



Variation in defensive traits against herbivores of native and invasive populations of *Carpobrotus edulis*

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Abstract The Enemy Release Hypothesis (ERH) predicts that the escape from natural enemies, such as specialist herbivores, may explain the invasiveness of some invasive alien plants, maximizing their investment in growth and reproduction. This release from natural enemies might decrease the investment in expensive defense mechanisms (i.e., digestibility reducers) against the attack of specialist enemies, whilst increasing the investment in defenses (i.e., cheap toxins) and tolerance against the attack of generalist herbivores, as exposed by the Shifting Defense Hypothesis (SDH). To test this, we conducted a greenhouse experiment to compare morphological and physiological traits of *Carpobrotus edulis*, collected in its native (South Africa) and introduced (Chile and Spain) ranges, attacked by the generalist spittlebug *Philaenus spumarius* and the specialist scale insect *Pulvinariella mesembryantheri*. Our

results do not support the ER and SD hypotheses. We found that *C. edulis* plants collected from native and introduced ranges showed no significant differences in growth and defensive compounds studied for both controls and those plants under attack by the generalist *P. spumarius* (i.e., showing no increase in biomass or changes in biochemical defenses). In contrast, the specialist herbivore *P. mesembryantheri* induced the production of total phenols and tannins and reduced the growth and survival of *C. edulis* plants. Overall, we found strong evidence that *C. edulis* is negatively affected by the attack of its specialist herbivore, but not by that of generalist herbivore, regardless of origin. The observed tolerance to generalist herbivores suggests the intriguing possibility of trait selection, allowing *C. edulis* plants to tolerate generalist herbivores more than specialists.

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Introduction

Plants present a diversity of defense mechanisms against herbivore attacks that can be divided into resistance and/or tolerance (Müller-Schärer et al. 2004). Resistance mechanisms confer plants the ability to prevent or minimize enemy attacks through constitutive and induced defenses (Strauss et al. 2002). Some constitutive defenses (e.g., cellulose, lignin, or tannins) are costly to produce since they are often produced in high concentrations to reduce the general digestibility of the plant (Wittstock and Gershenzon 2002). Some others (e.g., glucosinolates and alkaloids) may be toxic at low concentrations for many generalist herbivores, while adapted specialists are normally resistant to them and can even use them as recognition cues and feeding and oviposition stimulants (Oduor et al. 2011; Wang et al. 2012). Therefore, they aim to prevent the attack on both specialist and generalist herbivores (Müller-Schärer et al. 2004). Conversely, induced defenses (e.g., foliar-derived phenolic compounds) are produced in their response to herbivore damage (Kaplan et al. 2008) and allows plants to activate defenses only when attacked (Karban and Myers 1989). This strategy reduces defense costs and, therefore, optimizes the allocation of resources to growth and reproduction (Herms and Mattson 1992; Karban and Baldwin 1997). On the other hand, tolerance mechanisms allow plants to tolerate the negative impact of herbivore damage through compensatory growth, shifting biomass allocation, increasing photosynthetic rates, or reproduction after damage (Strauss and Agrawal 1999; Day et al. 2016). Tolerance is a non-specific defense trait; therefore, plants can compensate for the damage inflicted by a wide variety of antagonists (Rogers and Siemann 2002).

Humans have introduced non-native plant species to new areas for centuries (Pyšek et al. 2017), resulting in ecological and evolutionary shifts. When plants reach new regions, they are often released from their specialist natural enemies (i.e., Enemy Release Hypothesis; Keane and Crawley 2002). This may lead

to the absence of co-adapted herbivores, and therefore plants might reduce their investment in defense mechanisms, allowing them to maximize their investment in growth and reproduction (Blossey and Nötzold 1995). Consequently, this may affect the diversity of defense chemicals produced by invasive plants in the non-native range due to reduced herbivory pressure (Oduor 2022). Alternatively, the Shifting Defense Hypothesis (SDH) proposes that introduced plants might reduce their energy investment in the costly mechanisms that allow them to resist the attack of specialist herbivores (e.g., decreasing the production of constitutive defenses; Strauss et al. 2002). In contrast, increasing their investment in those mechanisms can allow them to resist and tolerate the attack by generalist herbivores (Bazzaz et al. 1987; Zhang et al. 2018).

Moreover, if any of the evolutionary changes predicted by SDH occur, it is not yet understood what happens when there is an intentional (e.g., biocontrol agents) or unintentional subsequent introduction of specialized natural enemies in the non-native range. Such introduction could potentially reverse evolutionary processes, favoring, again, the production of costly constitutive defenses (Rapo et al. 2010; Wheeler and Schaffner 2013). Over the last decades, many studies have focused on assessing switches in resistance and tolerance mechanisms in alien plants in their introduced ranges. However, only a few studies have evaluated these mechanisms simultaneously (Liu et al. 2004), which has been strongly advocated to advance our understanding of the factors influencing plant invasions (Lieurance and Cipollini 2013; Wang and Feng 2016). Here, we use the invasive plant *Carpobrotus edulis* (L.) N.E.Br. (Aizoaceae) as a case study to test whether *C. edulis* plants invest less in defense mechanisms in introduced than in their native ranges, especially in those areas where the specialist herbivore *Pulvinariella mesembryanthemi* (Vallot, 1829) (Hemiptera: Coccidae) is not present.

Carpobrotus edulis, a perennial succulent plant native to South Africa, is one of the most distributed and successful invasive plants worldwide (Campoy et al. 2018). This mat-forming clonal plant has been introduced to coastal regions in America, Australia, and Europe since the seventeenth century (Sotes et al. 2015; Campoy et al. 2018). In many of these regions, *C. edulis* was deliberately introduced to stabilize coastal dunes, becoming a problematic

invader (Campoy et al. 2018). Little is known about the genetic origin of the different populations and, despite their ability to hybridize, the number of hybrids present in the invaded ranges (Campoy et al. 2018). In introduced ranges such as Europe and North America, some authors recorded that *C. edulis* is attacked by both the generalist *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae) and the specialist enemy *P. mesembryanthemi* (Vallot 1829) (Hemiptera: Coccidae) (Washburn and Frankie 1981; Rodríguez et al. 2019, 2021a; Thompson 2021). *Philaenus spumarius* has a wide distribution along the Palearctic region and it has been introduced in North America, New Zealand, and Hawaii, with a clear presence in temperate coastal areas (Halkka et al. 1967). Therefore, it is not surprising that it also occurs in those areas invaded by *C. edulis*. *Pulvinariella mesembryanthemi* is native to South Africa and specific to the genus *Carpobrotus*. However, it has been unintentionally introduced to areas invaded by *C. edulis*. In the nineteenth century, Vallot (1829) described *P. mesembryanthemi* on cultivated ice plants in southern France, and approximately a century later, some specimens were recorded in the Royal Botanical Garden of Madrid in Spain (Gómez-Menor Ortega 1927). Similarly, the first record of this soft scale in the Americas was in the twentieth century in Argentina (Quintana 1956), and later on, Rojas (1965) mentioned its presence in Chile and it was recorded in California (Washburn and Frankie 1981). Despite its wide distribution, the presence of *P. mesembryanthemi* outside South Africa is limited by climatic conditions, pressure from predators, and low genetic variability (Vieites-Blanco et al. 2020b). In contrast, in its native range, it can be found ubiquitously on *C. edulis* (Vieites-Blanco et al. 2020a; Rodríguez et al. 2020).

Given that the specialist herbivore, *P. mesembryanthemi* is more ubiquitous in the native range of *C. edulis*, we hypothesize that *C. edulis* plants invest less in defense mechanisms in introduced than in their native ranges, especially in those areas where the specialist herbivore *P. mesembryanthemi* is not present. Aiming to test this hypothesis, we compare the growth and physiological activity and constitutive and induced defenses of *C. edulis* plants collected from native and introduced regions after exposing them to generalist (*P. spumarius*), specialist (*P. mesembryanthemi*), and no herbivores. We address

the following questions: Since the specialist herbivore *P. mesembryanthemi* is not widely distributed in the introduced regions, (1) would *C. edulis* plants collected in the non-native ranges present higher biomass and produce less constitutive defenses compared to plants collected in the native range? In the presence of generalist and specialist herbivores, (2) would *C. edulis* plants from introduced regions induce more biochemical defenses against generalist herbivores and less against specialist herbivores compared to plants from the native range? We expected to find an increased performance and decreased investment in defense mechanisms in *C. edulis* plants collected in introduced ranges compared to plants from the native range. Furthermore, we predicted that plants from introduced areas express more resistance and tolerance to the generalist and reduced performance and induction of digestibility reducers (i.e., phenols and tannins) to the specialist herbivore.

Materials and methods

Study species

Carpobrotus edulis has a weak inducible crassulacean acid metabolism (weak CAM-inducible) (Winter 1973; Earnshaw et al. 1987) and a mixed sexual and asexual breeding system (Suehs et al. 2004). It can spread horizontally through clonal propagation by producing internode connections that remain physiologically integrated (Roiloa et al. 2010; Fenollosa et al. 2016). As a result, *C. edulis* can successfully colonize large areas, forming monospecific stands (D'Antonio and Mahall 1991). During the invasion process, *C. edulis* modifies the characteristics of invaded ecosystems by altering plant-soil feedbacks (de la Peña et al. 2010; Novoa et al. 2014, 2020) and plant-herbivore interactions (Rodríguez et al. 2021a), disrupting plant-pollinator networks (Bartomeus et al. 2008) and displacing local flora (Novoa et al. 2012, 2013).

Philaenus spumarius, a cosmopolitan spittlebug insect, is native to the Palearctic region and was introduced in North America, New Zealand and Hawaii (Halkka et al. 1967). *Philaenus spumarius* appears to be univoltine (Halkka and Halkka 1990) and is one of the most polyphagous insects, with over 500 recorded hosts (Weaver and King 1954; Thompson

1994; Yurtsever 2000). The feeding versatility of this spittlebug has allowed it to feed without difficulty on plants with strong chemical defenses (Thompson 2021). In fact, nymphs feed on a wide variety of plants, including *C. edulis* (Rodríguez et al. 2019; Thompson 2021).

Pulvinariella mesembryanthemi, an oligophagous semi-parasitic soft scale, is native to South Africa (Miller and Miller 2003) and was unintentionally introduced in Australia, Europe, and North and South America (Washburn and Frankie 1981; Collins and Scott 1982; Miller and Miller 2003; Kondo and Gullan 2010; Franco et al. 2011). The distribution of *P. mesembryanthemi* coincides with its specific host, *C. edulis* and other species of the genus *Carpobrotus*. *Pulvinariella mesembryanthemi* can reproduce sexually and asexually by parthenogenesis (Washburn and Frankie 1981), and depending on the environmental conditions, it can be univoltine or bivoltine (Washburn and Frankie 1985). Adult males are winged, and females are sedentary, i.e., they have no wings and a shape similar to the larvae (Washburn and Frankie 1985). Its potential value as a biological control agent to manage *C. edulis* invasions has been reported; however, it has only recently been evaluated (Vieites-Blanco et al. 2019). Under greenhouse conditions, *P. mesembryanthemi* produces chlorosis in *C. edulis*

leaves, decreasing its biomass and photosynthetic performance, and can also cause the death of *C. edulis* (Vieites-Blanco et al. 2019; Núñez-González et al. 2021).

Seeds, insects and sand collection

We collected fruits of *C. edulis* from self-sustaining populations occurring in its native (i.e., Western Cape, South Africa), and introduced (i.e., Bio-Bio region, Chile and NW Iberian Peninsula, Spain) ranges during the respective fruiting seasons between January and July 2015, all of them on coastal dunes (Table 1). Although *P. mesembryanthemi* is present in both Chile and Spain, its distribution is very limited and environmental conditions dependent (Vieites-Blanco et al. 2020b). For this experiment, in Chile and Spain, we selected *C. edulis* populations where *P. mesembryanthemi* has not been observed (according to Amouroux et al. 2017; Kondo and Gullan 2010; Rodríguez et al. 2021a; Vieites-Blanco et al. 2020a). We collected one fruit per plant from at least 20 plants 5 m apart to avoid sampling the same clone in each population. We manually removed the seeds from the collected fruits and stored them in sealed plastic bags at 4 °C until the experimental set-up. We had seeds from 11 populations: three populations

Table 1 Locations (according to EPSG:4326–WGS84) and characteristics of the populations where the seeds, insects and sand were collected for the study

Material	Species	Range	Population	Coordinates	Collection date
Seeds	<i>Carpobrotus edulis</i> (L). N.E.Br. (Aizoaceae)	South Africa (native)	Strand	34°06'01"S 18°48'54"E	July 2015
			Kogel Bay	34°13'33"S 18°50'27"E	July 2015
			Hermanus	34°26'00"S 19°12'14"E	July 2015
		Chile (invasive)	Concón	32°54'42"S 71°30'87"W	January 2015
			Desembocadura	36°48'65"S 73°10'92"W	January 2015
			Horcón	32°42'35"S 71°29'50"W	January 2015
			Lenga	36°46'63"S 73°10'24"W	January 2015
		Spain (invasive)	A Lanzada	42°25'57"N 8°52'27"W	June 2015
			Ardia	42°28'16"N 8°51'23"W	June 2015
			Punta de Rons	42°29'55"N 8°52'56"W	June 2015
			Liméns	42°15'32"N 8°48'38"W	June 2015
Insects	<i>Philaenus spumarius</i> (Linnaeus, 1758) (Hemiptera: Aphrophoridae) <i>Pulvinariella mesembryanthemi</i> (Vallot, 1829) (Hemiptera: Coccidae)	Spain (native)	Baiona	42°07'23"N 8°51'06"W	March 2017
		Spain (non-native)	Tal de Abaixo	42°46'57"N 8°59'56"W	June 2016
Sand		Spain	Punta de Rons	42°29'55"N 8°52'56"W	July 2016

from South Africa, four from Chile, and four from Spain (Table 1). Additionally, we collected 50 individuals of the third instar of *P. spumarius* nymphs and 50 individuals of the fourth instar female of *P. mesembryanthemi* in Spain. They were present in a particular population feeding on *C. edulis* (Table 1). We transported the spittlebugs and scales in plastic boxes (i.e., 20 × 18 × 10 cm sized boxes with ten individuals per box) to the greenhouse for acclimatization. Insects were fed with *C. edulis* leaves and maintained for two days before infestation. Finally, we collected sand from a dune system in Spain inhabited by *C. edulis* (Table 1).

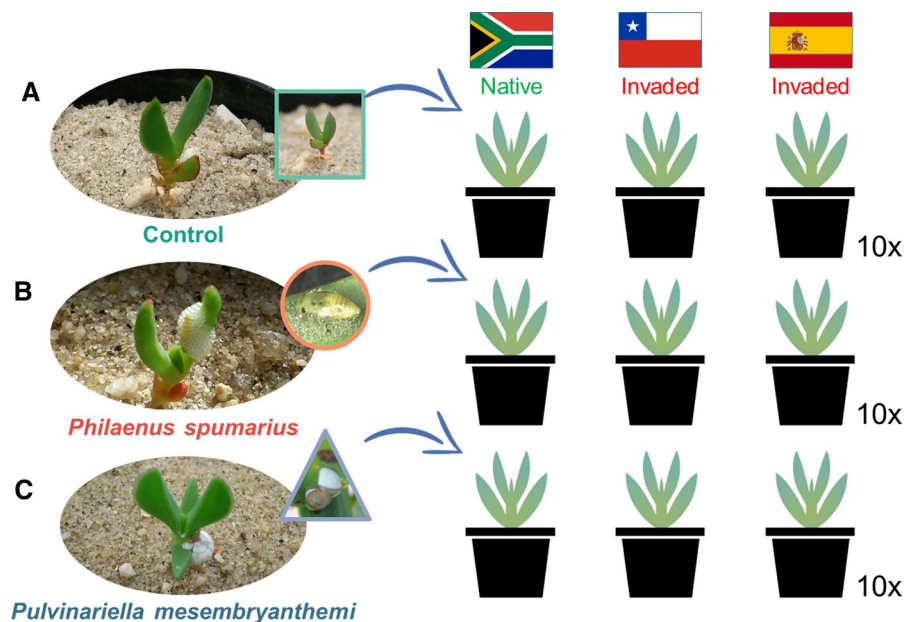
Experimental design

The experiment consisted of two crossed factors with three levels: origin (i.e., South Africa, Chile and Spain) and herbivory (i.e., generalist, specialist and control) (Fig. 1). In January 2017, we germinated seeds from all *C. edulis* populations in germination chambers at the University of Vigo (Spain). The seeds were placed on a thin layer of sand in Petri dishes (i.e., 14 cm diameter, 20 seeds per dish, ten dishes per population). We used periods of 12/12 h of light/dark and 25 °C/15 °C day/night temperatures. We watered each Petri dish with 2 mL of distilled water every two days. After 2 weeks, we randomly selected 90 seedlings per origin of similar size (30

seedlings from each South African population and 22–23 from each non-native population). Seedlings were then transplanted individually into 0.3 L round pots (i.e., 9 cm diameter top and 7 cm depth) filled with sand from the same dune system. Pots with seedlings were grown in a greenhouse at the University of Vigo under a natural photoperiod for three months to reduce the maternal environmental effects.

In April 2017, after three months of seedling establishment, we selected 30 seedlings per origin with similar sizes (i.e., height) and development stages (i.e., number of leaves) (10 seedlings from each South African population and 7–8 from each non-native population, separated in each respectively pots). Pots were labeled by origin (South Africa, Chile and Spain), without reference to the population of origin of the seedlings, and each week their position in the greenhouse was randomized. We then infested ten seedlings per origin with the correspondent herbivory treatment and the experiment continued for 90 days more. Overall, for each origin, we infested ten plants of *C. edulis* with one *P. spumarius* nymph per plant (i.e., generalist) and ten with one *P. mesembryanthemi* individual per plant (i.e., specialist), whilst ten individuals remained without herbivores (i.e., control). No dead insects were found during the experiment, so it was unnecessary to replace them, and neither was parthenogenesis found. The plants were grown in the greenhouse under natural photoperiod and watered

Fig. 1 Schematic of the experimental design including the different treatments for **A** control, **B** *Philaenus spumarius* (i.e., generalist herbivore) and **C** *Pulvinariella mesembryanthemi* (i.e., specialist herbivore) that were infested on invasive *Carpobrotus edulis* from different origins (South Africa, Chile and Spain)



with tap water once per week until harvesting. A plastic anti-insect net (i.e., threads: $20 \times 10 \text{ n}^\circ \text{ cm}$; shading: 20%) was placed around every pot (including controls) to prevent the entry of other herbivores and ensure the presence of each herbivore on the plant. Once a month, we assessed plant survival using a visual rating scale (i.e., 1–0) based on a dichotomous classification, where one indicates a living plant and zero a dead plant. The experiment lasted six months in total, including three months of seedling establishment and three months of insect infestation.

Measurements of plant traits

We measured plant traits of *C. edulis*, including physiological (photochemical efficiency determined by chlorophyll fluorescence), biometrical (total fresh and dry weight, shoot and root fresh and dry weight, number of whorls, leaf length, root length, root/shoot ratio and hydration) and biochemical (phenolic and tannin contents) variables, which allowed us to test the effect of herbivory on plant morphology and physiology for each origin.

We performed fluorescence measurements at a whole-plant level for all individuals one day before harvest using a Maxi Imaging PAM Chlorophyll Fluorescence System (Walz, Effeltrich, Germany). The F_v (variable fluorescence), F_0 (dark fluorescence yield) and F_m (maximal fluorescence yield) parameters were measured after the plants were kept in the dark. We obtained values for photochemical and non-photochemical quantum yields, including the effective quantum yield of the photosystem II (PSII) photochemical reactions (ϕ_{II}), regulated energy dissipation in the form of heat (ϕ_{NPQ}), non-regulated energy dissipation (fluorescence emitted) (ϕ_{NO}), maximum PSII efficiency (F_v/F_m), and the estimated electron transport rate (ETR). Following adaptation to darkness, when all the reaction centers were open, a saturation pulse of light was emitted to obtain the maximum PSII efficiency. The plants were then monitored during a five-minute exposure to actinic light ($110 \mu\text{mol m}^{-2} \text{ s}^{-1}$) to allow photosynthetic activity. Light saturation pulses of $2,700 \mu\text{mol m}^{-2} \text{ s}^{-1}$ were applied at intervals of 20 s to obtain the previously described photochemical and non-photochemical parameters.

At the end of the experiment, we carefully removed all individuals of *C. edulis* from the soil, harvesting

the shoot and root mass separately. We measured the fresh shoot and root weight, number of whorls, leaf length (one leaf per individual taken from the second apical whorl) and root length. We determined the total fresh weight (shoot+root weight) at the whole individual and the proportional weight allocated to roots (calculated as the root/shoot ratio). Hydration (H) was estimated as $H = (FW - DW)/DW$, where FW is the total fresh weight, and DW corresponds to the total dry weight (shoot+root weight) obtained after drying the samples at $60 \text{ }^\circ\text{C}$ until constant weight. During harvest, we cut one fresh leaf per individual (from the second apical whorl), powdered it with liquid N_2 in a mortar and pestle and immediately stored it at $-80 \text{ }^\circ\text{C}$. We then determined the total content in phenols and tannins using the Folin and Ciocalteu method (1927).

Statistical analyses

We conducted Generalized Linear Models (GLM) to test the effect of origin (i.e., South Africa, Chile and Spain) and herbivory (i.e., control, generalist and specialist) and their interaction on the following dependent variables of *C. edulis*: total fresh and dry weight, shoot and root fresh and dry weight, root/shoot ratio, hydration, number of whorls, leaf and root length, total phenols and tannins, F_v/F_m , ϕ_{II} , ϕ_{NPQ} , ϕ_{NO} , and ETR. Data for each dependent variable were checked for homogeneity of variance using Levene's test. Models were run with the most appropriate error family (i.e., Gaussian, gamma and Poisson) and their correspondent link function. The normality of residuals for each model was checked using the Shapiro–Wilk test. We identify and exclude from the results those variables highly correlated and redundant with other traits (i.e., total dry weight, hydration and number of whorls). Statistical differences between groups were analyzed using Tukey's post hoc test by comparing the least-squares means obtained from each GLM in the 'lsmeans' R package (Lenth 2016).

For the survival analysis (see Bewick et al. 2004 for details), we used the non-parametric Kaplan–Meier procedure to estimate the survival function (Kaplan and Meier 1958) and the log-rank test to identify significant differences in survival curves among treatments (Harrington and Fleming 1982). We used the Cox proportional hazards regression model to evaluate the relative effects of origin,

herbivory and their interaction on plant survival (Cox 1972). Survival analyses were conducted using the ‘survival’ R package v. 3.3-1 (Therneau 2022). All statistical analyses were performed using the software program R (R Core Team 2022; v. 4.2.1).

Results

Biometrical measurements

The attack by specialist herbivores significantly altered the performance of *C. edulis*, regardless of its origin (Table 2, Fig. 2; GLM, $p < 0.05$), reducing the shoot and root dry weight and leaf and root length. On average, *C. edulis* collected in Spain had a smaller root length (25% less) than *C. edulis* from South Africa and Chile (Fig. 2). We did not find significant differences in the root/shoot ratio (Table 2).

Biochemical measurements

The interaction between origin and herbivory treatments was statistically significant for both total phenols and tannins (Table 3; GLM, $p < 0.05$). *Carpobrotus edulis* from Chile and Spain, under the attack of specialist herbivores, had higher total phenols (by 2.2- and 2.6-fold higher, respectively) and tannins (2.6- and 3.3-fold higher, respectively) than the controls (Fig. 3). Furthermore, our results showed lower values of total phenols and tannins in the control plants from Spain, although only total phenols were significantly different from those of the other countries (Fig. 3). In contrast, we did not find differences between herbivory treatments of *C. edulis* collected in South Africa, although the average of total phenols tended to be higher for those attacked by specialist herbivores (Fig. 3).

Physiological measurements

All measured physiological parameters (F_v/F_m , ϕII , ϕ_{NPQ} , ϕ_{NO} , and ETR) were significantly different between the herbivory treatments (Table 4; GLM, $p < 0.05$). We also found a significant interaction between origin and herbivory for maximal photochemical efficiency (F_v/F_m). On average, *C. edulis* from Chile and Spain subjected to the attack of specialist herbivores had lower F_v/F_m (about 30% and

Table 2 Results from Generalized Linear Models (GLM) for effects of origin (South Africa, Chile and Spain) and herbivory (control, generalist and specialist) on shoot and root dry weight, root/shoot ratio, leaf and root length of *Carpobrotus edulis*

Biometrical measurements	Df, Res. Df	Deviance	Resid. dev.	$Pr(> Chi)$
<i>Shoot dry weight</i>				
Origin (O)	2, 87	1.351	105.135	0.273
Herbivory (H)	2, 85	49.930	55.205	0.001***
O x H	4, 81	3.638	51.567	0.136
<i>Root dry weight</i>				
Origin (O)	2, 87	3.224	129.715	0.066
Herbivory (H)	2, 85	74.604	55.111	0.001***
O x H	4, 81	1.716	53.394	0.576
<i>Leaf length</i>				
Origin (O)	2, 87	2.401	91.502	0.081
Herbivory (H)	2, 85	49.730	41.773	0.001***
O x H	4, 81	3.032	38.741	0.175
<i>Root length</i>				
Origin (O)	2, 87	1.379	38.368	0.003**
Herbivory (H)	2, 85	27.540	10.827	0.001***
O x H	4, 81	0.120	10.707	0.909
<i>Root/shoot ratio</i>				
Origin (O)	2, 87	0.797	39.854	0.409
Herbivory (H)	2, 85	1.629	38.225	0.161
O x H	4, 81	3.109	35.116	0.138

Models were carried out with the error family gamma and correspondent link function (except for leaf length: Gaussian). Df Degrees of freedom, Res. Df Residual degrees of freedom, Resid. Dev. Residual deviance

Asterisks indicate statistical differences **, $p < 0.01$; ***, $p < 0.001$. $n = 10$

15% less, respectively) than individuals subjected to generalist herbivores as the controls. In contrast, plants from South Africa had higher photochemical efficiency when attacked by specialized herbivores (Fig. 4). Our results also showed that ϕII and ETR were significantly lower for *C. edulis* individuals attacked by the specialist herbivore (about 26% and 15% less, respectively) than those attacked by the generalist herbivore the control (Fig. 4). Our models also indicated that the attack by the specialist

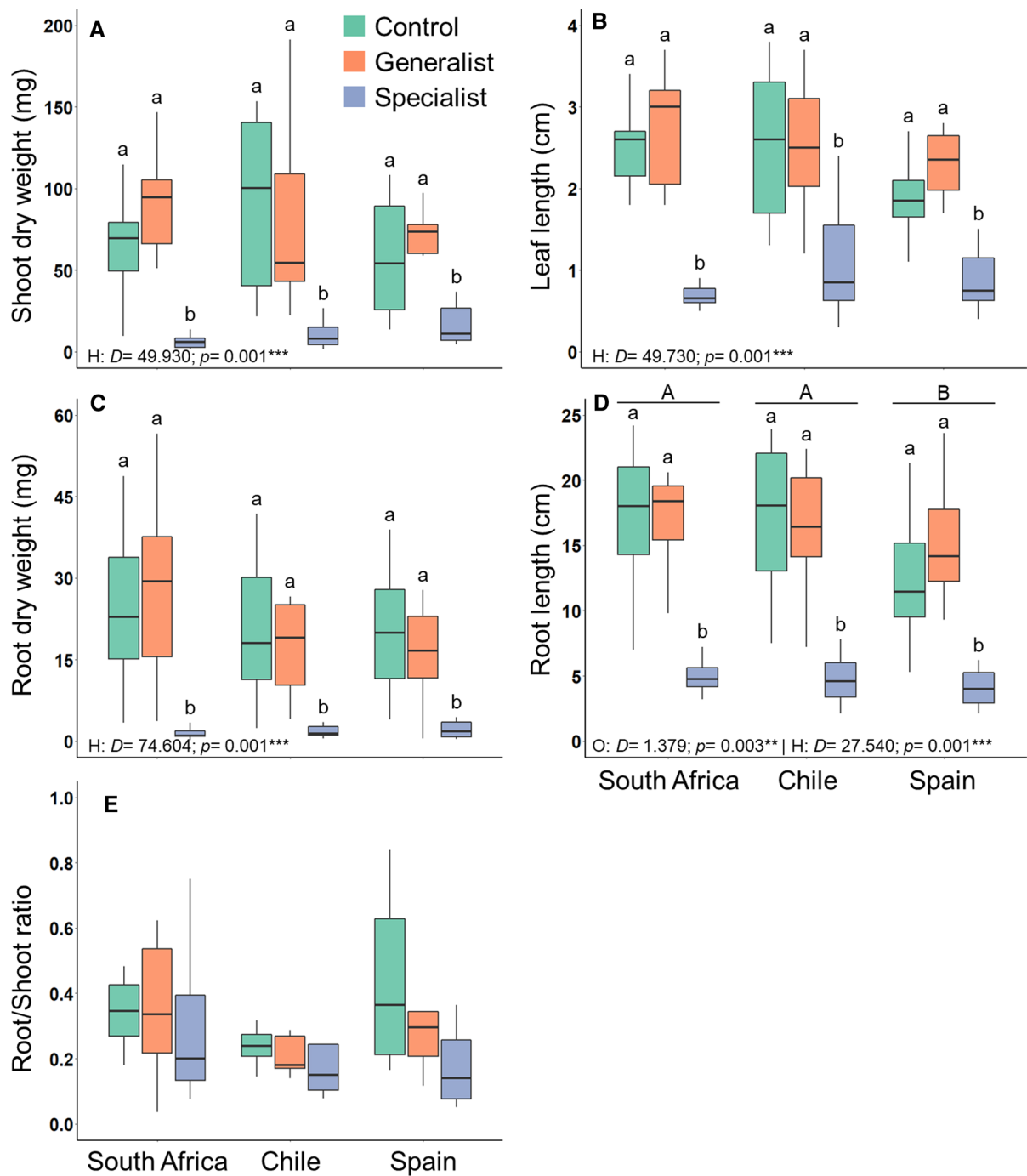


Fig. 2 Boxplots illustrating the differences for the effects of origin (O) (South Africa, Chile and Spain) and herbivory (H) (control, generalist and specialist) on biometrical measurements of *Carpobrotus edulis* (i.e., shoot dry weight (A), leaf length (B), root dry weight (C), root length (D), and root/shoot

ratio (E)). Different letters indicate statistically significant differences at $p \leq 0.05$ level using Generalized Linear Models followed by a Tukey's post hoc test, $n=10$. Refer to statistics in Table 2

Table 3 Results from Generalized Linear Models (GLM) for effects of origin (South Africa, Chile, and Spain) and herbivory (control, generalist, and specialist) on total phenols andtannins of *Carpobrotus edulis*. Models were carried out with the most appropriate error (gamma and Gaussian, respectively) and correspondent link function

Biochemical measurements	Total phenols				Tannins			
	Df, Res. Df	Deviance	Resid. dev.	<i>Pr(> Chi)</i>	Df, res. Df	Deviance	Resid. dev.	<i>Pr(> Chi)</i>
Origin (O)	2, 60	0.785	8.091	0.001***	2, 60	133.13	1806.54	0.001***
Herbivory (H)	2, 58	5.692	2.399	0.001***	2, 58	1024.11	782.43	0.001***
O x H	4, 54	0.981	1.418	0.001***	4, 54	281.62	500.81	0.001***

Df Degrees of freedom, *Res. Df* Residual degrees of freedom, *Resid. Dev.* Residual deviance

Asterisks indicate statistical differences **: $p < 0.01$; ***, $p < 0.001$. $n \leq 7$

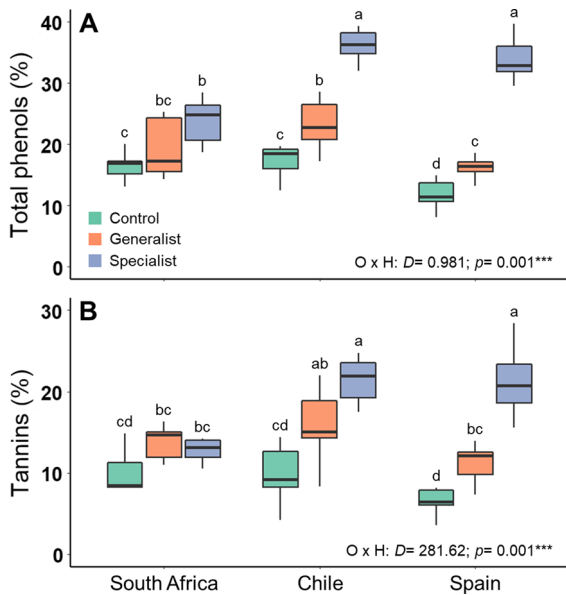


Fig. 3 Boxplots depicting the differences for the effects of origin (O) (South Africa, Chile and Spain) and herbivory (H) (control, generalist and specialist) on total phenols and tannins of *Carpobrotus edulis*. Different letters indicate statistically significant differences at $p \leq 0.05$ level using Generalized Linear Models followed by a Tukey's post hoc test, $n \leq 7$. Refer to statistics in Table 3

herbivore induced a higher ϕ_{NPQ} and ϕ_{NO} (about 24% and 28% more, respectively) compared to individuals that were not subjected to herbivore attack (i.e., the controls) (Fig. 4).

Survival

At the end of the experiment, regardless of plant origin, the survival of individuals under the attack of specialist herbivores was reduced by 70%

(Kaplan–Meier survival probability). In contrast, no plant mortality was observed for control individuals or individuals attacked by the generalist herbivore. The log-rank test revealed that the survival rates of *C. edulis* had a different response to herbivory ($\chi^2 = 78.2$, $df = 2$, $p < 0.001$). Moreover, the origin was not significant ($p = 1.000$), and there was no interaction between factors origin and herbivory. Cox proportional hazard regression only detected a herbivory effect (Wald = 9.97, $df = 2$, $p = 0.007$) (i.e., specialist herbivores significantly reduced the survival of *C. edulis*).

Discussion

Despite our initial expectations, our results did not support the ER and SD hypotheses. These hypotheses propose that non-native plant populations exhibit an increased growth and/or a decreased investment in defenses to the attack of specialist herbivores compared to plants from native ranges (Keane and Crawley 2002; Doorduyn and Vrieling 2011). Therefore, it is expected that those plants that grow fast in their native range may grow even faster in introduced distributions (Montesinos 2021). However, our control plants (i.e., plants not exposed to herbivores) showed similar growth, physiological activity and constitutive defenses between origins, except for a lower phenol content in the *C. edulis* plants from Spain (Fig. 3). From a biological point of view, the differences found in total phenol content between the controls are very subtle and a plausible explanation for our outcome can be attributable to the high phenotypic plasticity of *C. edulis* (Fenollosa et al. 2017; Rodríguez et al. 2021b). Furthermore, although all seeds were

Table 4 Results from Generalized Linear Models (GLM) for effects of origin (South Africa, Chile and Spain) and herbivory (control, generalist and specialist) on F_v/F_m , ϕII , ϕ_{NPQ} , ϕ_{NO} , and ETR of *Carpobrotus edulis*

Models were carried out with the error family Gaussian and link function = identity

Df Degrees of freedom, *Res. Df* Residual degrees of freedom, *Resid. Dev.* Residual deviance

Asterisks indicate statistical differences *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. $n \leq 10$

Fluorescence parameters	Df, Resid. Df	Deviance	Resid. dev.	$Pr(> Chi)$
F_v/F_m				
Origin (O)	2, 66	0.014	0287	0.006**
Herbivory (H)	2, 64	0.170	0.117	0.001***
O x H	4, 60	0.035	0.082	0.001***
ϕII				
Origin (O)	2, 66	0.023	0.406	0.069
Herbivory (H)	2, 64	0.116	0.290	0.001***
O x H	4, 60	0.034	0.256	0.092
ϕ_{NPQ}				
Origin (O)	2, 66	0.016	0.417	0.271
Herbivory (H)	2, 64	0.037	0.380	0.046*
O x H	4, 60	0.014	0.366	0.678
ϕ_{NO}				
Origin (O)	2, 66	0.001	0.096	0.393
Herbivory (H)	2, 64	0.039	0.056	0.001***
O x H	4, 60	0.007	0.049	0.058
ETR				
Origin (O)	2, 66	31.985	772.77	0.238
Herbivory (H)	2, 64	89.954	682.82	0.017*
O x H	4, 60	14.249	668.57	0.865

collected from coastal dunes populations and selected seedlings had similar size and development stage and were grown in a greenhouse under a natural photoperiod for three months to reduce maternal effects, obtaining seeds from plants growing naturally in the field could influence our observations (Hierro et al. 2022).

It is noteworthy that we found strong evidence to suggest that the attack of specialist herbivore *P. mesembryanthemi* affected the performance of all plants in a similar manner, regardless of their origin (Figs. 2 and 4). When *C. edulis* plants were exposed to the attack of *P. mesembryanthemi*, they all showed a reduction on the shoot and root dry weight, leaf and root length and survival rates, and a similar modification on their photosynthetic parameters. A plausible mechanism to explain this result when comparing two herbivores with the same feeding habits (i.e., *P. spumarius* and *P. mesembryanthemi*) could be the hypersensitive response of *C. edulis* due to the attack of the specialist herbivore (Radville et al. 2011). If this is the case, the plant could limit the spread of the detected damage and isolate the specialist herbivore, producing tissue necrosis and mortality. Although in our results these damages were not apparent, future

studies should explore this assumption to confirm whether this result could be compatible with a hypersensitivity response (Fernandes 1990; Fernandes and Negreiros 2001). Under the attack of *P. mesembryanthemi*, *C. edulis* plants reduced the efficiency of the photosystem II (ϕII) and ETR and increased the dissipation of regulated energy in the form of heat (ϕ_{NPQ}) and the non-regulated energy (ϕ_{NO}). In other words, *C. edulis* plants responded to the attack of their specialist herbivore by activating their photoprotective mechanisms, where some of the light energy captured by photosystem II was dissipated in the form of heat. This could promote the production of harmful by-products for the plant, which could inhibit photosynthesis (Kramer et al. 2004). Moreover, when exposed to the attack of their specialist enemy, *C. edulis* plants decreased their photochemical efficiency (F_v/F_m). This suggests that *C. edulis* individuals attacked by this natural enemy were defending themselves from the stress they are undergoing (Kramer et al. 2004), contributing to chlorophyll degradation and reducing plant growth, as previous studies also found (Vieites-Blanco et al. 2019; Núñez-González et al. 2021).

Despite similarities in biometric measurements and survival of *C. edulis* plants from different origins

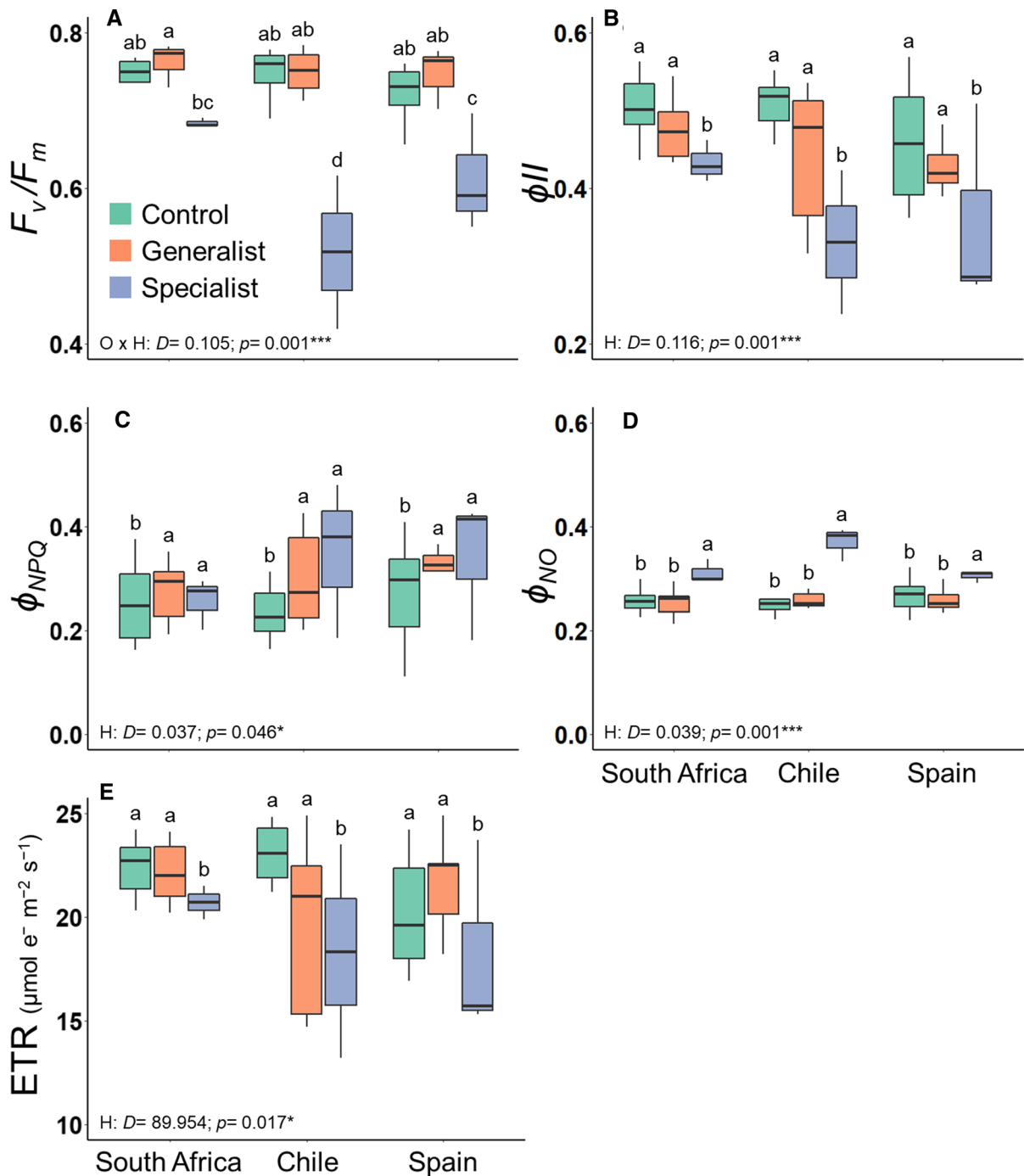


Fig. 4 Boxplots illustrating the differences for the effects of origin (O) (South Africa, Chile and Spain) and herbivory (H) (control, generalist and specialist) on fluorescence parameters of *Carobrotus edulis* (i.e., F_v/F_m , ϕ_{II} , ϕ_{NPQ} , ϕ_{NO} , and ETR).

Different letters indicate statistically significant differences at $p \leq 0.05$ level using Generalized Linear Models followed by a Tukey's post hoc test, $n \leq 10$. Refer to statistics in Table 4

when infested by the specialist herbivore *P. mesembryanthemi*, our results show differences between countries in the production of induced defenses of those plants exposed to the attack of this herbivore (Fig. 3). When plants from non-native populations (i.e., Chile and Spain) were attacked by *P. mesembryanthemi*, they produced a higher amount of phenols and tannins than those not exposed to herbivore or exposed to attack by the generalist herbivore *P. spumarius*. This trend was not observed in *C. edulis* plants from native populations (i.e., South Africa). This is probably because the host plant and its natural enemy have remained in constant coevolution, resulting in reduced growth due to attack but no increase in chemical defenses (Zhang et al. 2018). In our study system, this would mean that, under the attack of *P. mesembryanthemi*, *C. edulis* would shift its defense mechanisms by producing costly digestibility reducers while spending less energy on growth. This suggests that if *C. edulis* allocates more resources to defense mechanisms when attacked by *P. mesembryanthemi*, the plant will have a low growth rate (Huang et al. 2010; Züst and Agrawal 2017). These results concur with those obtained by Wan et al. (2019), who reported that when individuals of *Ambrosia artimisiifolia* L. from non-native populations were attacked by a specialist herbivore, they showed a higher defensive response. In that study, Wan et al. observed that enemy reassociation with a specialist herbivore can increase induced defense to specialists and reduce growth rates in the introduced range. Consequently, this may result in lower tolerance to specialists in the introduced genotypes (Lin et al. 2015; Liu et al. 2004). However, increased chemical defenses in individuals of the non-native range does not necessarily imply a greater diversity of chemical defense compounds (Oduor 2022).

Compensatory growth may play an important role in facilitating plant invasions (Ashton and Lerda 2008). Previous studies found that the attack of the generalist snail *Theba pisana* produced an increase in the biomass of *C. edulis* plants from the Iberian Peninsula (Rodríguez et al. 2018, 2021b). In contrast, our results do not support this for plants attacked by the generalist *P. spumarius* (Fig. 2). *Carpobrotus edulis* plants attacked by the generalist *P. spumarius* showed similar growth, survival rates, and photosynthetic capacity than those free from herbivores (i.e., controls), despite dissipating more light energy as heat (i.e., higher ϕ_{NPQ}). This result was independent of

origin (Fig. 4). A possible explanation is the presence of additional natural enemies (i.e., spittlebug species; see Rodríguez et al. 2020 for details) in the native range that are phylogenetically close to the generalist *P. spumarius* that *C. edulis* can tolerate. The higher ϕ_{NPQ} seen in plants attacked by *P. spumarius* could be explained because herbivore-damaged plants may use a higher proportion of absorbed light for photosynthesis due to the altered carbohydrate source-sink relationships caused by the attack of herbivores (Thomson et al. 2003). This energy-use efficiency, coupled with lower biomass construction costs, may facilitate its growth despite herbivore damage, as has been shown for other invasive plants (Ni et al. 2020). Consequently, tolerance to herbivores and invasion success in the introduced range may depend on the analogy of native generalist herbivores to those from its home range (Harvey et al. 2013) and the different defense strategies of invasive plants (Huang et al. 2010).

Pulvinariella mesembryanthemi is known to be present on areas invaded by *C. edulis* in many introduced regions (Vieites-Blanco et al. 2020b), where it could be used as a biocontrol agent (i.e., enemy reassociation; Wan et al. 2019). Based on our results, the use of *P. mesembryanthemi* as biocontrol of *C. edulis* under controlled conditions appears successful. This agrees with previous studies that reported the potential of *P. mesembryanthemi* as a biological control agent (Washburn and Frankie 1985; Vieites-Blanco et al. 2019; Núñez-González et al. 2021). Our results show strong evidence that the performance and survival of *C. edulis* is compromised by the attack of *P. mesembryanthemi*, suggesting that it could be a successful biocontrol. However, under natural conditions, defense levels of non-native populations could be recovered in a few years once the native specialist is present in the introduced range (Fukano and Yahara 2012; Sakata et al. 2014). And, the subsequent introduction of natural specialist enemies could reverse evolutionary processes (Rapo et al. 2010; Wheeler and Schaffner 2013). But this is yet to be studied for the different invasive species of the genus *Carpobrotus*.

Conclusions

Our results do not support the ER and SD hypotheses. Conversely, we can highlight that, regardless of

its origin, the performance and survival of the clonal invader *C. edulis* is significantly harmed by the attack of its specialist enemy, *P. mesembryanthemi*. This experiment cannot determine whether initial enemy release or reassociation has occurred, but currently, there is no evidence that origin affects the performance or susceptibility to generalist or specialist herbivores. Although more exploration of the defense mechanisms responsible for the invasion success of *C. edulis* is necessary, the present study provides evidence that the attack by the generalist *P. spumarius* do not differentially affect the growth observed in *C. edulis*. This suggests the intriguing possibility that the phenotypic plasticity of this plant favor it under stress conditions when attacked by generalist herbivores. Further research is needed to unravel the role of enemies reassociation in the levels of resistance and tolerance of *C. edulis* plants. Studies adding more details on defense mechanisms and increased competitive ability (for example, testing the evolution of increased competitive ability (EICA) hypothesis; Blossey and Notzold 1995; Oduor et al. 2017) would also be needed, as well as field experiments to confirm that we are not underestimating the shifts in defense mechanisms of the invasive *C. edulis* in its introduced range.

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Author contributions JR designed the methodology and led the writing of the manuscript; JR and GS conceived the ideas; JR, AN, GS, AP and LG collected the seeds, and JR the insects; JR performed the experiment, collected, and analyzed the data. All authors contributed to the drafts, agreed on the content and gave final approval for publication.

Availability of data and materials The datasets generated during and/or analyzed during the current study are available in the FigShare repository. <https://doi.org/10.6084/m9.figshare.14604486.v1>

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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