

Short communication

Release from soil pathogens plays an important role in the success of invasive *Carpobrotus* in the Mediterranean

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Abstract

Introduced plant species can become locally dominant and threaten native flora and fauna. This dominance is often thought to be a result of release from specialist enemies in the invaded range, or the evolution of increased competitive ability. Soil borne microorganisms have often been overlooked as enemies in this context, but a less deleterious plant soil interaction in the invaded range could explain local dominance.

Two plant species, *Carpobrotus edulis* and the hybrid *Carpobrotus X cf. acinaciformis*, are considered major pests in the Mediterranean basin. We tested if release from soil-borne enemies and/or evolution of increased competitive ability could explain this dominance. Comparing biomass production in non-sterile soil with that in sterilized soil, we found that inoculation with rhizosphere soil from the native range reduced biomass production by 32% while inoculation with rhizosphere soil from the invaded range did not have a significant effect on plant biomass. Genotypes from the invaded range, including a hybrid, did not perform better than plants from the native range in sterile soil. Hence evolution of increased competitive ability and hybridization do not seem to play a major role. We conclude that the reduced negative net impact of the soil community in the invaded range may contribute to the success of *Carpobrotus* species in the Mediterranean basin.

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1. Introduction

Many plant species have been introduced into areas where they previously did not occur. Most of these fail to establish or only establish locally, however a few species become dominant and displace the natural vegetation (Williamson and Fitter, 1996). Several hypotheses explaining this dominance have been proposed. Three of these are enemy release, (Elton, 1958; Keane and Crawley, 2002), evolution of increased competitive ability (Blossey and Notzold, 1995), and hybridization (Ellstrand and Schierenbeck, 2000). These hypotheses are often tested in isolation and rarely in combination although they are not mutually exclusive and partly depend upon each other.

Most studies on enemy release of invasive species focus on the role of aboveground herbivores (Maron and Vila, 2001; Agrawal et al., 2005) but attention on the soil community has increased recently as the feedback between plants and soil organisms has proven to be an important driver in ecosystem dynamics (Klironomos, 2002; Kardol et al., 2006). Plant–soil feedback is an important determinant of plant density. Plants that quickly accumulate pathogens are found at low densities in natural communities, while plants that accumulate pathogens slowly, or are not strongly affected by these pathogens, are often locally dominant (Klironomos, 2002).

Plants can undergo genetic changes after introduction resulting in more vigorous genotypes in the invaded range (Bossdorf et al., 2005). If an invasive species is released from its enemies, the benefit of defence mechanisms is diminished and the balance in the trade-off between defence and growth or reproduction (Herms and Mattson, 1992) may shift towards

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growth and reproduction. Therefore these plants have a better competitive ability than, and may out compete, plants that invest more in defence mechanisms. This hypothesis is known as the evolution of increased competitive ability or EICA (Blossey and Notzold, 1995). This will result in increased performance in the absence of pathogens, e.g. in sterilized soil, compared to plants from the native range.

A third factor that may play an important role in the success of invasive species is hybridization (Ellstrand and Schierenbeck, 2000). Hybridization can result in heterosis and hybrid vigour resulting in a more competitive phenotype (Wolfe et al., 2007). Hybridization can also increase genetic variation and thus facilitate EICA.

These hypotheses apply to the highly invasive species *Carpobrotus edulis* (L.) N.E. Br. and the hybrid *C. X cf. acinaciformis* (*C. edulis* x *C. acinaciformis* (L.) L. Bolus) of the Aizoaceae. These are invasive in the Mediterranean basin and are threatening native species by the formation of large, monospecific mats excluding native species (D'Antonio and Mahall, 1991; Draper et al., 2003; Vila et al., 2006). Related species are invasive in areas with a Mediterranean climate around the world (Suehs et al., 2006).

We predicted that, 1) the inoculation with soil from the native range has a stronger negative effect than inoculation with soil from the invaded range. This would support the enemy release hypothesis, 2) plants from the invaded range have a higher biomass production on sterilized soil than the plants from the native range. This would support the increased competitive ability hypothesis.

2. Materials and methods

2.1. Study species

Carpobrotus edulis, *C. acinaciformis* and their hybrid, *C. X cf. acinaciformis*, are succulent perennials with strong clonal growth and creeping vine-like branches that root at internodes. Branches can grow up to 1 m per year, overgrowing and replacing surrounding vegetation (D'Antonio, 1993; Acosta et al., 2006). Both species originate from South Africa and were introduced in order to stabilize sand dunes and road sides (Vila and D'Antonio, 1998) in the western Mediterranean basin since the early 1800s (Gouffe De La Cour, 1813).

Rhizosphere soil was collected from three populations of *C. edulis*. A typical native population near East London, South Africa (SA 1) and two populations from southern Spain, one near Cadiz, 10 km from the seashore (Spain 1) and another near Malaga next to the Mediterranean seashore (Spain 2). For every population 10 plants were selected. Distance between selected plants was between 15 and 800 m. Under each plant approximately 1.5 kg of soil was collected. Soil was collected no deeper than 15 cm. below the soil surface. These samples were homogenized into an aggregate sample per population. Seeds from the same populations were collected. Additional seeds, but not soil, were collected from a second population near Cape Town, South Africa (SA 2) and two populations on Bagaud island (Hyères archipelago, France); one consisting of

pure *C. edulis* (France 1) and one of the genetically more diverse hybrid *C. X cf. acinaciformis* (France 2) (Suehs et al., 2004, 2006).

2.2. Experimental setup

Soils and seeds were moved to Wageningen, The Netherlands, where the experiment was performed. Half of each soil sample was autoclaved (3 h at 121.5 °C) resulting in three sterilized and three non-sterilized inocula. The inocula were mixed with bulk soil (a sterilized mixture of sandy loam soil and river sand) in a 1 to 4 w:w ratio and homogenized. This resulted in inoculated soils and soils that received a similar but sterilized inoculum as a control for the abiotic effects of the inoculum. Pots with a diameter of 14 cm and a height of 12 cm were filled with 1000 g of this mixture.

Seeds were germinated in sterilized river sand. Two weeks after germination, similar sized seedlings were selected and transplanted into the pots. As the seeds from one of the populations in South Africa (SA 1) did not germinate, these were excluded from the experiment, but the soil from this population was used. The five remaining progenies, one South African, two Spanish and two French, were grown in soils with six different inocula, two Spanish and a South African and their respective sterilized controls. These five progenies and six soils resulted in 30 treatments. Every treatment was replicated five times, in five randomized complete blocks, a total of 150 pots each containing one seedling. Dead seedlings were replaced with similar sized seedlings during the first two weeks after planting. Pots were brought to the same weight, 1150 g (20% soil moisture), by watering twice a week. Plants were grown for 12 weeks in a conditioned greenhouse with day/night temperatures of 25 °C/20 °C for 16/8 h. Plants were moved within blocks twice a week and blocks shifted position every two weeks.

After twelve weeks, plants were harvested and separated into roots, shoots and dead material. Roots were cleaned by rinsing with water, and all parts were dried at 70 °C for at least 48 h before weighing. Total nitrogen and phosphorous content of soil samples were measured (Novozamsky et al., 1984) and available nitrogen, phosphorous and potassium content of soil samples were measured using a 0.01 M CaCl₂ extraction (Houba et al., 1986).

2.3. Data analysis

To calculate the effect of the soil inoculum the relative difference in biomass between plants grown in inoculated soil and plants grown in soil with the same but sterilized inoculum is used. These calculations were made using plants grown in the same block.

Data were analysed using a general linear model (GLM), with origin of the inoculum and progeny as independent factors, including their interaction. One-sample *t*-tests were used to test for significant effects of inoculation. To test if seedling death was randomly distributed over the treatments probability of the found and extremer combinations were calculated. The biomass

of the different progenies at the end of the experiment was compared using ANOVA and a Tukey B *post hoc* test. Potential differences in nutrient concentration between treatments were analysed using GLM (including Tukey B *post hoc* tests) with sterilization and inoculum origin as independent factors. All analyses were carried out using SPSS 12.0.1 (SPSS inc., Chicago, USA).

3. Results

Soil from the native range had a stronger negative influence on biomass than soil from the invaded range ($F_{2,55}=4.105$, $P=0.0054$) irrespective of the location where the progeny was collected ($F_{4,55}=0.561$, $P=0.692$). The effect of inoculation did not differ between progenies ($F_{8,55}=0.973$, $P=0.467$).

Inoculating with South African soil reduced plant biomass production by $31.9\pm 6.7\%$, ($t_{24}=4.737$, $P<0.001$) in comparison with the sterilized South African control. Inoculation with the two Spanish soils did not significantly reduce biomass production in comparison with their respective sterilized controls ($7.1\pm 4.4\%$; $t_{23}=1.620$, $P=0.119$ for Spain 1 and $3.3\pm 10.0\%$; $t_{24}=0.316$, $P=0.754$ for Spain 2) (Fig. 1).

During the experiment five seedlings died. Of these, four were growing in inoculated South African soil and one in sterilized Spanish soil. This distribution differs significantly from random mortality (exact $P<0.01$). The three seedlings that died in the first two weeks of the experiment were replaced; the two that died after two weeks were not replaced.

Plant biomass in sterilized soil did differ between progenies, ($F_{4,65}=5.836$, $P<0.01$). Biomass production of plants of South African and Spanish origin was significantly higher than the French hybrid *C. X cf. acinaciformis* (Tukey B; $\alpha=0.05$) (Fig. 2). Biomass production of the French *C. edulis* was intermediate.

Soil nutrient contents did not differ between the soils with different inocula, except for total phosphorous, which was significantly higher in soils with a South African inoculum (Tukey B, $P<0.05$). Sterilization did not affect any of the

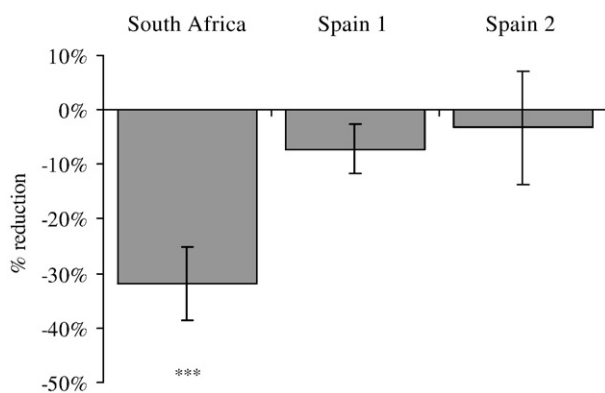


Fig. 1. Average reduction (\pm s.e.) in biomass production of *Carpobrotus edulis* and *C. X cf. acinaciformis* between non-sterilized and sterilized soil for soils of South African and Spanish origin. Values are averaged over all genotypes, genotype itself did not have an effect and did not interact with soil origin ($P>0.05$). (***) indicates difference from 0, $P<0.001$).

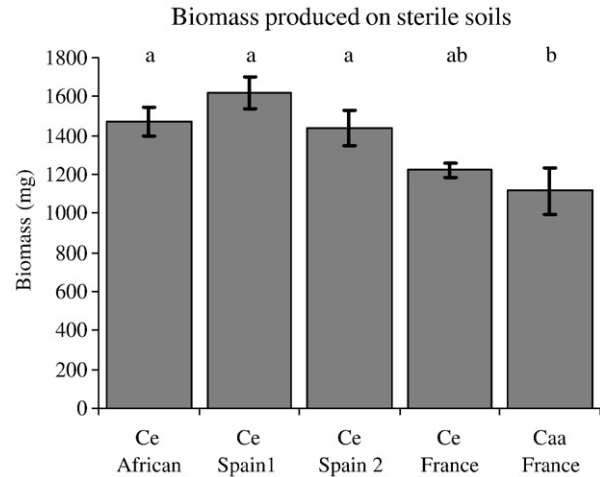


Fig. 2. Average dry biomass (\pm s.e.) produced after 12 weeks for plants of different origin grown in sterilized soils. Letters indicate statistically similar subsets (Tukey HSD $\alpha=0.05$). Ce=*Carpobrotus edulis* (L.) N.E. Br., Caa=*Carpobrotus X cf. acinaciformis* (*C. edulis X C. acinaciformis* (L.) L. Bolus).

measured nutrients, including available and total phosphorous ($P>0.05$).

4. Discussion

Biomass production was strongly reduced when plants were inoculated with soil from the native range while there was no significant effect of inoculation with soil from the invaded range. This supports the hypothesis that *Carpobrotus* is released from soil-borne enemies in the Mediterranean.

The difference between soil from the native and invasive range can be removed by sterilization, this indicates that the African inoculum contains a soil community with a strong net negative effect on plant growth. This can be explained as a stronger pathogenic activity in the African soil, as a result of greater density or stronger virulence of pathogens acting on *Carpobrotus* seedlings. We did not find evidence for the EICA hypothesis in this species. The plants from the invaded range performed equally well as or worse than plants from the native range in sterilized soil. However, considering the small sample size this does not prove absence of EICA. We also did not find any support for hybrid vigour but this conclusion is limited as we have only sampled a single hybrid population.

Differences in the effect of the soil community between native and invaded range can be ecologically significant. Release from soil pathogens is thought to explain the dominance of some invasive plant species (Klironomos, 2002). Soil pathogens have been shown to influence succession and plant competition (Van der Putten et al., 1993; Klironomos, 2002; Kardol et al., 2006). Therefore a release from soil-borne pathogens can partly explain the dominant behavior of *C. edulis* in the invaded range.

Autoclaving of soil is known to release nutrients under some circumstances and this can confound the sterility effect (Troelstra et al., 2001). We used inocula in a sterile bulk soil to dilute this effect and controlled for differences by measuring

nutrient contents in the resulting soils. The soils did not differ in nutrients except for the concentration of total phosphorous, this being higher in soils with the South African inoculum. There were no differences between non-sterilized and sterilized soils, therefore confounding effects as previously mentioned can be excluded.

We acknowledge that no generalizations are possible with the limited number of sampled sites. But as typical sites have been used and soil samples are aggregates collected throughout the populations it is possible that these results are of common occurrence and may represent a general phenomenon.

We conclude that release from growth reducing soil-borne factors, such as soil pathogens, plays an important role in the invasiveness of *C. edulis* in the Mediterranean, while we did not find support for evolution of increased competitive ability.

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