area and filmed different inflorescences with a video camera for 10 minutes once a week for 8 weeks. The censuses started at 9:00 and finished at 19:00 (local time). We alternated the different inflorescences filmed (two from the invaded area and two from the non-invaded zone) during the study day. The 10 minutes samples were analysed in the laboratory and we noted, only for *Linepithema humile* and *Camponotus cruentatus* (Latreille), the two species most abundant in both areas, the time spent by each species collecting nectar and the number of ants that touched the anthers or the stigma. The percentages of those two measures were compared using a chi-square test.

During the census we counted the number of ants on the cyathia of each inflorescence of the plant (each plant of *E. characias* can have 1 to 50 inflorescences). The percentage of occupied inflorescences (arcsin√x transformed) (with worker ants presence on the cyathia) and the number of worker ants on the cyathia of the inflorescences (only for occupied inflorescences), during flowering in both areas, was compared using a paired t-test.

RESULTS

Ant pollen load

Only 2 ant species were collected on the cyathia of the inflorescences in the invaded area: *L. humile* and *P. pygmaea*. On the other hand, in the non-invaded area we collected 8 species of ants. All species carried pollen, but for *E. characias*, *Camponotus cruentatus* carried more pollen than the other species (Table I) (one-way ANOVA. F = 48.47, $p < 0.001$, and post-hoc HSD Tuckey test $p < 0.05$). For *E. biumbellata*, *C. cruentatus* and *Camponotus piceus* (Leach) carried more pollen than *P. pygmaea* and *L. humile* (Table I) (one-way ANOVA. F = 19.32, $p < 0.001$, and Tukey HSD post-hoc test $p < 0.05$).

Ant activity on the inflorescences

We observed 36 individual ants of *C. cruentatus* on the cyathia of *E. characias* inflorescences. They foraged on 291 cyathia, collected nectar from 237 and touched the anthers or the stigma on 261. We observed 158 individual ants of *L. humile* on the cyathia of the inflorescences, also foraging on 291 cyathia, but collecting nectar from 130 and touching the anthers or the stigma on 89. The percentage of cyathia where *C. cruentatus* collected nectar was higher than for *L. humile* (Fig. 1) ($X^2 = 31.20$, $p < 0.001$, d.f = 1). In addition, the percentage of cyathia where *C. cruentatus* workers touched the anthers or the stigma, independently the ants collected nectar or not, was higher than the same percentage for *L. humile* (Fig. 1) ($X^2 = 84.52$, $p < 0.001$, d.f = 1).

During the flowering season we examined 941 inflorescences of *E. characias* in the invaded area; the Argentine ant occupied 405. In the non-invaded area we examined 974 inflorescences; *C. cruentatus* occupied 213. The percentage of inflorescences with Argentine ant presence in the invaded area (73.04 ± 9.31, mean percentage ± se) was the same than the percentage of inflorescences with

Table I. – Number of pollen grains of *Euphorbia characias* and *E. biumbellata* carried by workers of Argentine ants and native ant species.

	E. characias			*E. biumbellata*		
	N	Mean	se	N	Mean	se
Linepithema humile Mayr	170	0.37	0.06	25	0.44	0.21
Camponotus cruentatus (Latr.)	94	52.06	5.57	37	38.84	6.32
Plagiolepis pygmaea (Latr.)	40	0.20	0.07	42	0.17	0.12
Crematogaster scutellaris (Olivier)	33	1.21	0.69	-	-	-
Formica gagates Latr.	19	0.84	0.46	-	-	-
Formica subrufa Roger	6	1.67	0.99	-	-	-
Camponotus piceus (Leach)	-	-	-	5	42.80	21.57
Formica cunicularia Latr.	3	-	-	-	-	-
Lasius lasioides (Emery)	1	-	-	-	-	-

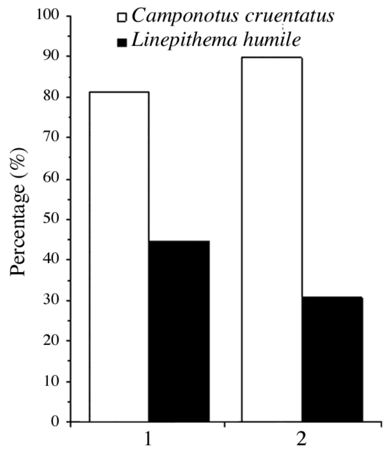

Fig. 1. – 1, Percentage of flowers where *Camponotus cruentatus* and *Linepithema humile* collected nectar; 2, Percentage of flowers where these species touched the anthers or the pistils.

C. cruentatus in the non-invaded area (42.91 ± 11.14, mean percentage ± se) (t = 2.254, p = 0.065, d.f = 6). The number of Argentine worker ants per inflorescence during the flowering (9.75 ± 2.47 ant workers, mean ± se) was higher than for *C. cruentatus* (1.82 ± 0.14 ant workers, mean ± se) (t = 3.358, p = 0.015, d.f = 6).

DISCUSSION

Invaded areas by the Argentine ant around the world areas suffer a drop in the native ant populations (Bond & Slingsby 1984, Human & Gordon 1996, Holway 1998, Suarez *et al.* 1998). The Mediterranean basin is not an exception (Gómez *et al.* 2003). In Doñana National Park (Spain) the richness and diversity of native ant species around houses was similar to that in natural habitats, but when the Argentine ant became the dominant species, there was considerably lower native ant species diversity and richness (Carpintero *et al.* 2003). In our case, on the cyathia of *Euphorbia characias* and *E. biumbellata* we only found two ant species in the invaded area, the Argentine ant and *Plagiolepis pygmaea*. On the other hand, we found eight species in the non-invaded area.

Ants are usual visitors of flowers in the Mediterranean basin (Bosch *et al*. 1997), and several species, including the genus *Camponotus*, are good pollinators (Garcia *et al.* 1995, Gómez *et al.* 1996). *C. micans* (Nylander) is, for example, the principal summer pollinator of *Lobularia maritima* (L.) Desv. (Gómez 2000). *C. cruentatus* and *C. piceus* have some characteristics typical of good pollinator species: they are nectarivorous and omnivorous species (Espadaler & Gómez 1996); they don’t have metapleural glands (Hölldobler & Engel-Siegel 1984); they are a big ant species (6-14 and 3.4-7 mm, respectively); they carried pollen grains; and, *C. camponotus* often touched the anthers or the stigma of *E. characias*. On the other hand, the Argentine ant is an omnivorous ant whose diet can be divided into two distinct food groups: animal and insect prey and liquid foods such as honeydew (Vega & Rust 2001). It is a little ant (2.5-3 mm) and it touched the anthers or the stigma of *E. characias* three times less frequently than the *C. cruentatus*, it didn’t carry pollen grains of *E. characias* and *E. biumbellata*, and it has metapleural glands.

In California, Argentine ants find food and recruit in higher numbers than the native ant species, and they forage for longer periods throughout the day (Human & Gordon 1996). In our case we observed the same first pattern: the number of Argentine worker ants climbing the cyathia of the inflorescences was higher than the number of native worker ants.

In an herbaceous Mediterranean plant community the native ants are more active from May to September (Bosch *et al.* 1997). In the invaded zone, the Argentine ant was more active at the onset and in the middle of the flowering of *E. characias*, dropping its activity in the start of April. On the other hand, *C. cruentatus* started its activity in the end of March and increased it during the flowering of *E. characias* and until the end, when most plants in the area were flowering, and when *E. characias* had more flowers.

The Argentine ant displaced all native ants, except *P. pygmaea*, that probably exploited the nectar opportunistically. But species such as *C. cruentatus* or *C. piceus* could be important pollinators of several plants. The seed-set depends on pollination (Herrera 1987, Lyons *et al.* 1989, Dafni 1992, Kearns & Inouye 1993) and the Argentine ant invasion could alter the natural pollination of two Mediterranean shrubs. In the long term, this process could potentially lead to some changes in the composition of plant communities of the invaded areas.

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