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High Invasive Pollen Transfer, Yet Low Deposition on Native Stigmas in a *Carpobrotus*-invaded Community

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• *Background and Aims* Invasive plants are potential agents of disruption in plant–pollinator interactions. They may affect pollinator visitation rates to native plants and modify the plant–pollinator interaction network. However, there is little information about the extent to which invasive pollen is incorporated into the pollination network and about the rates of invasive pollen deposition on the stigmas of native plants.
• *Methods* The degree of pollinator sharing between the invasive plant *Carpobrotus affine acinaciformis* and the main co-flowering native plants was tested in a Mediterranean coastal shrubland. Pollen loads were identified from the bodies of the ten most common pollinator species and stigmatic pollen deposition in the five most common native plant species.
• *Key Results* It was found that pollinators visited *Carpobrotus* extensively. Seventy-three per cent of pollinator specimens collected on native plants carried *Carpobrotus* pollen. On average 23 % of the pollen on the bodies of pollinators visiting native plants was *Carpobrotus*. However, most of the pollen found on the body of pollinators belonged to the species on which they were collected. Similarly, most pollen on native plant stigmas was conspecific. Invasive pollen was present on native plant stigmas, but in low quantity.
• *Conclusions Carpobrotus* is highly integrated in the pollen transport network. However, the plant-pollination network in the invaded community seems to be sufficiently robust to withstand the impacts of the presence of alien pollen on native plant pollination, as shown by the low levels of heterospecific pollen deposition on native stigmas. Several mechanisms are discussed for the low invasive pollen deposition on native stigmas.

Key words: Alien plant, *Carpobrotus aff. acinaciformis*, competition for pollinators, invasion, Mediterranean shrubland, plant-pollinator network, pollen loads, pollinator visits, stigma.

INTRODUCTION

Biological invasions caused by the intentional or accidental introduction of alien species are threatening the conservation of biodiversity through the local displacement of native species, changes in community structure and the modification of ecosystem function (Vitousek, 1994; Enserink *et al.*, 1999). It has long been established that alien plants can interfere with native plants through direct competition for abiotic resources (i.e. soil nutrients, water, space and light) (Levine *et al.*, 2003). In addition, biological invasions are increasingly viewed as potential agents to disrupt mutualistic interactions (Richardson *et al.*, 2000; Mitchell *et al.*, 2006; Traveset and Richardson, 2006), possibly resulting in changes in pollen transfer dynamics and subsequent plant reproductive success (Bjerknes *et al.*, 2007).

Whether an entomophilous invasive plant facilitates or competes for pollinators and ultimately for pollen, depends on how pollinators respond to the temporal and spatial changes in resource availability (Knight *et al.*, 2005), as well as the interference that invasive pollen may cause on native stigmas. Invasive plant species may change pollination patterns in many ways, such as through the decline of certain pollinator species, the disappearance of certain plant–pollinator interactions or the increase in exotic pollinators (Morales and Aizen, 2002; Olesen *et al.*, 2002; Lopezaraiza-Mikel *et al.*, 2007; Bartomeus *et al.*, 2008). These changes in the pollinator community may result in increased or decreased visitation rates to native species (Chittka and Schürkens, 2001; Brown *et al.*, 2002; Moragues and Traveset, 2005). Changes in visitation rates may also modify pollen transfer patterns from pollinators to stigmas. Low conspecific pollen and high invasive pollen deposition on native species could decrease the native plant seed set (Chittka and Schürkens, 2001; Brown *et al.*, 2002; Moragues and Traveset, 2005, Larson *et al.*, 2006).

However, studies describing the events underlying potential competition for pollinators between invasive and native plant species are scarce (Knight *et al.*, 2005). Given the complexity of the structure of plant–pollinator interactions, the effects of invaders appear to be context-specific and thus remain difficult to predict (Bascompte *et al.*, 2003; Bascompte and Jordano, 2007; Blüthgen *et al.*, 2007). Competition for pollinator services require (*a*) pollinator sharing between alien and native plants, (*b*) alien pollen transfer to the body of pollinators, (*c*) substantial alien pollen deposition on the stigmas of native plants, and (*d*) chemical or mechanical interference of alien pollen with native pollen. Additionally, (*e*) pollinators might mechanically lose large amounts of native pollen during visitation to alien plants, especially if they visit native and alien species during single foraging

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bouts, due to the rubbing of animal's body against different parts of the alien flower.

In this study, the potential competition for pollinators between an invader plant with large, pollen-rich flowers, *Carpobrotus affine acinaciformis* (*Carpobrotus* hereafter), and the main co-flowering native plants is analysed in a Mediterranean shrubland. Previous studies have shown that *Carpobrotus* could facilitate pollinator visitation to some plants, but compete with others (Moragues and Traveset, 2005). Invaded communities attracted more pollinators than non-invaded communities (Bartomeus *et al.*, 2008). Thus, *Carpobrotus* acted as a 'magnet' species to native plants. This pattern has also been observed in *Impatiens glandulifera*-invaded sites in the UK (Lopezaraiza-Mikel *et al.*, 2007). However, due to its profuse pollen production per flower, *Carpobrotus* could also potentially alter the network of pollen distribution in the community. In this study, pollinator visitation rates to *Carpobrotus* and coexisting native plant species, pollinator pollen loads and pollen deposition on the stigmas of native plants were measured. The following questions were asked: (*a*) What is the extent of pollinator sharing between native and invader species? (*b*) Do pollinators carry invasive pollen and are there differences among pollinator species in this regard? (*c*) Do pollinators carry more invasive pollen compared with heterospecific pollen from other native species? (*d*) Do pollinators visiting native plants carry invasive pollen? (*e*) Is invasive pollen deposited on native stigmas? *Carpobrotus* flowers produce large amounts of pollen, have an unspecialized morphology and pollen presentation, and are visited by a wide array of generalized pollinators (Bartomeus *et al.*, 2008). Thus, our hypothesis is that *Carpobrotus* pollen is well integrated in the plant-pollination network and we expect significant pollen deposition on native stigmas.

MATERIALS AND METHODS

Study area

The study area is located in a coastal Mediterranean shrubland invaded by *Carpobrotus* in the Natural Park of Cap de Creus (Catalonia, north-east Spain). The community is dominated by shrubs (*Pistacea lentiscus*, *Juniperus communis*, *Erica arborea*, *Lavandula stoechas*, *Rosmarinus officinalis* and *Cistus* spp.) and annual herbs (*Sonchus tenerrimus* and *Helianthemum guttatum*). *Carpobrotus* is the only invasive plant species. The area is characterized by cool, wet winters and warm, dry summers. Mean temperatures of the coldest (January) and hottest (August) months in 2006 were 6 °C and 23 °C, respectively, and the annual precipitation was 450 mm (www.meteocat.com).

Invasive species studied

Carpobrotus (Aizoaceae) are crawling succulent chamephytes with fast clonal growth that have been introduced from South Africa into almost all Mediterranean regions. In Spain, they were introduced for gardening and soil fixation at the beginning of the 20th century (Sanz-Elorza *et al.*, 2006). Introgressive hybridization is common in *Carpobrotus* (Vilà *et al.*, 2000). In the study area, *Carpobrotus* are probably hybrids of *C. edulis* and *C. acinaciformis.* The nomenclature of Suehs *et al.* (2004) is followed and the study plants are referred to as the hybrid complex *Carpobrotus affine acinaciformis*. Besides asexual reproduction, *Carpobrotus* has a generalist pollination system and a facultative outcrossing mating system (Vilà *et al.*, 1998; Suehs *et al.*, 2005). It flowers from early April to late May. The flowering peak is very spectacular with an average 16 flowers m^{-2}. *Carpobrotus* flowers measure 8–10 cm in diameter, being the largest flowers in the study community. *Carpobrotus* accounts for 39 % of the plant cover representing 9·75 % of the floral units in the community. This site would be representative of a medium level of invasion.

Pollinator visitation rates

In spring 2005, an invaded coastal shrubland was selected where two parallel 50-m permanent transects were positioned. To avoid over-sampling of the most abundant plant species, pollinator counts were limited to a total of six observation areas per flowering species. In each observation area, the study focused only on one plant species. The observation areas were about 30 × 30 cm and were randomly located along transects at 2-m intervals. To fully randomize sampling along transects, we started each day's sampling at a different random initial point within transects. On every sampling day, pollinator visits were recorded at each observation area during 4-min periods. Sampling was conducted every 2 weeks (six times in total), encompassing the entire flowering period of the invasive plant. In total each plant species was sampled during 144 min. It is thought that by sampling several observation areas per species during short periods of time the chance of finding more pollinator species increased than if fewer areas were sampled for longer periods. No zero value per species and sampling day was ever found. The sampling protocol allowed the time spent per plant species to be standardized compared with classical transect walks. Previous extensive surveys indicated that this sampling intensity was sufficient to characterize the pollinator community (Bartomeus *et al.*, 2008). Sampling was conducted from 0900 h to 1900 h on non-windy, sunny days with temperatures higher than 15 °C.

The study focused on pollinator visits to *Carpobrotus* and the five most abundant flowering native plant species: *Cistus monspeliensis* (Cistaceae, 18 % cover), *Cistus salvifolius* (Cistaceae, 13 %), *Lavandula stoechas* (Lamiaceae, 7 %), *Cistus albidus* (Cistaceae, 6 %) and *Sonchus tenerrimus* (Asteraceae 5 %). Taken together, these five native species represented 49 % of the plant cover and 66 % of the flower abundance and received more than half of the pollinator visits to the community (Bartomeus, 2005). Data on the complete plant-pollinator network can be found in Bartomeus *et al.* (2008). All pollinators in the area were native. Visitation data are reported on the ten most common pollinators (five bee and five beetle species), accounting for 76 % of the total visits recorded. All ten species visited both the invader and some of the native target species (Bartomeus, 2005). These taxa include two social bees – *Apis mellifera* (Apidae, 7·4 % visits) and *Bombus terrestris* (Apidae, 2·8 %), three solitary bees: *Andrena* sp. (Andrenidae, 13·8 %), *Anthidium sticticum* (Megachilidae, 8·1 %) and *Halictus gemmeus* (Halictidae, 1·9 %) – and five beetles – *Oxythyrea funesta* (Scarabaeidae, 8·8 %), *Cryptocephalus* sp. (Chrysomelidae, 2·7 %), *Mordella* sp. (Mordellidae, 5·2 %), *Oedemera* spp. (including *O. flavipes*, *O. lurida* and *O. nobilis*; Oedemeridae, 7 %) and *Psilothrix* sp. (Dasytidae, 8 %). The chi-square test was used to compare the visitation frequency (i.e. total number of insects observed on a plant species during all sampling period) of beetles and bees to *Carpobrotus versus* native species.

Pollinator pollen loads

In spring 2006, pollinators were collected throughout the *Carporbrotus* flowering period. For each plant–pollinator interaction observed at least 15 pollinator individuals were caught. In total, 474 pollinators were collected (298 on native plant species and 176 on *Carpobrotus*). All the native plants on which pollinators were collected were at a maximum of 5 m from a flowering *Carpobrotus*. To avoid pollen contamination among specimens, pollinators were caught in individual, clean vials with cotton and a few drops of ethyl acetate. Two pollen samples were later obtained from each individual by gently rubbing small pieces of fuchsin-stained gelatine on their bodies (Kearns and Inouye, 1993). One pollen sample was taken from the ventral part and the other from the dorsal part of the pollinator. Pollen samples were mounted on microscope slides, and all pollen grains were identified and counted at ×400 magnification. Pollen identification was based on a reference collection of the main native species of the study area.

This method did not allow the total pollinator pollen loads to be accurately quantified, but, because the same sampling effort was applied to each individual, the number of pollen grains in the samples was used as an estimation of pollen load density. Identified pollen grains were grouped into three categories: conspecific (pollen from the plant species on which the pollinator was caught), heterospecific (pollen from other native plant species) and invasive (*Carpobrotus*) pollen.

Differences between pollinator taxa were compared in pollen species richness and in pollen loads. To assess the degree of incorporation of invasive pollen into the pollination network, differences in the percentage of *Carpobrotus* pollen loads were compared between pollinator taxa. Specimens caught on native plants were analysed separately from specimens caught on *Carpobrotus*. For each pollinator taxon, the percentage of invasive pollen carried by individuals collected on *Carpobrotus* and by individuals collected on native species was compared. Differences in conspecific pollen loads across pollinator taxa visiting native plants were also tested. For each pollinator taxon, the percentage of the dominant heterospecific native pollen and invasive pollen was compared. Finally, to describe the general pattern of pollinator pollen loads when visiting different native plant species, differences in conspecific pollen loads across visited plants and differences in invasive pollen loads across native species were investigated. One-way ANOVAs were used to test differences between pollinator taxa and plant species. For all ANOVAs, *post hoc* Fisher tests were conducted to assess pair-wise differences. Contrasts within pollinator taxa were conducted with *t*-tests.

Stigma pollen loads

Thirty stigmas (one from each of 30 individuals) were collected per plant species through the plant flowering period. Flower buds were marked, and stigmas were collected on the day after the maximum receptivity according to the literature [Bosch (1992) for *Cistus* spp.; Devesa *et al.* (1986) for *L. stoechas*] and personal observations. Stigmas were squashed on microscope slides with fuchsin-stained gelatine and identified at ×400. Sometimes, pollen grains were clumped or masked by stigma tissue, so that accurate pollen counts were not feasible. In general, pollen in the peripheral parts of the stigma was easier to identify and count, but it was decided to sample all stigmas to reduce the spatial bias on pollen load. Thus, for each pollen type (conspecific, heterospecific, invasive), five abundance categories were established: absent (no pollen grains), present (only one pollen grain); low (<20 % of the total pollen grains representing approx. 20–40 pollen grains); moderate (20–70 %); and high (>70 %). Differences in frequency of pollen abundance categories on stigmas were compared between heterospecific and invasive pollen with chi-square tests. A different chi-square test was used for each abundance category.

RESULTS

Visitation rates

A total of 323 (51 % of the total survey on the community) visits were recorded to target native plants and 172 (35 % of the total) to *Carpobrotus*. All ten target pollinator taxa visited *Carpobrotus* and at least one of the target native species (Table 1). Bee and beetle visitation frequency differed between *Carpobrotus* and native plants ($\chi^2 = 79{\cdot}03$, $P < 0{\cdot}0001$). Bees were more often recorded on native species than on *Carpobrotus*, except for *Bombus terrestris* that visited *Carpobrotus* flowers almost exclusively. In contrast, beetles tended to favour *Carpobrotus* over natives, except for *Cryptocephalus* sp., that visited mostly *Sonchus tenerrimus*.

Pollinator pollen loads

A total of 139 063 pollen grains was identified from the bodies of the collected pollinators. The average number of pollen species (including species other than our five target species and *Carpobrotus*) per individual pollinators was 3·17 and ranged from one to eight with a mode of three. Pollen loads and pollen species richness differed among pollinator species ($F_{9,464} = 11{\cdot}27$, $P < 0{\cdot}0001$; $F_{9,464} = 26{\cdot}32$, $P < 0{\cdot}0001$, respectively; Table 2).

TABLE 1. *Percentage of visits by the ten most abundant pollinator species to the invader* Carpobrotus *and the five most abundant native plant species*

	Bees					Beetles				
	Andrena sp.	*Anthidium sticticum*	*Apis mellifera*	*Bombus terrestris*	*Halictus gemmeus*	*Cryptocephalus* sp.	*Mordella* sp.	*Oedemera* spp.	*Oxythyrea funesta*	*Psilothrix* sp.
Carpobrotus aff. acinaciformis	5·41	11·36	17·50	93·33	20·00	33·33	61·54	32·91	38·46	56·41
Cistus albidus	13·51	11·36	5·00	6·67				8·86	7·69	
Cistus monspeliensis	22·97				20·00			32·91	25·00	2·56
Cistus salvifolius	21·62		5·00				23·08	10·13	23·08	17·95
Lavandula stoechas	4·05	68·18	37·50		60·00				1·92	
Sonchus tenerrimus	31·08	2·27				66·67	15·38	15·19	1·92	23·08

Seventy-three per cent of the pollinators collected on native plants carried *Carpobrotus* pollen grains, and, on average, pollinators visiting native plants carried 23·38 ± 4·02 % (mean ± s.e.) invasive pollen. However, there were significant differences among pollinator species in the percentage of invasive pollen loads ($F_{9,288} = 7·73$, $P < 0·0001$, Fig. 1; only individuals caught on native plants included). The beetles *Oxythyrea funesta*, *Psilothrix* sp. and the solitary bee *Anthidium sticticum* were the pollinators that carried more *Carpobrotus* pollen (Table 2). In general, when visiting native plants, beetles carried a higher proportion of *Carpobrotus* pollen than bees (33 % vs. 19 %, *t*-test = 3·75, $P = 0·002$).

Pollinators visiting *Carpobrotus* carried mostly *Carpobrotus* pollen, but there were significant differences among pollinator species ($F_{9,170} = 3·96$, $P < 0·001$; Fig. 1), with *A. mellifera*, *B. terrestris* and *Psilothrix* sp. scoring highest. As expected, pollinators collected on *Carpobrotus* carried more *Carpobrotus* pollen than pollinators collected on native plant species (all *t*-test $P < 0·05$), except for *Oxythyrea funesta* for which differences were not significant (*t*-test = 1·99, d.f. = 14, $P = 0·07$).

Overall, the percentage of conspecific pollen (55·49 ± 5·32) on the bodies of the pollinators visiting native plants was higher than the percentage of heterospecific (21·03 ± 2·96) and invasive pollen (23·38 ± 4·02; $F_{2,288} = 5·07$, $P < 0·001$). There were significant differences among pollinators in conspecific pollen loads ($F_{9,288} = 4·17$, $P < 0·001$) which ranged from 19 % in *Anthidium sticticum* to 79 % in *Cryptocephalus* sp. (Table 2).

For each pollinator species, differences were analysed between the dominant heterospecific native pollen and *Carpobrotus* pollen loads. It was found that while *Andrena* sp. and *Cryptocephalus* sp. carried more pollen of the dominant heterospecific species than invasive pollen (paired *t*-test = 2·00, d.f. = 74, $P = 0·05$; $t = 4·02$, d.f. = 14, $P < 0·001$, respectively), *Oxythyrea funesta* and *Psilothrix* sp., carried more invasive pollen than the dominant heterospecific native pollen (paired *t*-test = 4·31, d.f. = 74, $P < 0·001$; $t = 3·22$, d.f. = 49, $P < 0·006$, respectively). There were no significant differences for the other pollinator taxa (paired *t*-test, all $P > 0·2$).

There were significant differences in the percentage of conspecific pollen carried by pollinators depending on the plant species on which they were collected ($F_{5,464} = 20·91$, $P < 0·001$). *Sonchus tenerrimus* and *Carpobrotus* were the species whose pollinators carried a higher percentage of conspecific pollen. The percentage of invasive pollen loads carried by pollinators varied depending on the native species on which they were collected

TABLE 2. *Mean (± s.e.) number of pollen species, pollen grains counted, and percentage of conspecific, heterospecific native with indication of the most dominant and invasive (*Carpobrotus*) pollen carried by bees and beetles collected on the five most abundant native plant species (see Table 1) at a site invaded by* Carpobrotus

Pollinators	Order	Pollen species	Pollen grains	Conspecific	Heterospecific	Dominant heterospecific	Invasive
Andrena sp.	Bee	2·84 ± 0·10^{b}	461·42 ± 91·46^{ab}	60·76 ± 4·93^{a}	25·25 ± 4·63	24·51 ± 4·44	13·99 ± 1·80^{a}
Anthidium sticticum	Bee	6·57 ± 0·39^{c}	181·64 ± 24·43^{b}	18·76 ± 7·73^{c}	41·70 ± 6·72	25·06 ± 4·74	39·54 ± 10·57^{b}
Apis mellifera	Bee	3·08 ± 0·24^{ab}	716·92 ± 118·54^{a}	58·67 ± 9·16^{a}	23·68 ± 5·23	14·48 ± 2·36	17·65 ± 4·72^{a}
Bombus terrestris	Bee	4·33 ± 0·54^{a}	889·92 ± 170·36^{a}	69·61 ± 6·21^{a}	19·32 ± 5·36	12·82 ± 4·32	11·07 ± 4·67^{a}
Halictus gemmeus	Bee	2·94 ± 0·22^{b}	376·19 ± 54·16^{bc}	49·88 ± 6·30^{ab}	28·88 ± 5·85	23·07 ± 4·47	21·24 ± 4·16^{a}
Cryptocephalus sp.	Beetle	2·50 ± 0·27^{b}	49·60 ± 14·70^{b}	78·60 ± 6·20^{a}	10·31 ± 5·08	9·14 ± 4·35	11·07 ± 3·50^{a}
Mordella sp.	Beetle	1·25 ± 0·63^{b}	4·75 ± 2·06^{b}	67·50 ± 23·58^{a}	17·53 ± 2·50	10·33 ± 3·58	14·07 ± 4·57^{a}
Oxythyrea funesta	Beetle	3·69 ± 0·12^{ab}	340·46 ± 53·19^{bc}	39·90 ± 4·01^{b}	12·90 ± 2·37	10·20 ± 1·78	47·19 ± 5·64^{b}
Oedemera spp.	Beetle	2·33 ± 0·09^{b}	47·18 ± 6·74^{b}	57·48 ± 4·21^{a}	18·27 ± 3·10	15·50 ± 2·84	24·20 ± 2·85^{a}
Psilothrix sp.	Beetle	2·19 ± 0·15^{b}	26·79 ± 7·82^{b}	53·69 ± 6·94^{ab}	12·46 ± 2·68	10·21 ± 2·24	33·83 ± 6·91^{ab}
Mean		3·02 ± 0·47	309·49 ± 97·57	55·48 ± 5·32	21·03 ± 2·96	16·64 ± 1·59	23·38 ± 4·02

Different letters indicate significant differences within a column.

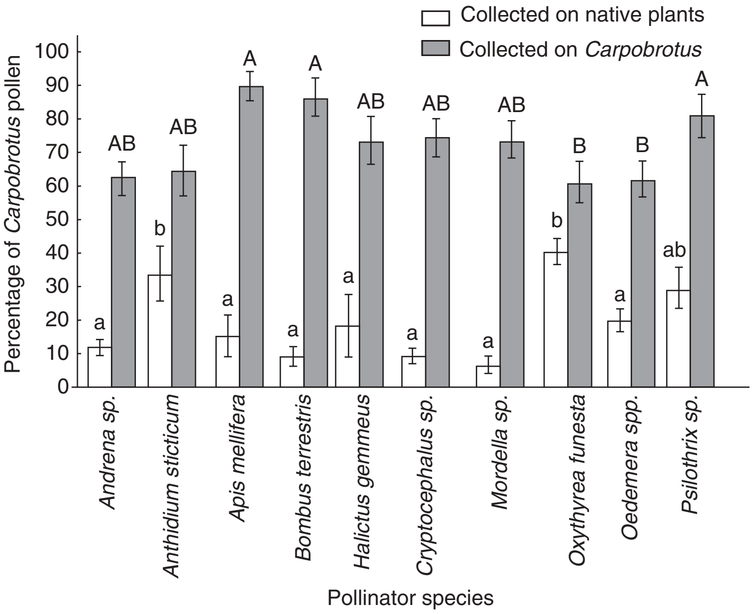

FIG. 1. Percentage (mean ± s.e.) of *Carpobrotus* pollen loads from the bodies of the ten most abundant pollinator species collected on native plant species (open columns) and on the invader *Carpobrotus* (shaded columns). Different letters indicate significant differences between pollinators for native species (lower case) and *Carpobrotus* (upper case).

($F_{4,288} = 12\cdot06$, $P < 0\cdot001$). Pollinators collected on *Sonchus tenerrimus* carried less invasive pollen than pollinators collected on the other plant species (Fig. 2).

Stigma pollen loads

Almost all stigmas were fully covered with pollen. Per stigma, $2\cdot13 \pm 0\cdot1$ pollen species were found. Plant species differed in pollen species richness ($F_{4,145} = 14\cdot99$, $P < 0\cdot001$), ranging from 1·5 in *Lavandula stoechas* to 3·5 in *Cistus albidus.* For all species, conspecific pollen was the most common type. Heterospecific and invasive pollen were found on all plant species, but were never abundant.

Of the 150 native stigmas sampled, 36 % had invasive pollen. Taking into account all plant species, frequencies of heterospecific pollen abundance categories were different from frequencies of invasive pollen abundance categories (all χ^2, $P < 0\cdot0001$). Heterospecific pollen counts were mostly in the low and moderate abundance categories, whereas invasive pollen counts were mostly in the absence and presence categories; i.e. *Carpobrotus* pollen was less abundant than total heterospecific pollen (Table 3).

DISCUSSION

Carpobrotus flowers produce large amounts of readily accessible pollen and attract a wide range of pollinator species (Suehs *et al.*, 2005; Bartomeus *et al.*, 2008). At the study site, there was a substantial overlap in pollinators between *Carpobrotus* and the most abundant native plants in the community. *Carpobrotus* pollen was efficiently transferred to the bodies of all pollinators, supporting the hypothesis that invasive pollen is well integrated in the plant-pollination network (Memmott and Waser, 2002). All ten pollinator species studied carried *Carpobrotus* pollen (albeit in low numbers) when collected on native plant species. However, stigma pollen loads contained mostly conspecific pollen and invasive pollen was only rarely found on native plant stigmas. Therefore, even if *Carpobrotus* is the most abundant plant species in the community, produces large amounts of pollen compared with native species, and shares generalist pollinators with the most abundant native plant species, the likelihood of invasive pollen interfering with conspecific native pollen appears to be low.

Both bees and beetles mainly carried pollen from the plant on which they were caught. However, there were differences in pollen loads and in pollen species identity between pollinator species. This could be related to body size and morphology (e.g. presence of hairs) and preferences for certain flower traits (Adler and Irwin, 2006). Bees, which are viewed as the most efficient pollinators (Proctor *et al.*, 1996), carried more pollen than beetles and visited native flowers more frequently than invasive flowers. Among bees caught on native plants, *Carpobrotus* pollen was always less abundant than the dominant heterospecific pollen. Beetles accounted for a high proportion of visits, and some beetles (*Oxythyrea funesta*) carried large quantities of *Carpobrotus* pollen. Beetles are typically viewed as poor pollinators (Proctor *et al.*, 1996), although in Mediterranean ecosystems they are very abundant floral visitors (Dafni *et al.*, 1990; Bernhardt, 2000). However, beetles spend long periods of time on each flower they visit, and therefore visit fewer flowers than bees (Bosch, 1992). *Oxythyrea funesta* and *Psilothrix* sp. were observed spending the night inside closed *Carpobrotus* flowers. Individuals of these two beetle species caught on native

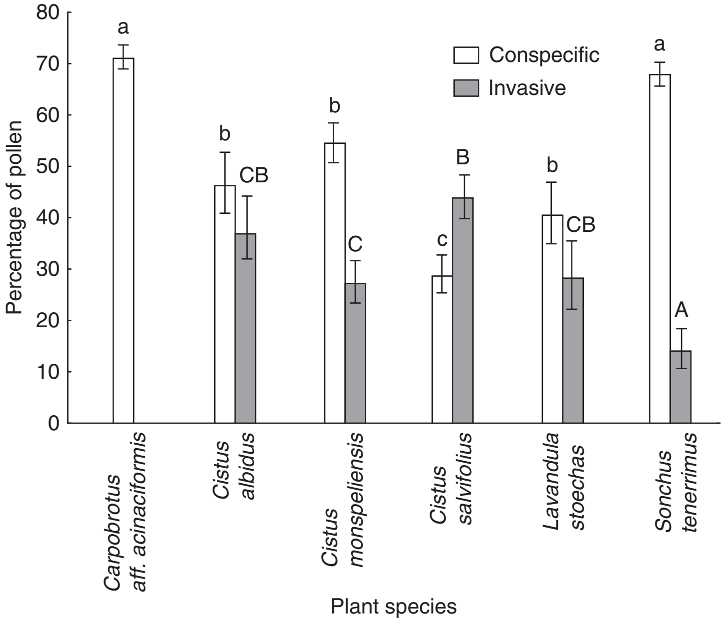

FIG. 2. Percentage (mean ± s.e.) of conspecific (open columns) and invasive pollen (shaded columns) loads on pollinators collected on different plant species at an invaded *Carpobrotus* site. Different letters indicate significant differences between plant species for conspecific pollen (lower case) and invasive pollen (upper case).

plants carried more *Carpobrotus* pollen than any other heterospecific pollen and, overall, carried more *Carpobrotus* pollen than bees.

All stigmas were fully covered with pollen. As with pollinator pollen loads, stigmas had mostly conspecific pollen. Neither heterospecific nor invasive pollen was present in high proportions. Even *Cistus salvifolius*, whose pollinators carried more invasive than conspecific pollen, had stigmas thoroughly covered with conspecific pollen. Several mechanisms may contribute to prevent high levels of *Carpobrotus* pollen deposition on native plant stigmas. First, pollinator fidelity (floral constancy) is widely reported for most pollinator groups, including bees and beetles (De los Mozos and Medina, 1991; Goulson *et al.*, 1997; Goulson and Wright, 1998; Gegear and Thomson, 2004) and plays a very important role in conspecific pollen transfer. Secondly, due to differences in flower morphology and stigma position, pollinators carry different pollen species on different body parts (Ambruster *et al.* 1994); for example, disc-shaped flowers such as *Carpobrotus* or *Cistus* deposit their pollen on the ventral parts of pollinators, while labiate flowers, such as *Lavandula stoechas* deposit their pollen on the dorsal part of the pollinator's body. Thirdly, there are differences in temporal pollen presentation among plant species; for example, *Carpobrotus* flowers last a few days, open late in the day and close at night, whereas anthesis occurs in *Cistus* spp. early in the morning and flowers senesce the same day by early afternoon (Bosch, 1992). This might explain that, although it was observed that pollinators carried more pollen from *Carpobrotus* than from *Cistus*, pollen deposition on *Cistus* stigmas takes place before pollinators get highly loaded with *Carpobrotus* pollen. Fourthly, beetles have a low flower visitation rate, and even though they are abundant on flowers (including *Carpobrotus*), their contribution to pollination might be low (Bosch, 1992).

Low *Carpobrotus* pollen transfer from pollinators to native flower stigmas was previously described to occur in the Balearic Islands (Moragues and Traveset, 2005). In this same study, the experimental addition of a mixture of *Carpobrotus* and conspecific pollen on the stigmas of native emasculated flowers caused no negative effect on the seed set (Moragues and Traveset, 2005). Chemical pollen interference between pollen of distantly related genera is rare (Heslop-Harrison, 2000; Brown and Mitchell, 2001) and might not occur between *Carpobrotus* and coflowering native species.

Moreover, the presence of *Carpobrotus* not only did not decrease visitation rates to native plants but even resulted in an increase in pollinator visitation to some native plants (Moragues and Traveset, 2005; Bartomeus *et al.*, 2008). Whether this 'magnet' effect also contributes to low competition between the invader and native species remains to be explored. To ascertain competition for pollination further, the male function of native plants should be explored by analysing the presence of native pollen loads on stigmas in the invader, differences in conspecific pollen loads between invaded and uninvaded communities, and most importantly, it should be explored whether native plants are pollen-limited and if native seed set differs between invaded and uninvaded sites. So far, the present findings suggest that there is low potential for pollination between the invader and native species.

Plant–pollinator interactions are generalized, with most plants receiving visits from several pollinators and most pollinators visiting several plants (Jordano, 1987; Waser *et al.*, 1996). This property facilitates the integration of invasive plants into the pollination network (Memmott

TABLE 3. *Mean (± s.e.) number of pollen species on the stigmas of the five most abundant plant species at an invaded* Carpobrotus *site, and percentage of stigmas with various abundance categories of conspecific, hetrospecific native and invasive* (Carpobrotus) *pollen*

		Conspecific					Heterospecific					Invasive				
Plant species	Pollen species	Absent	Present	Low	Moderate	High	Absent	Present	Low	Moderate	High	Absent	Present	Low	Moderate	High
Cistus albidus	3·6 ± 0·29	0	0	0	0	100·0	0	10·00	75·0	15·0	0	45·0	0	45·0	10·0	0
Cistus monspeliensis	1·6 ± 0·16	0	0	0	10·0	90·0	60·00	3·40	36·7	0	0	86·6	0	13·3	0	0
Cistus salvifolius	2·3 ± 0·20	0	0	0	0	100·0	32·20	16·1	45·1	6·4	0	41·9	25·8	25·8	6·5	0
Lavandula stoechas	1·5 ± 0·16	0	16·7	33·3	10·0	40·0	46·40	20·0	33·3	0	0	90·0	10·0	0	0	0
Sonchus tenerrimus	2·2 ± 0·19	0	0	0	6·7	93·3	33·30	23·3	36·7	6·7	0	60·0	20·0	13·3	6·7	0
Total natives	2·2 ± 0·20	0	3·3	6·7	5·3	84·7	34·38	14·6	45·4	5·6	0	64·7	11·2	19·5	4·6	0

Absent, No pollen; Present, 1 pollen grain; Low, <20 % of the total pollen load; Moderate, 20–70 % of the total pollen load; High, >70 % of the total pollen load.

and Waser, 2002; Lopezaraiza-Mikel *et al.*, 2007). At the same time, this low specialization appears to make plant-pollination networks robust and resilient to changes in pollinator and plant composition (Memmott *et al.*, 2004). While plants and pollinators have probably co-evolved within generalized networks (Jordano, 1987; Jordano *et al.*, 2003; Bascompte *et al.*, 2006), they have developed effective mechanisms to ensure successful pollination (Knight *et al.*, 2005; Blüthgen *et al.*, 2007). The incorporation of *Carpobrotus* species is unlikely to result in the collapse of the pollination network via competition for pollinators, at least at the current invader abundance. It is not known whether there is a density-dependent effect between floral density and visitation rate as classically postulated by Rathcke (1983). Recently, manipulative plant density experiments have shown that invasive plants disrupt pollinator services to native plants only at high densities while at low densities their effect is neutral or positive (Muñoz and Cavieres, 2008).

Carpobrotus grow very fast as a mat-forming plant. Competition for space and soil resources may be of greater importance to the local persistence of native plants (Vilà *et al.*, 2006) than competition for pollinators. It is envisaged that even at higher *Carpobrotus* abundance, native plants would suffer from competition for space before *Carpobrotus* flower abundance could increase competition for pollinators.

In summary, invasive plants may negatively affect plant communities in many ways (Levine *et al.*, 2003), and some invasive species have been found to have an impact on the pollination of native plants (Chittka and Schürkens, 2001; Brown *et al.*, 2002). In the present system, the invader was found to be highly integrated in the pollen transport network because all pollinators carried invasive pollen, but deposition of *Carpobrotus* pollen on stigmas of native plants was low and since *Carpobrotus* and native plants are phylogenetically non-related, decreasing the chances of stigma interference, it is believed that the invaded plant-pollinator community is robust enough to prevent competition for pollination services.

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