Pollen transfer from invasive *Carpobrotus* spp. to natives – A study of pollinator behaviour and reproduction success

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ABSTRACT

The influence of invasive plant species on native community composition is well-documented, but less is known about underlying mechanisms. Especially scarce is knowledge about effects on biotic interactions such as relationships between native plants and their pollinators. In this study we investigate if pollen transfer from the invasive and highly pollen productive *Carpobrotus* spp. affects seed production and/or seed quality in three native species. We monitored pollinator movements and pollen loads on pollinators and native stigmas, and in a field pollination experiment we investigated the effect of invasive pollen on reproduction. Invasive pollen adhered to pollinators, pollinators switched from *Carpobrotus* spp. to natives, invasive pollen was transferred to native stigmas, and it affected seed production in one species. Although all possible steps for interference with seed production were found to be qualitatively taken, invasive pollen has probably little impact on the native community because the frequency of invasive pollen transfer to natives was low. However, pollination interactions may change with plant abundance and our study provides evidence that pollen transfer from *Carpobrotus* spp. to natives does occur and have the potential to affect seed production. We found the species identity of shared pollinators to be of importance, higher flower constancy and lower capacity of pollen adherence are likely to result in less invasive pollen transfer.

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1. Introduction

Biological invasions are recognized as one of the main drivers of biodiversity loss, and virtually all ecosystems are affected by accidentally or intentionally introduced species (Heywood, 1989; Wilcove et al., 1998; Levine and D'Antonio, 2003). Invasive plants are well known to change community composition and ecosystem processes (e.g. Parker et al., 1999; Brooks et al., 2004) but the mechanisms behind these changes are relatively poorly understood (Levine et al., 2003). Moreover, when mechanisms have been studied, the main focus has been on direct competition for abiotic resources such as space, water and nutrients (Levine et al., 2003). More studies of indirect effects and the altering of ecological relationships such as breaking of native mutualisms are needed for further understanding of the processes operating in invaded systems (Traveset and Richardson, 2006; White et al., 2006). Invasive plants are often visited by native pollinators (e.g., Memmot and Waser, 2002), suggesting that they may interfere with native plant species by using their pollinators. Negative effects of invasive plants on pollination success (reviewed in Bjerknes et al., 2007) can be mediated by a reduction in pollination quantity if the invasive plant is more attractive to pollinators (Chittka and Schürkens, 2001; Brown et al., 2002; Ghazoul, 2004; Moragues and

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Traveset, 2005; Larson et al., 2006; Totland et al., 2006), or by a reduction in pollination quality if interspecific pollen transfer (IPT) occur. Studies have shown IPT between natives to cause a reduced female reproductive success (e.g., Galen and Gregory, 1989; Caruso and Alfaro, 2000; but see Waites and Ågren, 2004), but only a few studies have investigated IPT between invasive and native species (Grabas and Laverty, 1999; Moragues and Traveset, 2005; Larson et al., 2006). IPT can affect female reproductive success by three main mechanisms: (i) chemical interference (allelopathy) when heterospecific pollen exude chemicals inhibiting pollen germination, pollen tube growth, stigma receptivity, or ovule development (Sukhada and Jayachandra, 1980; Thomson and Andrews, 1982; Murphy, 2000), (ii) mechanical interference when conspecific pollen is prevented from reaching the stigma surface (Rathcke, 1983; Waser and Fugate, 1986; Galen and Gregory, 1989) and (iii) hybridization when the number of ovules available for legitimate pollination is reduced through fertilization with other species (Ellstrand et al., 1999).

Pollinator behaviour is influenced by absolute and relative abundance of flowering species (e.g., Stephens and Krebs, 1986; Rust, 1990; Rasheed and Harder, 1997), by the abundance of different pollinator species (Inouye, 1978; Bowers, 1986), and by weather conditions. Pollinator populations also exhibit strong dynamics at a local scale (Roubik, 2001). Temporal variability in number of invasive pollen grains deposited on native stigmas, as found by Larson et al. (2006), is therefore highly expected. The amounts of invasive pollen transferred to native stigmas depend on the frequency of pollinator switching from the invasive to the native, the amount of invasive pollen adhering to switching pollinators, and the number of pollen grains actually transferred to native stigmas during each visit. In the present work we monitor pollen transfer from the invasive Carpobrotus spp. to three native species, and we examine the effects of invasive pollen on seed production and seed quality in a field experiment.

2. Methods

2.1. Study species and sites

Carpobrotus edulis and C. affine acinaciformis and hybrids between them (hereafter referred to as Carpobrotus spp.) are South African and belong to the Aizoaceae. The taxa are widespread around the Mediterranean basin and considered a threat to several plant species on Mediterranean islands (e.g., Suehs et al., 2001, 2005; Hulme, 2004; Vilà et al., 2006). The showy flowers contain hundreds of stamens and produce large amounts of pollen (Blake, 1969). Three native species sharing main pollinators with the invasive (Padrón and Traveset, unpublished data) were selected for our study: Asphodelus aestivus Brot. (Liliaceae), Dorycnium hirsutum L. (Fabaceae), and Helichrysum stoechas L. (Asteraceae). The study was performed from May to July 2006 at two field sites on north-east Majorca. A. aestivus and D. hirsutum were studied in a semi-natural grassland in the S'Albufera Natural Park (39°48′3.03″N; 3°6′57.58″E) whereas H. stoechas was studied in a dune environment in Son Serra de Marina (39°43′53.34″N; 3°13′56.94″E).

2.2. Observations of pollinator behaviour

Pollinator movements from Carpobrotus spp. to the natives were observed during 15 min periods on calm and sunny days. Pollinators visiting natives were identified and classified into arriving from (1) a conspecific individual (2) a Carpobrotus spp. flower or (3) an unknown pollen source. The number of observed Carpobrotus spp. flowers was twice as high as the number of conspecific individuals and was divided by two before compared to arrivals from conspecifics. For D. hirsutum and H. stoechas 31 observation efforts at 10 sites were made, and for A. aestivus 11 observations at two sites were made. A Wilcoxon matched paired tests was used to test if a pollinator species arrived more often from a conspecific than from a Carpobrotus spp. flower (each comparison being made within one observation effort). Differences in behaviour between pollinator species were analyzed by a Fischer exact test.

2.3. Pollen loads on pollinators and stigmas

A reference collection was made of pollen from flowering species within 100 m from the sites where pollinators and stigmas were collected (see below). Pollen grains were measured (30 pollen per species) and photographed at 400× magnification. Pollen from Carpobrotus spp. and the three native species could be distinguished from pollen from other species, with the exception of H. stoechas which could not always be separated from Asteriscus maritimus, and D. hirsutum which could often, but not always be separated from D. pentaphyllum.

To estimate Carpobrotus spp. pollen load on pollinators that have recently visited a Carpobrotus spp. flower, ten individuals of each species observed to switch were collected whilst visiting Carpobrotus spp. flowers. To estimate pollen loads of pollinators arriving to a native species, 10 individuals of each switching pollinator species were caught whilst arriving at a D. hirsutum flower. One switching pollinator (Polistes gallicus) was not collected since this species was rare at both sites. Pollinators were caught in individual vials to avoid pollen contamination among individuals, and were stored in a freezer. Pollen was removed from the pollinators by filling the vials with isoton and shaking them in a vortex after an hour of soaking. This process was repeated until less than 10% of the total pollen number found in the sample of the first removal was found, and the mean pollen number for all samples corresponding to one individual pollinator was summed up. Number of pollen grains of each pollen species was estimated by using Lycopodium spore tablets (batch 483216 from Lund University, Sweden, one spore tablet containing an average of 18,583 spores). The use of Lycopodium spore tablets to calculate pollen concentrations is a standard method in Quaternary palynology (Maher, 1997). A spore tablet was mixed with the pollen sample to a perfect suspension with a vortex, a drop of perfect suspension was extracted and pollen grains and spores were counted under the microscope. The number of pollen grains of a certain pollen species in the sample was then calculated as "number of pollen grains in the drop × (18,583/the number of spores in the drop)". This was repeated five times for each sample and the average was used as the estimate of total pollen number in that sample.

For pollinators caught in *Carpobrotus* spp. flowers the pollen was classified into *Carpobrotus* spp. pollen and other pollen. For pollinators caught whilst arriving at *D. hirsutum* individuals, pollen was as far as possible identified to species level. Differences among pollinator species in total and proportion of *Carpobrotus* spp. pollen load were analyzed by 1-ANOVAs and Mann–Whitney *U*-tests. Total number of *Carpobrotus* spp. pollen was log-transformed to fulfil the requirements of the ANOVA. For pollinators caught on *D. hirsutum*, the number of *D. hirsutum* and *Carpobrotus* spp. pollen were compared to the number of the most common other pollen species by Kruskal–Wallis tests.

Stigmas were collected from eight individuals per species, all growing closer than 1 m to a *Carpobrotus* spp. flower. Under the microscope, 10 (*A. aestivus*), 15 (*D. hirsutum*), and 15 (*H. stoechas*) stigmas per individual were inspected for invasive, conspecific, and other pollen species. Counts included self-pollen since flowers were not emasculated. Stigmas from *A. aestivus* and *D. hirsutum* were stored in alcohol in separate vials. For *H. stoechas* it was not possible to collect individual flowers, so whole flower heads were collected and stigmas were later removed under a stereoscope in the lab. Number of conspecific pollen was compared to number of pollen from *Carpobrotus* spp. using a Mann–Whitney *U*-test.

2.4.   Effect of Carpobrotus pollen on seed set and seed quality

We performed three pollination treatments in the field to test if *Carpobrotus* spp. pollen had a negative effect on seed set or seed quality of the natives. Three pollen samples were used: (1) pollen from conspecific individuals, (2) pollen from *Carpobrotus* spp., and (3) a mixture of conspecific pollen and *Carpobrotus* spp. pollen. Flowers exposed to natural pollination served as control. Buds were bagged to prevent pollination before treatment and after treatment flowers were open to natural pollination. The ability of spontaneous self-pollination was investigated by bagging buds and removing the bags after flowers had wilted. The conspecific pollen was gathered in Petri dishes from five individuals at least 30 m from the target individual, and transferred with fine paint brushes within 15 min to ensure viability. Fifteen individuals of each species were subjected to the treatments, and treatments were blocked within individuals. Target and pollen donor individuals were situated in an area without *Carpobrotus* spp. flowers, so we expect no natural transfer of invasive pollen to these individuals. In *A. aestivus* each treatment was randomly assigned to seven single flowers. In *D. hirsutum* each treatment was assigned to two flower-heads. The flower-heads were randomly selected; those located close to ground or "inside" the bush were not considered. In *H. stoechas* each treatment was applied to three randomly selected groups of flower-heads, and treatments were applied twice since all florets in a flower-head were not open at the same time. In total, 420 *A. aestivus* flowers, 874 *D. hirsutum* flowers, and 1907 *H. stoechas* flower-heads were monitored for fruit and seed set. Fruit heads of *D. hirsutum* and *H. stoechas* were bagged when the fruits were almost ripe to prevent seed loss. The seeds were weighted and counted in the lab. A randomized block mixed-model ANOVA with individual as a random and treatment as a fixed factor was used to analyze effects of the pollination treatments on fruit set, seed production, seed number per fruit, and seed weight. Pollination treatments were compared to open controls by planned comparisons. Fruit set in *A. aestivus* was very low and seed predation high, thus seed number per fruit and seed weight could not be analyzed. Fruit set was analyzed as number of fruits/number of pollinated flowers per individual, i.e. there were no replicates of fruit set within individuals and therefore the model did not include an interaction term between treatment and individual. For *D. hirsutum* the analyses of seed number per fruit and seed weight were made on means for fruit-heads, and fruit set was analyzed on flower to fruit ratios for fruit-heads. One individual showed very low fruit set and was omitted from the analyses of seed number per fruit to avoid problems with too may zeros in the data material, but was included in the analysis of fruit set. For *H. stoechas* the analyses of seed production and seed weight were made on means for groups of flowerheads. All ANOVAs fulfilled assumptions of homogeneity of the variances (Cochran C test) and normally distributed residuals. All analyses were performed with STATISTICA v 6.0.

3.   Results

3.1.   Pollinator movements

A. aestivus was visited by six, *D. hirsutum* by 15, and *H. stoechas* by 19 pollinator species, and total number of visits was 59 for *A. aestivus*, 538 for *D. hirsutum* and 478 for *H. stoechas* (Appendix A). Only a few of the pollinator species were observed to move directly from *Carpobrotus* spp. to the natives (Table 1). For all three plant species, any switching pollinator species was always arriving more often from a conspecific individual than from a *Carpobrotus* spp. flower (Wilcoxon matched paired test $p < 0.05$ for all species combinations, Table 1). *Rhodanthidium septemdentatum* arrived from *Carpobrotus* spp. to *D. hirsutum* significantly more often than did *Apis mellifera* (Fischer exact $p < 0.01$). For *A. aestivus*, the number of observed events of pollinators arriving from the invasive was too low to admit any analysis. All switching pollinators were important in terms of visit frequencies to the natives. *A. mellifera* and *R. septemdentatum* performed 81% of the visits to *A. aestivus*. These two insects and *Polistes gallicus* made 93% of the visits to *D. hirsutum*, whilst the beetle *Oedemera* sp. was the second most common pollinator to *H. stoechas*, making 26% of the visits.

3.2.   Pollen loads on pollinators and stigmas

There was a significant difference in *Carpobrotus* spp. pollen load size among the three species caught whilst foraging in *Carpobrotus* spp. flowers (ANOVA: df = 2, $F = 11.9$, $p < 0.01$), *Oedemera* sp. carried less pollen than *R. septemdentatum* and *A. mellifera* (Fig. 1A). *R. septemdentatum* arriving to *D. hirsutum* carried both a higher total number of *Carpobrotus* pollen (ANOVA df = 1, $F = 6.14$, $p < 0.05$, Fig. 1B) and a higher proportion of *Carpobrotus* pollen than did *A. mellifera* to *D. hirsutum* flowers (2% (range 0–36%) vs. 0.2% (range 0–6%), respectively; Mann–Whitney *U*-test $U = 17$, $p < 0.05$). This is consistent with the finding that the former moved more often from the inva-

Table 1 – Arrival frequencies for pollinator species switching between *Carpobrotus* spp. and three native plant species

Native species	Switching pollinator	Arrivals from unknown pollen source	Arrivals from conspecific	Arrivals from *Carpobrotus* spp.
A. aestivus	*A. mellifera*	25	18	3 (1.5)
	R. septemdentatum	0	0	2 (1)
D. hirsutum	*A. mellifera*	120	139	12 (6)
	R. septemdentatum	116	31	24 (12)
	P. gallicus	35	18	4 (2)
H. stoechas	*Oedemera* spp.	70	43	12 (6)

As the number of observed *Carpobrotus* spp. flowers was always twice as high as the number of possible conspecific pollen donors, the adjusted number is shown in brackets.
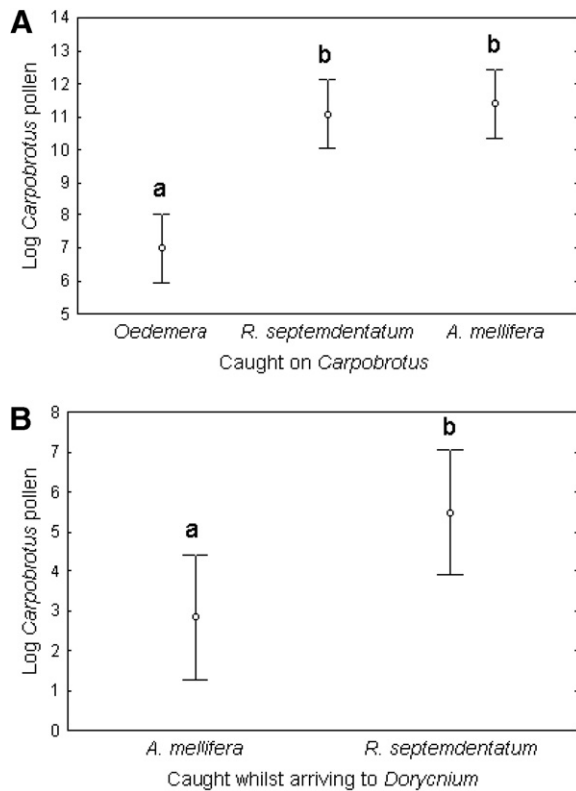

Fig. 1 – Differences in total *Carpobrotus* spp. pollen load (A) among three pollinator species caught in *Carpobrotus* spp. flowers, and (B) between two pollinator species caught while arriving to the native *D. hirsutum*. Different letters above bars indicate significant differences according to a Tukey HSD test (*p* < 0.005). Vertical bars denote 0.95 confidence intervals around least squares means.

sive to the native. With respect to pollen from other plant species, there were no large differences between the bee species, each bee species carried one pollen species not carried by the other species but shared the rest of the pollen species (Appendix B). On *R. septemdentatum*, there was a significant difference in the number of different pollen species (Kruskal–Wallis $H = 18.21$, $p < 0.001$). *Echium sabulicola* pollen was present in the same amounts as *D. hirsutum* pollen and it was more common than *Carpobrotus* spp. pollen (Fig. 2A). Also on *A. mellifera* there was a significant difference in the number of different

pollen species (Kruskal–Wallis $H = 22.34$, $p < 0.001$). *Trifolium campestre* pollen was present in the same amounts as *Carpobrotus* spp. pollen but in much lower levels than *D. hirsutum* pollen (Fig. 2B).

The number of *Carpobrotus* spp. pollen grains found on native stigmas was always much lower than the number of conspecific pollen grains (Mann–Whitney U-test $p < 0.001$ for all natives, Table 2). Conspecific pollen counts include self-pollen to some degree, at least for *D. hirsutum* and *H. stoechas* in
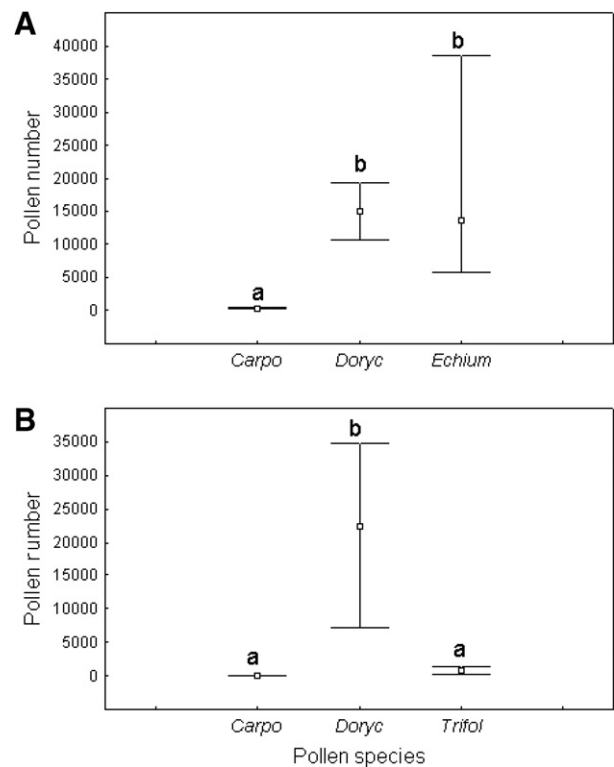
Fig. 2 – Average number of *Carpobrotus* spp. pollen, *D. hirsutum* pollen and the other most common pollen species found on (A) *R. septemdentatum* and (B) *A. mellifera* caught whilst arriving at *D. hirsutum* flowers. Carpo = *Carpobrotus* spp., Doryc = *D. hirsutum*, Trifol = *Trifolium campestre*. Different letters above bars indicate significant differences according to multiple comparisons of mean ranks for all groups (*p* < 0.05). Vertical bars denote the 25–75 percentiles around medians.

which anthers and stigmas are close to each other, but since absolute numbers of *Carpobrotus* spp. pollen was very low and the total amount of other pollen were just as high or even slightly higher as than *Carpobrotus* spp. pollen (Table 2), the results indicate that transfer of *Carpobrotus* spp. pollen to native stigmas was low.

3.3. Effect of Carpobrotus spp. pollen on seed set and quality

Spontaneous self-fertilization in *D. hirsutum* and *A. aestivus* appeared to be nil, as bagged flowers produced no fruits at all, thus both species needed a pollinator agent for seed production. By contrast, bagged *H. stoechas* flowers produced seeds in some cases, but much less than open control flowers (3.7 vs. 16.9 achenes per flower head respectively; Mann–Whitney U-test $U = 93$, $p < 0.01$). The pollination treatments had an effect on fruit set in *A. aestivus* and on seed production in *D. hirsutum* and *H. stoechas* (Table 3). In *A. aestivus* and *D. hirsutum*, supplementary pollinated flowers produced more fruits and seeds per fruit than open controls, indicating pollen limitation, but these species were not affected by *Carpobrotus*

spp. pollen (Fig. 3a and b). *H. stoechas*, which was pollen limited too, was the only native negatively affected by *Carpobrotus* spp. pollen (Fig. 3c). There was no effect of pollination treatment on seed weight in *D. hirsutum* or *H. stoechas*, and no effect on fruit set in *D. hirsutum* (Table 3).

4. Discussion

There is a low risk that transfer of *Carpobrotus* spp. pollen will reduce seed production in the native species, since very low amounts of invasive pollen were found on native stigmas. However, our study shows that such impact could potentially become a problem because invasive pollen adhered on pollinators, pollinators moved from the invasive to natives, pollen was transferred to native stigmas, and invasive pollen affected seed production in one of three species. Pollinator behaviour change with factors such as flower abundance and composition of the plant community (e.g. Stephens and Krebs, 1986; Rust, 1990; Rasheed and Harder, 1997), and the ratio of *Carpobrotus* spp. flowers to native flowers was rather low at our study sites due to a former governmental removal program. If that ratio increases, the frequency of movements from *Carpobrotus*

Table 2 – Number of conspecific, Carpobrotus spp. and other species pollen grains on stigmas of three native species growing in close vicinity (<1 m) from a *Carpobrotus* spp. flower

Species	Conspecific pollen		Carpobrotus pollen		Other pollen	
	Range	Mean	Range	Mean	Range	Mean
A. aestivus	0–70	9.3	0–4	0.27	0–6	0.34
D. hirsutum	0–1208	122	0–1	0.04	0–120	5.8
H. stoechas	2–228	48	0–4	0.048	0–120	5.8

Table 3 – Effect of pollination treatments (conspecific pollen, mixed pollen, invasive pollen and open controls) on reproductive success in *D. hirsutum*, *A. aestivus* and *H. stoechas*

| Species	Variable	Source	df	MS	F	p
A. aestivus	Fruit set	Individual	14	0.02	1.20	0.31
		Treatment	3	0.07	5.05	0.005
		Error	42	0.01		
D. hirsutum	Seed number	Individual	13	2.30	2.72	0.007
		Treatment	3	3.39	4.03	0.01
		Individual * treatment	39	0.85	1.27	0.22
		Error	47	0.67		
	Fruit set	Individual	14	0.42	5.23	0.001
		Treatment	3	0.20	2.55	0.07
		Individual * treatment	42	0.08	1.38	0.12
		Error	61	0.06		
	Seed weight	Individual	13	1.67	6.99	0.001
		Treatment	3	0.21	0.85	0.47
		Individual * treatment	49	0.25	1.78	0.03
		Error	47	0.14		
H. stoechas	Seed number	Individual	14	68.67	2.15	0.03
		Treatment	3	153.55	4.81	0.006
		Individual * treatment	42	31.90	1.71	0.01
		Error	120	18.67		
	Seed weight	Individual	14	0.0001	2.73	0.0006
		Treatment	3	0.0002	1.53	0.22
		Individual * treatment	42	0.001	1.38	0.009
		Error	118	0.00009		
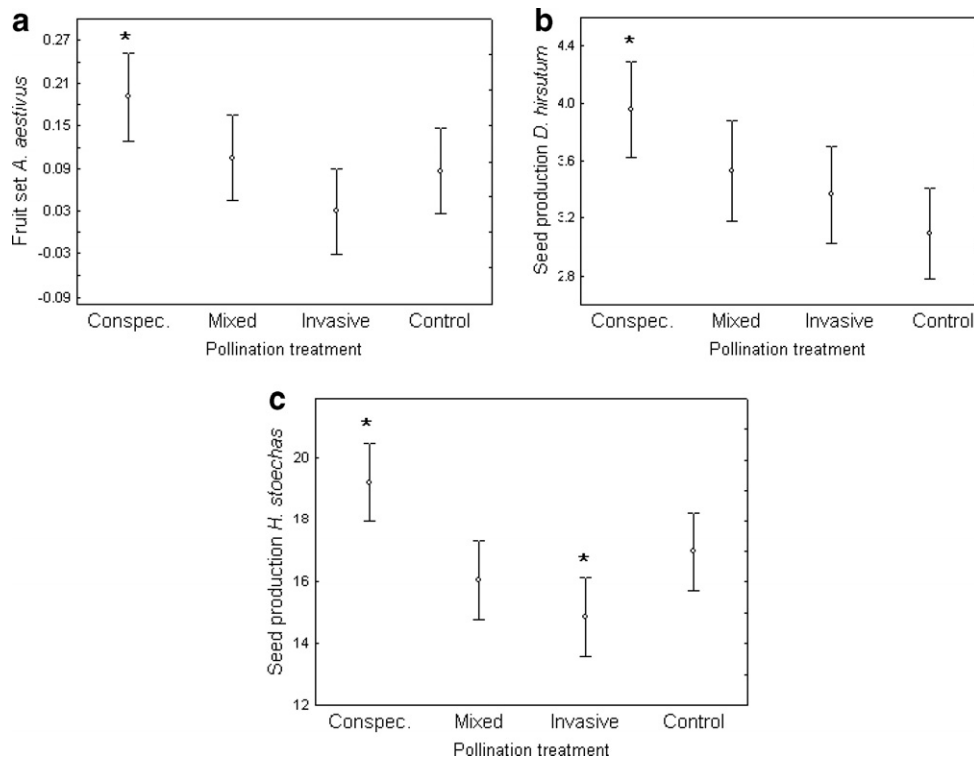
Fig. 3 – Effect of pollination treatments on (a) fruit set in *A. aestivus*, (b) seed production per fruit in *D. hirsutum*, and (c) seed set per fruit-head in *H. stoechas*. Conspec. = conspecific outcross pollen, Mixed = a mixture of conspecific and *Carpobrotus* spp. pollen, Invasive = *Carpobrotus* spp. pollen, Control = open pollination. Flowers were exposed to natural pollination after the treatments. Treatment bars with an asterisk (*) differ significantly from the control according to planned comparisons ($p < 0.02$). Vertical bars denote 0.95 confidence intervals around least square means.

spp. to natives may increase as well. In areas where the spreading of *Carpobrotus* spp. is uncontrolled the species often covers large areas and produces large amounts of flowers (Suehs et al., 2004). It is also worth noticing that although only one of three species showed a significant negative effect of invasive pollen on seed production, all three native species follow the same trend where fruit set, seed set, or seed production are highest for the legitimate treatment, lower for the mixed treatment and lowest for the treatment with pure *Carpobrotus* spp. pollen. This indicates that all species may be sensitive to *Carpobrotus* spp. pollen, but the power of our data might be too low to detect significant differences. A possible sensitivity to invasive pollen transfer is further corroborated by the finding that seed production in all natives was pollen limited. In general, pollen limitation should make natives more susceptible to any impact from invasive species on pollination interactions (Ghazoul, 2005; Traveset and Richardson, 2006).

The species identity of shared pollinators was important, because pollinator species differed in flower constancy and in capacity of carrying invasive pollen. The solitary bee *R. septemdentatum* switched more often from the invasive to the native than the social bee *A. mellifera*, and also carried a higher proportion of *Carpobrotus* spp. pollen when arriving to a native. This is consistent with the knowledge that individual workers of social bees often specialize on one flower resource at a time, whereas generalist solitary bees which are single providers for a nest more often switch between plant species during the same search bout (Williams and Tepedino, 2003). The beetle

Oedemera sp. carried less *Carpobrotus* spp. pollen than the furry bee species, and will thus be less likely to cause IPT. We did not make any attempt to map pollen placement on bodies although such spatial segregation of pollen may reduce IPT (Grabas and Laverty, 1999). However, all pollinator species were observed to carry *Carpobrotus* spp. pollen on both ventral and head parts, and the stigmas in native flowers were positioned in a manner much likely to be contacted by these parts of the insects.

The negative effect of *Carpobrotus* spp. pollen on seed production in *H. stoechas* could be caused by stigma clogging or by pollen allelopathy. We consider stigma clogging as the most likely mechanism, since no negative effect was found in the mixed treatment which would be expected if allelopathy occurred. The reason for stigma clogging not to affect seed production in the mixed treatment could be due to an "order effect", where stigma clogging was severe enough to affect seed production only when invasive pollen was transferred first, but not when conspecific and invasive pollen was transferred at the same time. Such 'order effects' have previously been reported in controlled pollinations between two native plants (Caruso and Alfaro, 2000). Alternatively, stigma clogging might be a problem only when there is pollen limitation. Seed production in *H. stoechas* was shown to be pollen limited under natural pollination conditions and the stigmas receiving the "pure" *Carpobrotus* spp. treatment received conspecific pollen only under natural pollination conditions, therefore we cannot separate a potential "pollen limitation effect" from an "order effect".

Interspecific pollen transfer is regarded as an important factor in the structuring of plant communities (Rathcke, 1983), and has been suggested to cause evolution of interspecific pollen-flow reducing mechanisms such as separation in flowering times and morphological adaptations for specific pollen placement (Waser, 1983; Campbell, 1985; Stone et al., 1998; review in Palmer et al., 2003). Thus a new species not co-evolved with the plant community would be expected to cause much IPT. However, the few studies made on pollen transfer from invasive to native species provide equivocal evidence. Both low (Moragues and Traveset, 2005; Larson et al., 2006) and high (Grabas and Laverty, 1999) levels of invasive pollen have been found on native stigmas, and controlled pollination treatments have shown both negative (Brown and Mitchell, 2001) as well as neutral effects (Moragues and Traveset, 2005) of invasive pollen on seed production. Frequent pollinator switching and lower seed set in the native was found by Brown et al. (2002), but this study did not separate between a pollen transfer effect and an effect of reduced visit frequency. At our study site, interspecific pollen transfer from some natives seemed to be at least as frequent as from the invasive. It is possible that the potential of invasive plants to affect pollination interactions lies more in their capacity to achieve high abundances rather than in specific plant traits such as showiness or high pollen or nectar production, since pollinator behaviour can be greatly affected by abundance of the flowering resource (Brian, 1954; Inouye, 1978; Bowers, 1986).

In conclusion, only a few studies have examined the effects of an invasive plant on fruit and seed set or on seed quality of native species in the recipient ecosystem. We provide evidence that insects do switch directly from the invasive to natives, that pollen is transferred to native stigmas, and that this invasive pollen is able to affect seed production, although this depends upon the native species. The implication for management is that in areas where *Carpobrotus* spp. is highly abundant pollen transfer can affect seed production in natives and that effects are likely to be species specific, depending on the recipient native plant species and on the species identity of shared pollinators.

Acknowledgements

Appendix A

Pollinator species visiting *Dorycnium hirsutum*, *Asphodelus aestivus*, and *Helichrysum stoechas*. The pollinators were classified into arriving from conspecific individuals, *Carpobrotus* spp. flowers or an unknown pollen source. The study took place at two field sites on Mallorca, S'Albufera nature reserve (*D. hirsutum* and *A. aestivus*) and Son Serra de Marina (*H. stoechas*); nomenclature follows Fauna Europaea (www.faunaeur.org).

Pollinator species on *D. hirsutum*	Total visits	No. visits arriving from unknown pollen source	No. visits arriving from a conspecific	No. visits arriving from *Carpobrotus*
Andrena sp. 1	2	2		
Andrena sp. 3	3	2	1	
Anthocharis cardamines	2	2		
Anthophora sp. 1	13	10	3	
Apis mellifera	271	120	139	12
Pieris brassicae	3	2	1	
Sarcophagidae sp. 1	1	1		
Sarcophagidae sp. 2	3	3		
Halictus sp.	4	3	1	
Melecta sp.	1	1		
Oedemera sp.	1	1		
Polistes gallicus	57	35	18	4
Rhodanthidium septemdentatum	171	116	31	24
Syrphidae sp. 1	3	2	1	
Syrphidae sp. 2	3	1	2	
Total	538			
Pollinator species on *A. aestivus*				
Antophora sp. 1	2	2		
Apis mellifera	46	25	18	3
Sarcophagidae sp. 2	2	2		
Polistes gallicus	6	6		
Rhodanthidium septemdentatum	2			2
Unidentified small fly	1	1		
Total	59			

Appendix A – *continued*

Pollinator species on *D. hirsutum*	Total visits	No. visits arriving from unknown pollen source	No. visits arriving from a conspecific	No. visits arriving from *Carpobrotus*
Pollinator species on *H. stoechas*				
Andrena sp. 1	4	3	1	
Andrena sp. 2	95	37	58	
Apis mellifera	36	20	16	
Colletes sp.	139	56	83	
Dasyscolia ciliata	3	1	2	
Eristalis tenax	4	3	1	
Cerambycidae sp. 1	1	1		
Sarcophagidae sp. 1	13	13		
Sarcophagidae sp. 2	5	3	2	
Sarcophagidae sp. 3	7	5	2	
Gymnosoma sp.	4	3	1	
Lucilia sp.	6	4	2	
Oedemera sp.	125	70	43	12
Osmia sp.	5	3	2	
Polistes gallicus	9	8	1	
Polyommatus icarus	7	6	1	
Rhagonycha fulva	6	5	1	
Rhodanthidium septemdentatum	2	2		
Unidentified small fly	7	5	2	
Total	478			

Appendix B

Number of pollen of different species found on individuals of *Apis mellifera* and *Rhodanthidium septemdentatum* caught whilst arriving at *Dorycnium hirsutum* flowers. Field site: S'Albufera nature reserve, Majorca

Individual	*Carpobrotus* spp.	*D. hirsutum*	*Centaurium erythraea*	*Convolvulus althaeoides*	*Cistus salviifolius*	*Echium sabulicola*	*Galactites tomentosa*	*Lotus corniculatus + L. cytisoides*	*Trifolium campestre*	Unidentified
Apis mellifera										
1	53	84,627	154	0	0	0	0	241	948	0
2	0	7086	0	0	0	0	0	126	462	0
3	35	3590	0	0	0	0	0	46	0	0
4	0	25,391	0	0	0	0	0	351	996	0
5	108	14,320	50	0	0	0	108	57	166	133
6	362	5858	0	0	0	0	0	158	291	438
7	330	49,983	0	0	0	0	34	1695	9644	162
8	0	34,802	0	0	0	0	137	108	2021	575
9	79	22,732	0	0	0	426	0	79	528	52
10	0	22,095	0	0	0	423	0	0	1430	287
Rhodanthidium septemdentatum										
1	0	19,261	0	0	0	11,321	0	0	48	0
2	366	46,724	0	0	139	78,007	0	317	751	1485
3	606	11,099	0	0	0	20,588	0	2203	252	461
4	6100	10,674	0	0	153	14,452	1561	8432	520	464
5	520	19,507	0	25,909	0	12,761	0	0	302	373
6	339	7707	0	0	26	5729	0	782	242	195
7	293	13,948	0	432	0	1204	0	77	604	46
8	321	19,386	0	7472	460	38,537	291	552	1737	444
9	315	16,101	0	5305	307	85,352	808	1781	0	286
10	77	7070	0	343	0	1278	0	38	2267	859

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