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Original Article

Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae)

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Abstract

We have studied the influence of the Argentine ant, *Linepithema humile*, on the pollination of *Euphorbia characias*, a deciduous insect-pollinated shrub. The observations were made in two adjacent areas (invaded and non-invaded by *L. humile*) in a Mediterranean cork-oak forest. In the invaded area, *L. humile* has replaced most of the native ants that climb up this plant's inflorescences. Five native ant species were detected in the non-invaded areas and only one in the invaded area. The number of visitors to infested inflorescences (1.54 ± 1.86 visitors/10 min observation) was lower than in non-infested inflorescences in the invaded area (3.74 ± 4.19 visitors/10′), and in the non-invaded areas (4.16 ± 5.00 visits/10′). For several species of flower-visiting insects, no differences were detected between the time spent in the flowers and the number of flowers visited in the two areas, except for *Eristalis tenax*, a dipteran which visited more flowers (15.2 ± 11.1 flowers visited/10′) and spent more time (9.4 ± 5.8 sec) in the non-invaded area than in the invaded area (7.8 ± 8.2 flowers visited/10′ and 5.3 ± 2.1 sec, respectively). The relative representation of insect orders in the two areas was not different. A significant reduction in fruit-set and seed-set was detected in the invaded area. These results suggest that the Argentine ant may greatly affect the reproductive success of components of the Mediterranean flora.

Keywords: Argentine ant; biological invasion; *Euphorbia characias*; *Linepithema humile*; pollination

1. Introduction

Pollination is one of the most important types of interaction between plants and animals in ecosystems because it is a key process in the sexual reproduction of most angiosperms and can affect directly the plant reproduction success (Dafni, 1992; Kearns and Inouye, 1993). Most animal–plant interactions are not species-specific relationships (Dafni and O'Toole, 1994; Gomez and Zamora, 1999; Herrera, 1995; Zamora, 2000). For example, flowers of Mediterranean plant species are usually visited by insects belonging to three or more orders (Herrera, 1996). Among plants visited by many pollinator species, the relative contribution of each pollinator to plant reproduction is determined by variation in both pollinator and plant traits (Pellmyr and Thompson, 1996). The presence of different pollinators with different efficiencies influences the seed-set and the quality of descendants (Herrera, 2000). This is an important issue, if we consider the possible variation in the assemblages of pollinators, due to the presence and spread of invasive introduced species.

The Argentine ant is one of the most invasive species documented. Native to South America, it has invaded all Mediterranean ecosystems of the world (Suarez et al., 2001). Two supercolonies of Argentine ants are present in a 6000-km stretch of the Mediterranean and Atlantic coast of southern Europe (Espadaler and Gómez, 2003; Giraud et al., 2002). In the invaded areas the Argentine ant's presence usually exerts a negative influence on invertebrate biodiversity (Cole et al., 1992; Holway, 1998; Human and Gordon, 1997). These changes can influence ecological processes such as pollination and seed dispersal, two key processes for the reproductive success of plants. Several studies examine the effect of the Argentine ant on seed dispersal by ants in invaded zones (Christian, 2001; Carney et al., 2003; Gómez and Oliveras, 2003; Gómez et al., 2003) but few studies have examined the impacts of this invader on the pollination. A reduction in the

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diversity and abundance of insects in *Protea nitida* inflorescences infested by *L. humile* has been observed in South African fynbos (Visser et al., 1996), and Argentine ant workers attack nests of pollinators (Buys, 1990).

The main objective of this work is to document and discuss the impact of the Argentine ant invasion on the pollination of *Euphorbia characias* (L.) by comparing pollination in invaded and non-invaded areas. This work is also the first to our knowledge to document which insects visits and potentially pollinate this plant species. Other studies of pollination in *Euphorbia* species have focussed on *Euphorbia albomarginata*, *E. capitellata*, *E. hyssopifolia* in Arizona and New Mexico (Ehrenfeld, 1979) and on the shrub *E. dendroides* on the island of Cabrera (Balearic Islands) (Traveset and Sáez, 1997).

We studied (a) the number and diversity of visitors and pollinators, (b) behavioral aspects of visitors and pollinators (time spent in each flower, number of flowers [cyathia] visited and distance moved between visited flowers), and (c) fruit-set, i.e., the proportion of flowers that set fruit, and seed-set, i.e., the number of viable seeds produced by fruit.

2. Material and methods

2.1. Study area

This study was carried out between February and July 2002 in the Serra Llonga, on the southern limit of Gavarres Massif (NE Spain) (41° 49′ N, 3° 00′ E). The study area was 4 km from the Mediterranean coast. The climate in this region is Mediterranean subhumid, with a mean of 627 mm of annual rainfall. Invaded and non-invaded zones are situated at 250 m elevation and the vegetation is open cork-oak secondary forest dominated by *Quercus suber* (L.), *Quercus ilex* (L.), and *Pinus pinaster* (Ait.).

Euphorbia characias (L.) is a deciduous shrub 30–100 cm tall, growing in scrub, cork-oak and holm-oak forests (Bolòs et al., 1993). The genus *Euphorbia* has simple flowers gathered in a single structure: the cyathium. Each cyathium consists of 5 male flowers, each with only 1 stamen, a pedicel, and no perianth, surrounding an apical female flower, also with pedicel and without perianth. This grouping of unisexual flowers in a bisexual inflorescence surrounded by colored petaloid appendages and nectaries is functionally equivalent to a hermaphrodite flower (Strasburger et al., 1994).

2.2. Diversity and abundance of flower visitors

During the flowering season of *E. characias*, we performed a focal study and a scan study in a zone with invaded and non-invaded areas next to each other. Concerning the focal study the observations were focused on the inflorescence. We chose 5 plants in each zone and filmed different inflorescences with a video camera for 10 min once a week (one day a week), for 8 weeks. The censuses started at 9:00 h and finished at 19:00 h (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 samples filmed were watched in the laboratory and the insect visitors that touched the flowers' anthers or pistils were noted. The number of visits per flower and the number of flowers visited every 10 min were compared between the two areas with paired *t*-tests (each pair correspond to each study day). All visitors were identified and for each visitor we noted the number of flowers visited, the time spent in each flower visited, and the distance moved between visits to consecutive flowers in the same inflorescence. As the data were obtained through the filmed sample, the number of visits was for half of each inflorescence.

Concerning the scan study, we also noted all visitors to the plants (adult plants of *E. characias* can have from 1 to 50 inflorescences) during the video camera censuses. The different species of insect visitors were collected and categorized in to orders and the proportion of species belonging to each order was compared between the two areas using a chi-square test.

In order to check a possible "repellent" effect of the presence of the Argentine ant on the visitors, we counted the number of visitors to the following groups of plants:

- visitors in the non-invaded areas (96 inflorescences);
- visitors to plants infested by the Argentine ant (47 inflorescences) and ;
- visitors to plants in the invaded zone, but on which workers of the Argentine ant were absent (46 inflorescences).

The number of visitors (log-transformed) was analyzed using a two-way ANOVA with plant groups and days as fixed effects. Differences in numbers of visitors found between groups of plants could be due to variation in the density of *E. characias* shrubs. To explore this possibility, we counted the number of *E. characias* individuals in three 15 × 15 m plots at each zone and the density were compared with a one-way ANOVA.

2.3. Fruit-set and seed-set

To compare the fruiting efficiency and the seed-set mediated by different types of pollinators in the invaded and non-invaded zones, we developed a pollinator exclosure experiment. Before the flowering period, we applied four different exclusion treatments to four inflorescences per plant. We chose 10 *E. characias* plants in each zone, invaded and non-invaded, and applied the following treatments:

- Exclosure of flying insects: the inflorescences were covered with a nylon bag.
- Exclosure of ants and other non-flying insects: the inflorescence bases were coated with colorless and odorless non-poisonous glue (Ratimur®).
- Exclosure of all insects: the inflorescences were covered with a nylon bag and coated with glue at the base.
- Control: all insects could access the inflorescence.

The number of flowers in each inflorescence and the number