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Evidence for enemy release and increased seed production and size for two invasive Australian acacias

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Summary

1. Invasive plants are hypothesized to have higher fitness in introduced areas due to their release from pathogens and herbivores and the relocation of resources to reproduction. However, few studies have tested this hypothesis in native and introduced regions. A biogeographical approach is fundamental to understanding the mechanisms involved in plant invasions and to detect rapid evolutionary changes in the introduced area.

2. Reproduction was assessed in native and introduced ranges of two invasive Australian woody legumes, *Acacia dealbata* and *A. longifolia*. Seed production, pre-dispersal seed predation, seed and elaiosome size and seedling size were assessed in 7–10 populations from both ranges, taking into account the effect of differences in climate.

3. There was a significantly higher percentage of fully developed seeds per pod, a lower proportion of aborted seeds and the absence of pre-dispersal predation in the introduced range for both *Acacia* species. *Acacia longifolia* produced more seeds per pod in the invaded range, whereas *A. dealbata* produced more seeds per tree in the invaded range. Seeds were bigger in the invaded range for both species, and elaiosome: seed ratio was smaller for *A. longifolia* in the invaded range. Seedlings were also larger in the invaded range, suggesting that the increase in seed size results into greater offspring growth.

4. There were no differences in the climatic conditions of sites occupied by *A. longifolia* in both regions. Minimum temperature was higher in Portuguese *A. dealbata* populations, but this difference did not explain the increase in seed production and seed size in the introduced range. It did have, however, a positive effect on the number of pods per tree.

5. *Synthesis*. *Acacia dealbata* and *A. longifolia* escape pre-dispersal predation in the introduced range and display a higher production of fully developed seeds per fruit and bigger seeds. These differences may explain the invasion of both species because they result in an increased seedling growth and the production of abundant soil seedbanks in the introduced area.

Key-words: *Acacia dealbata*, *Acacia longifolia*, alien species, elaiosome, enemy release, invasive plants, legume, reproduction, seed size

Introduction

The size and number of seeds produced and their dispersal are critical factors in a species' ability to establish and invade new ranges (Leishman *et al.* 2000). Once established, plants in invasive populations often produce a higher number of viable seeds than plants in the native range (Mason *et al.* 2008; Herrera, Raymond & Nicholas 2011). Higher reproductive output might be explained by release from pathogens and herbivores in the introduced region that leads to a higher availability of resources for growth and reproduction and specifically by release from seed predators (Blossey & Nötzold 1995; Keane & Crawley 2002; Wandrag *et al.* 2013). As seed size is highly correlated with maternal and offspring fitness (Harper, Lovell & Moore 1970), changes in seed mass over time during invasion may potentially enhance the competitiveness of introduced species and contribute to

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invasiveness. Overall, there seems to be a trend for seed mass to be higher in the introduced range of exotic species than in their native range (Daws *et al.* 2007), although exceptions do occur (Mason *et al.* 2008). Recent studies have showed differences in seed mass between ranges for both herbaceous (*Centaurea solstitialis*, Graebner, Callaway & Montesinos 2012 and Hierro *et al.* 2013; *Echium plantagineum*, Konarzewski, Murray & Godfree 2012; *Lupinus polyphillus*, Sõber & Ramula 2013) and woody species (*Cytisus scoparius*, Buckley *et al.* 2003).

Australian *Acacia* species (Fabaceae, Mimosoideae, subgen. Phyllodineae (DC.) Ser) exemplify invaders, due to their spread and persistence in different ecosystems world-wide (Stock, Wienand & Baker 1995; Richardson & Rejmánek 2011). *Acacia* species produce large quantities of hard-coated, heat-tolerant and long-lived seeds. The seeds have long dormancy and are able to form extensive and persistent soil seedbanks (Richardson & Kluge 2008; Marchante, Freitas & Hoffmann 2010), and their germination is stimulated by heat and/or smoke (Auld 1986 and references therein; Kulkarni, Sparg & Van Staden 2007). All these characteristics contribute to the persistence of Australian *Acacia* in the invaded ranges (Milton & Hall 1981; Richardson & Kluge 2008).

Invasiveness may be enhanced by greater seed output or seed size in the introduced range because these differences can speed up naturalization and spread. But it is unknown whether seed production and seed traits differ between populations of native and non-native *Acacia* species. A biogeographical approach is fundamental to understanding the mechanisms that are involved in plant invasions (Hierro, Maron & Callaway 2005); however, few studies document differences between *Acacia* species in native and introduced ranges (Birnbaum *et al.* 2012 and Crisóstomo, Rodríguez-Echeverría & Freitas 2013 for rhizobial symbionts; Wandrag *et al.* 2013 for seed dispersal and predation; Harris *et al.* 2012 for genetic variability; and Birnbaum *et al.* 2014 for root fungi). Thus, this study aims to compare seed production, seed traits and early seedling size of two highly invasive Australian *Acacia* species, *A. dealbata* Link. and *A. longifolia* (Andr.) Willd. Seed production, pre-dispersal seed predation, seed and elaiosome mass, and seedling size were assessed both in native Australian and in non-native Portuguese ranges. Specifically, we asked whether there are biogeographical differences between these regions and whether these differences explain the degree of invasion for *A. dealbata* and *A. longifolia* in their non-native ranges.

Materials and methods

PLANT SPECIES

Acacia dealbata and *A. longifolia* are the most widespread Australian *Acacia* species growing in Portugal. These species were intentionally introduced in Portugal during the first half of the 20th century for forestry, soil stabilization and gardening (Castroviejo *et al.* 1999). In its native range, *A. dealbata* occurs on the tablelands and western slopes of New South Wales, Victoria and eastern Tasmania (Maslin 2001; May & Attiwill 2003). It occupies areas under oceanic climate with annual rainfall over 500 mm and usually at altitudes from 350 to 1000 m above sea level (May & Attiwill 2003). *Acacia longifolia* is native to south-eastern Australia (Orchard & Wilson 2001) and generally occurs at locations with more than 550 mm of annual rainfall (Muyt 2001) in coastal and forested areas. Two subspecies are recognized in Australia (Whibley 1980): the coastal subspecies, *sophorae* and a more forest/woodland subspecies, *longifolia*, both of which may occur in Portugal. *Acacia longifolia* subsp. *longifolia* usually occurs more towards the hinterlands of coastal districts and is generally replaced by *Acacia longifolia* subsp. *sophorae* nearer to the sea. However, in some localities, the two subspecies appear to grade spatially into one another. While we largely sampled from the coastal subspecies, some samples in Australia were from the woodland subspecies. Both *Acacia* species can be found in all regions of mainland Portugal. *Acacia dealbata* grows mainly in mountain ranges and roadsides, and *Acacia longifolia* occurs mainly in coastal sand dunes from central and northern Portugal. The *Acacia longifolia* habit may vary from a spreading shrub to small tree, whereas *Acacia dealbata* occurs more frequently as a tree. The flowering and fruiting phenology in both native and invaded ranges of both species is provided in Supporting Information, Appendix S1, Supporting Information.

STUDY SITES AND SAMPLING DESIGN

This study was performed in a total of 34 populations from the native range in Australia and the invaded range in Portugal. Sampling was done during the peak of the fruiting season in each region. In Australia, sampling was conducted in New South Wales (NSW), Victoria (VIC) and Canberra (ACT) during December 2012. Sampling in Portugal covered the entire country during July 2013. In both countries, we carried out a north–south transect along the coast to sample ten populations of *A. longifolia* and a north–south transect inland to sample seven populations of *A. dealbata* (Table 1). Populations more than 20 km apart had no history of disturbance in the past 15–20 years and no sign of anthropogenic disturbance or strong damage by herbivores. In each population, ten healthy-looking individual trees (1.5–3 m high for *A. longifolia* and 3–5 m high for *A. dealbata*) were selected at least 5 m apart to avoid sampling closely related individuals.

To characterize the estimated reproductive output (seed production per tree), the total number of pods was counted along three branches selected at random in three different equidistant positions around the canopy of each plant. Sampled branches were at heights of about 1–1.5 m for *A. longifolia* and 1.8 m for *A. dealbata*. Along each sampled branch, we counted the number of fully developed, aborted or preyed upon seeds per pod, in three unopened pods. Estimated reproductive output was calculated by multiplying the average number of fully developed seeds per pod per tree by the estimated number of pods per tree. The number of branches per plant was estimated by counting all branches with pods in one-quarter of the canopy.

Up to forty diaspores (seeds plus elaiosome) were collected from each plant in each population and stored in paper bags under cool dry conditions. Diaspores were dried at 50 °C for 48 h before being weighed. Thirty healthy-looking diaspores per tree (i.e. no holes in testa, no discoloration) were individually weighed to the nearest milligram with and without elaiosome to estimate the allocation to total seed mass and elaiosome mass, respectively, from which elaiosome–seed mass ratio (ESM) for each species in each region was calculated.

Table 1. Populations of *A. dealbata* and *A. longifolia* studied in Australia and Portugal and respective climatic data for the 12 months previous to sampling

Populations				Coordinates	Elevation (m)	Precipitation (mm month^{-1})	T_{max} (°C)	T_{min} (°C)
Acacia dealbata	Australia	Wangaratta, VIC	WR	−36.355608, 146.347556	151	861.4	21.9	7
		Chiltern-Mount Pilot National Park, VIC	MP	−36.251967, 146.671194	527	877.6	22.4	8.4
		Point Hut – Picnic Area, ACT	PH	−35.452607, 149.035300	665	905.8	20.0	6.0
		Discovery Drive – ACT	DH	−35.407106, 148.983884	649	898.0	20.0	6.0
		Murraguldrie State Forest, NSW	UM	−35.471492, 147.638839	460	820.4	22.2	8.2
		Dalton Rd – Gunning, NSW	GN	−34.745315, 149.194470	567	826.5	21.6	5.5
		Lake George – Remembrance Driveway, NSW	LG	−35.003507, 149.380664	701	868.7	19.2	5.3
	Portugal	Parque Nacional Peneda-Gerês	PG	41.739139, −8.156389	503	1140.3	17.2	8.1
		Caldas da Felgueira, Viseu	F	40.485956, −7.869428	250	948.3	19.8	9.9
		Albergaria a Velha, Aveiro	AV	40.679919, −8.467778	106	1062.2	20.2	11.2
		Serra da Lousã, Coimbra	L	40.102567, −8.233697	392	944.9	20.0	10.7
		Pinhal, Coimbra	PC	40.208969, −8.400917	79	980.3	20.7	11.6
		São Luís, Alentejo	SL	37.669017, −8.654328	143	650.5	21.7	13.2
		Serra de Monchique, Algarve	M	37.289633, −8.554669	271	612.9	21.1	13.1
Acacia longifolia	Australia	Puckeys Estate Reserve, NSW	PK	−34.398344, 150.90360	10	1255.2	20.9	14
		Shellharbour, NSW	SH	−34.585590, 150.87310	62	658	22.1	11
		Gumley Reserve, NSW	GH	−34.850500, 150.74924	8	1029.2	21.9	10.7
		Jervis Bay, NSW	JB	−35.135539, 150.75571	45	906	20.1	13.5
		Zane Grey Tourist Park, Bermagui, NSW	BG	−36.429550, 150.08331	17	1057.8	19.7	12.8
		Tathra Beach, NSW	TH	−36.711830, 149.97722	7	1062	20.6	9.6
		Eden, NSW	ED	−37.056310, 149.91152	9	986.1	20.6	9.6
		Mallacoota, VIC	ML	−37.606030, 149.72130	27	1201.4	19.2	10.9
		Lind National Park, VIC	LD	−37.575410, 149.02150	256	1180.7	17.8	8.8
		Woodside Beach, VIC	WD	−38.548220, 146.97706	7	773.8	19.5	8.5
	Portugal	Moledo, Caminha	MO	41.853139, −8.865444	10	1554.3	18.1	10.1
		Parque Natural do Litoral Norte, Apúlia	AP	41.503353, −8.785197	4	1403.2	19.6	11.1
		Torreira, Estarreja	TR	40.766483, −8.699694	13	1221.4	20.8	11.9
		Tocha, Cantanhede	TO	40.326308, −8.795967	41	1093.8	21.1	12.2
		Pinhal, Coimbra	PC	40.208969, −8.400917	79	980.3	20.7	11.6
		São Pedro de Moel, Marinha Grande	SPM	39.763478, −9.023911	41	905.8	20.9	12.3
		Costa da Caparica, Lisboa	CP	38.617264, −9.214975	8	792.2	20.9	13.2
		Lagoas de Santo André, Alentejo	SA	38.028203, −8.819600	27	758.5	21.8	12.8
		Porto Covo, Alentejo	EPC	37.879594, −8.745447	106	748.7	21.8	12.9
		Bensafrim, Algarve	BF	37.163497, −8.743456	53	725.6	21.3	13.8

Six seeds from each plant in each population were randomly selected from the pool of seeds collected and used to analyse differences in seedling growth between plants from each range in an experiment conducted in a glasshouse at the Botanical Garden of University of Coimbra (Portugal). To induce germination, seeds of *A. longifolia* were mechanically scarified and seeds of *A. dealbata* were soaked in hot water and left overnight. Each seed was sown in a 125-cm^3 pots filled with a mixture (1:1 vol) of grit sand and commercial soil. Pots were placed in a randomized design in the glasshouse. Plants were watered every 3 days and kept for 12 weeks under natural photoperiod and at average temperature of 16.6 °C (T_{min} 4 °C – T_{max} 24 °C). Plants were then harvested, dried at 50 °C for 48 h, and weighed to the nearest mg. Mean seedling weight was calculated for each species and population.

Data on monthly mean maximum and minimum temperatures and rainfall for each population were obtained from KNMI Climate Explorer (http://climexp.knmi.nl) and Bureau of Meteorology of Australian Government (http://www.bom.gov.au/). Data were obtained for the last 30 years to describe the climate for each population (see Appendix S2) and for the 12 months before sampling to analyse the effect of climatic conditions on pod and seed production (Table 1).

DATA ANALYSES

Differences in mean minimum temperature (T_{min}), maximum temperature (T_{max}) and precipitation between Australia and Portugal for values in the previous year before seed collection were tested with a Welch *t*-test.

For each species, generalized linear mixed models were used to test the effect of range (and climatic data for *A. dealbata*) on the total number of seeds per pod and the proportion of seeds viable, aborted and preyed upon using individual nested within population as a random factor. The total number of seeds per pod was analysed using a Poisson distribution and a log link function. The proportion of viable and aborted seeds was analysed using a binomial distribution and a log link function. The proportion of seeds preyed upon was analysed using a zero-inflated Poisson and a log link function.

Generalized mixed models were used to test the effect of region (and climatic data for *A. dealbata*) on number of pods per branch, number of pods per tree and the reproductive output. The number of pods per branch was analysed using a Poisson distribution, a log link function and tree as a random factor. The number of pods per tree was analysed using a Poisson distribution, a log link function and population as a random factor. The estimated reproductive output was analysed using a negative binomial distribution, log link function.

Linear mixed models were used to test the effect of region (and climatic data for *A. dealbata*) on seed and elaiosome weight, elaiosome–seed mass ratio and seedling weight using tree nested within population as a random factor.

For *A. dealbata,* GLMM and LMM models using region and minimum temperature as fixed factors for all response variables were compared using AICc values.

All analyses were conducted separately for each plant species using the packages lme4 (Bates *et al.* 2013), MuMIn (Barton 2013), pscl (Jackman 2015) and MASS (Venables & Ripley 2002) in R v 3.0.2. (R Core team 2013). Different models for fixed factors were compared using the AIC value.

Results

Overall, mean annual precipitation, mean T_{min} and mean T_{max} were very similar for the populations of both species in Australia and in Portugal (Appendix S2 and Table 2).

Significant differences in climatic variables between countries were found only for mean T_{min} in the populations of *A. dealbata* (6.61 °C in Australia and 11.09 °C in Portugal, $P < 0.001$, Table 2). Thus, minimum temperature was included as a factor in the analysis of seed variables for *A. dealbata* although climatic data were not included in further analyses for *A. longifolia*.

DIFFERENCES IN SEED PRODUCTION AND SEED TRAITS FOR *A. DEALBATA*

The difference in AICc values between fitted and null models for the total number of seeds per pod ($n = 1277$) revealed that neither region nor minimum temperature were good predictors of this variable (Fig. 1a, Table 3). The variability of the total number of seeds per pod was better explained by the properties of population/individuals than by those factors. When analysing the proportion of fully developed, aborted and preyed upon seeds, the models containing either region or region and minimum temperature as fixed factors were equivalent. The most parsimonious decision was taken, and models were fitted only with region as fixed factor (Appendix S3). This procedure revealed a contrasting effect of the region of origin on the proportion of each type of seeds, which was positive for mature seeds and negative for aborted and preyed upon seeds (Fig. 1b). The percentage of mature seeds was higher among Portugal populations, whereas the percentage of aborted and preyed upon seeds was higher in Australia (Fig. 1b).

The number of pods per branch was not significantly different between countries. However, the estimated number of pods per tree and estimated reproductive output were significantly higher in Portugal than Australia with the estimated reproductive output being sixfold larger in the invaded range (Table 4). The difference in AIC values between fitted and null models for the number of pods per branch and tree revealed that the best model to explain the results included region and minimum temperature as explanatory factors (Table 3). For the reproductive output, the models containing region or region and minimum temperature as fixed factors were equivalent. The most parsimonious decision was taken, and models were fitted only with region as fixed factor (Table 3, Appendix S3).

For *A. dealbata*, seed weight was clearly correlated with the region of origin: seed size was 23% greater in the invaded region ($n = 4089$; Table 3 and Appendix S2, Fig. 2a). The differences in AICc values between fitted models for elaiosome weight (Fig. 1a) and elaiosome:seed mass ratio (Australia: $n = 2021$, 0.053 ± 0.0006 and Portugal: $n = 2087$, 0.047 ± 0.004) were very small; thus, the null model was selected as the most parsimonious solution for both variables (Fig. 2a, Table 3).

A significant effect of region was also found for the biomass of 3-month seedlings *(*$n = 337$, $t = 4.021$, $P = 0.0017$). Seedlings from seeds collected in the invaded region, Portugal, were 32% larger than seedlings originating from seeds collected in the native range, Australia (Fig. 1b).

DIFFERENCES IN SEED PRODUCTION AND SEED TRAITS FOR *A. LONGIFOLIA*

No differences in climate occur between the native and invaded ranges. Consequently, for *A. longifolia,* only the effect of region was used to analyse the studied variables.

Table 2. Values (mean ± SE) for annual precipitation (mm/year) and mean maximum and minimum temperature (°C) for all populations in each region for the 12 months previous to sampling. Differences in these values between regions were tested with a Welch's *t*-test

		Precipitation	T_{max}	T_{min}
A. dealbata	Australia	865.49 ± 12.35	21.03 ± 0.48	6.61 ± 0.47
	Portugal	905.62 ± 75.48	20.09 ± 0.54	11.09 ± 0.67
	P value	0.6177	0.2241	0.0002
A. longifolia	Australia	1011.02 ± 59.84	20.23 ± 0.40	10.95 ± 0.61
	Portugal	1029.56 ± 92.86	20.71 ± 0.35	12.19 ± 0.34
	P value	0.8689	0.3792	0.0953

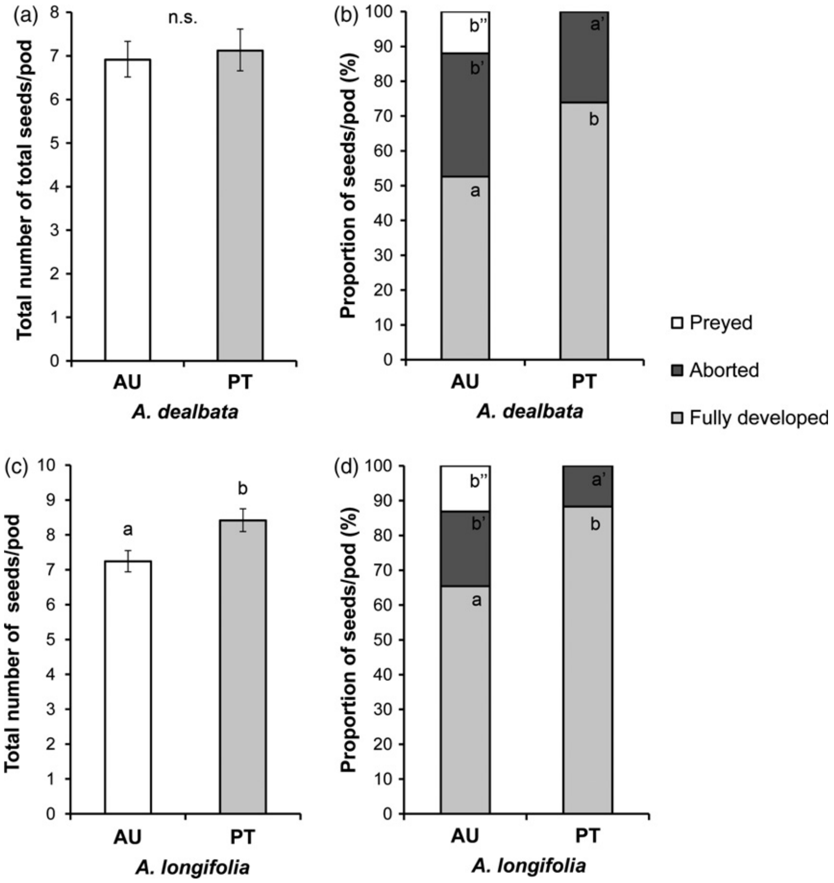

Fig. 1. Results for the seeds per pod survey for *Acacia dealbata* and *A. longifolia* in Australia (AU) and Portugal (PT). a, c Total number of seeds per pod; b, d proportion of seeds per pod (%). Values are given as model-adjusted back-transformed least-square means and 95% confident intervals. For *A. longifolia*, significant differences ($P < 0.05$) of least-square means are indicated by different letters between regions. For *A. dealbata*, different letters indicate that the best model includes region as a predictor.

Table 3. Comparison of alternate models based on AICc values for seed traits of *A. dealbata*. The best model (lowest AICc) is indicated in bold face. Equivalent models (delta AIC < 2) are indicated in italics

	Region	$T_{\min}$	Region + $T_{\min}$	Null model
Seed weight	**−37661.5**	37656.7	−37659.3	−35326.17
Elaiosome weight	*−56385.8*	*−56384.5*	*56383.8*	***−56385.6***
Elaiosome–seed mass ratio	−19610.3	−19509.9	−19596.44	**−19619.3**
Number of seeds per pod	5894.1	5894.3	5896.1	**5892.3**
Viable seeds	**4679.2**	4689.5	*4680.9*	4687.8
Aborted seeds	**4600.4**	4603.8	4602.1	*4601.8*
Seeds preyed upon	**1591.6**	1622.8	*1593.3*	1623.4
Pods per branch	*3363.9*	*3363.8*	3365.9	***3362.7***
Estimated pods per tree	3004.12	3006.25	**2992.6**	3062.7
Estimated reproductive output	***3030.66***	3064.5	*3029.5*	3062.7

Significant differences were found in the total number of seeds per pod ($n = 1705$, $P = 0.00925$, $F = 6.774$) with higher values found in Portugal (Fig. 1c). Region was a significant factor also for the number of total, aborted and preyed seeds produced per pod (Fig. 1d). The percentage of fully developed seeds per pod was higher in Portugal than in Australia ($n = 1705$, $P < 0.0001$, $z = 6.352$). The percentage of aborted and preyed upon seeds per pod was higher in Australia than in Portugal ($P < 0.0001$, $z = -4.70$ and $P = 0.00839$, $z = -2.636$).

The values obtained for total number of pods per branch ($P = 0.293$, $z = -1.05$, $F = 1.1047$), estimated number of pods per tree ($P = 0.613$, $z = 0.505$, $F = 0.2553$) and estimated reproductive output ($P = 0.218$, $z = 1.233$) were not significantly different between countries (Table 4).

Significant differences were detected in seed weight between countries ($P = 0.0032$, $F_{1.18} = 11.5729$ respectively): larger seeds (up to 20%) were produced in Portugal, but no differences were found in elaiosome weight between plants from the two countries ($n = 6081$, $P = 0.749$, $F_{1.18} = 0.105$; Fig. 2c). However, the elaiosome–seed mass ratio was significantly higher for plants in Australia (Australia: $n = 3046$, 0.24 ± 0.012 and Portugal: $n = 3035$, 0.16 ± 0.001; $t = -6.856956$; $P < 0.0001$).

A significant effect of region of origin was found for the biomass of *A. longifolia* 3-month-old seedlings ($n = 587$, $P = 0.0001$, $F_{1.18} = 23.6002$). Seedlings from seeds collected in the invaded range (Portugal) were 28% bigger than those from seeds collected in the native range (Australia; Fig. 2d).

Table 4. Characterization of the *Acacia* species studied for fruit display and estimated reproductive output (estimated number of seeds per tree) in native and invasive areas. Values are given as means and standard error of the mean followed by sample size in parenthesis. See text and Table 3 for differences between regions

		Australia	Portugal
A. dealbata	Pods per branch	18 ± 1 (420)	18 ± 1 (420)
	Estimated pods per tree	2369 ± 518 (142)	10 765 ± 3876 (142)
	Estimated reproductive output	10 829 ± 3001 (142)	62 735 ± 24 084 (142)
A. longifolia	Pods per branch	16 ± 1 (566)	14 ± 1 (566)
	Estimated pods per tree	6132 ± 1322 (190)	6662 ± 1264 (190)
	Estimated reproductive output	38 650 ± 10 507 (190)	50 427 ± 9577 (190)

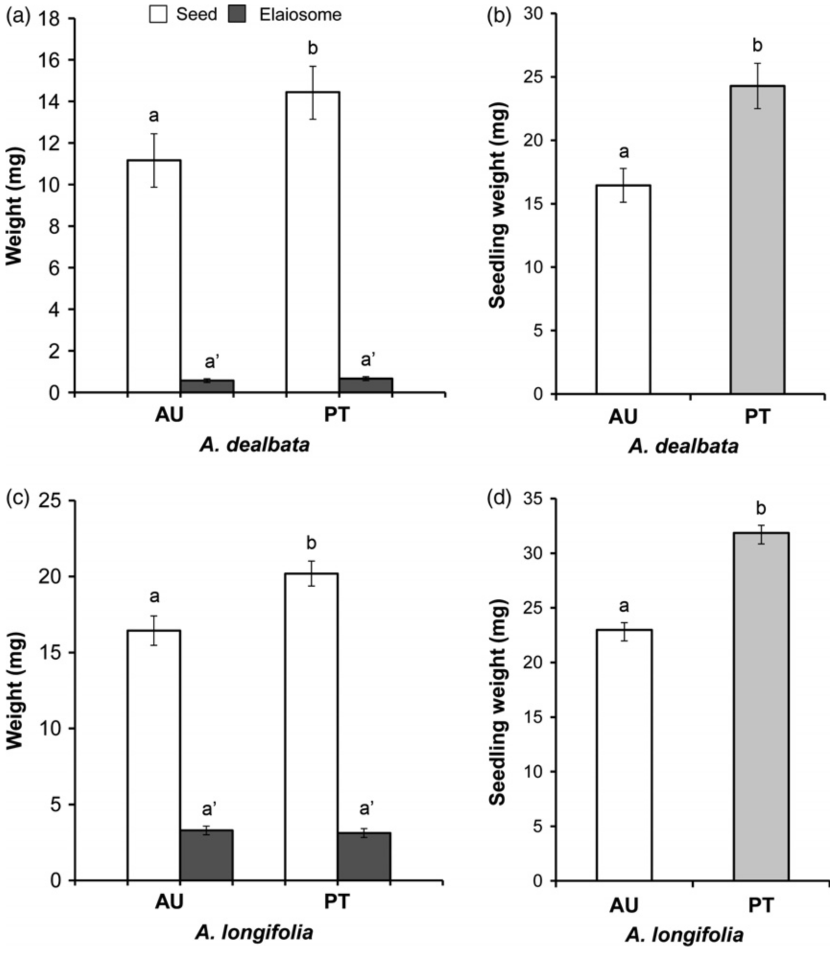

Fig. 2. Results for the seed survey for *Acacia dealbata* and *A. longifolia* in Australia (AU) and Portugal (PT) a, c seed and elaiosome weight (mg); b, d seedling weight (mg). Values are given as model-adjusted back-transformed least-square means and 95% confident intervals. For *A. longifolia*, significant differences ($P < 0.05$) of least-square means are indicated by different letters between regions. For *A. dealbata*, different letters indicate that the best model include region as a predictor.

Discussion

Invasive plants are often taller (Blossey & Nötzold 1995), produce more seeds (Noble 1989; Groves & Paynter 1998) and live longer (Rees & Paynter 1997) in their introduced range when compared with the native range. However, few biogeographical studies have been undertaken for invasive woody species to detect a consistent pattern (Thébaud & Simberloff 2001). Our study presents an intercontinental comparison of the reproductive output of two world-wide invasive woody species. Our data show that both species of *Acacia* have higher performance in the introduced range, which could be crucial in an invasion. Key traits for invasive populations of *A. dealbata* and *A. longifolia* were the production of a significantly lower proportion of aborted seeds and the absence of pre-dispersal predation, as well as the production of larger seeds in the invasive range that result in larger seedlings. The absence of trade-offs between seed size and seed production for both species is apparent. Trade-offs between seed size and seed number are common (Jakobsson & Eriksson 2000), but more difficult to find in long-lived plants with complex life history (Venable 1992; Koenig *et al.* 2009).

Crucial differences between regions were the lower percentage of aborted seeds and the lack of pre-dispersal seed predation in the introduced range, which resulted in a higher proportion of developed seeds per pod. For both species, seed abortion was as much as 12% greater in native populations than in invasive populations. This difference may represent an important characteristic that enables invasion because more seeds result in more opportunities for successful colonization and establishment (Clarke, Lewis & Horvath 2001; Westoby *et al.* 2002). In addition, *A. longifolia* plants in the invaded area produced significantly more seeds per pod than plants in the native area. These results corroborate previous studies that found higher seed production of *A. longifolia* in non-native regions (Weiss & Milton 1984; as cited in Noble 1989; Correia, Castro & Rodríguez-Echeverría 2015). Interestingly, the estimated average number of seeds per tree for *A. dealbata* was higher in Portugal than in the native range. No significant differences were found in the number of seeds per pod or the number of pods per branch for *A. dealbata* between regions, but the number of branches with pods per tree was higher in Portugal than in Australia. This difference could be partially explained by the higher minimum temperature experienced by *A. dealbata* populations in Portugal because temperature can affect flower production, pollen viability and pod production in some *Acacia* species (Sedgley 1985; Kendrick & Knox 1997; Beck-Pay 2012). Alternately, between-region differences in reproductive traits of *A. dealbata* plants could also explain this result. Previous studies have shown that *A. dealbata* makes a huge investment in flower production in the invaded area and a significantly higher reproductive success than other Australian *Acacia* species in Portugal due to a large production of flower heads per flowering branch and per tree (Correia *et al.* 2014). This difference in performance between regions could also be explained by the selection for more vigorous and showy varieties by forestry services during the introduction of this species (Richardson 1998).

Acacia longifolia and *A. dealbata* plants from the invasive Portuguese populations produced significantly larger seeds than plants from Australian populations. Differences in seed size between plants in native and non-native ranges have been described for invasive herbaceous species (e.g. *Centaurea solstitialis* L. (Hierro *et al.* 2013), *Echium plantagineum* L. (Konarzewski, Murray & Godfree 2012) and *Lupinus polyphyllus* Lindl. (Sõber & Ramula 2013) and for the woody legume *Cytisus scoparius* (L.) Link (Buckley *et al.* 2003). Large seeds are advantageous under a variety of unfavourable conditions (e.g. highly competitive or low-light environments) during establishment (Leishman *et al.* 2000; Paz & Martínez-Ramos 2002). Also, a positive correlation between seed size and seedling size has been found for some Australian acacias (Correia *et al.* 2014). Our study, using a large number of populations, corroborates this correlation, but the actual relevance of larger seed size to an invasion remains to be elucidated.

Several hypotheses (not mutually exclusive) can explain the higher number and size of seeds produced by alien plants in invaded areas (Mason *et al.* 2008). Our data suggest that the increase in the total or relative allocation to reproduction could be the result of the release from natural enemies (Crawley 1987) as predicted by the evolution of increased competitive ability hypothesis (Blossey & Nötzold 1995) and shown for other invasive woody legumes (Groves & Paynter 1998). The absence of specialist herbivores can avoid maternal selection against larger seeds (e.g. Agrawal 2001; Buckley *et al.* 2003). Also, reduced pathogen pressure can result in larger parent plants (Rees & Paynter 1997), which are able to sustain a higher seed output (seed size and number; Venable 1992). Artificial selection during introduction or rapid evolution in the new regions could also render greater seed production and/or larger seeds (Carroll & Dingle 1996; Sakai *et al.* 2001; Hänfling & Kollman 2002; Lee 2002; Stockwell, Hendry & Kinnison 2003; Maron *et al.* 2004). Finally, invasive species might occupy more favourable environments in the invaded range, which allow a higher investment in growth and/or reproduction (Crawley 1987; Erfmeier & Bruelheide 2004). For example, invasive populations of *Lythrum salicaria* are more fecund than populations in the native area only when growing in nutrient-rich habitats (Edwards, Adams & Kvìt 1998). We did not measured soil fertility, although soil nutrient levels could certainly influence reproductive output. This explanation seems unlikely as the number of populations sampled covered a large geographical area and variety of soils and the studied plants are large long-established trees. Furthermore, we found no differences in climate or no significant consistent effect of climatic variables on the measured traits. Thus, our data suggest that changes in reproductive output between native and invaded areas for these Australian *Acacia* species are explained by biological mechanisms but not by climatic differences. These results support the importance of biotic interactions in biological invasions by woody plants (Mitchell *et al.* 2006; Haugo, Bakker & Halpern 2013), although other studies have shown that post-invasive evolution might be driven by climate (Colomer-Ventura *et al.* 2015).

The ratio of elaiosome size to seed size can influence seed removal by ants (Gunther & Lanza 1989; Oostermeijer 1989; Hughes & Westoby 1992). Selection could optimize the trade-off between seed size and elaiosome size as this outcome represents the compromise between the resources invested in the seed (embryo, endosperm and seed coat) vs. the amount invested in dispersal. The elaiosome–seed mass ratio for *A. longifolia* was significantly higher in Australia than in Portugal, suggesting that a higher investment in ant reward is not a factor for seed dispersal in the invaded region. Accordingly, recent studies indicate that myrmecochory is proportionally less important in Portugal than in Australia (D. Montesinos, M. Correia, S. Castro, K. French & S. Rodríguez-Echeverría, unpubl. data). Interestingly, although seed size was higher for *A. dealbata* in Portugal, no differences were found for the elaiosome–seed mass ratio.

Several mechanisms have been proposed to explain the high invasiveness of Australian *Acacia* species. The invasive spread of Australian *Acacia* in Portugal is intimately linked to these species association with below-ground mutualisms (mycorrhizal fungi and symbiotic nitrogen-fixing bacteria) that enhance plant nutrition and growth and may have a positive effect on seed number and size (Rodríguez-Echeverría *et al.*

2009; Rodríguez-Echeverría *et al.* 2012; Crisóstomo, Rodríguez-Echeverría & Freitas 2013). *A. dealbata* and *A. longifolia* can also displace native species by either releasing phytotoxins (Ens, French & Bremner 2009; Lorenzo *et al.* 2010; Lorenzo, Pereira & Rodríguez-Echeverría 2013) or through competition particularly under limited nutrient availability (Peperkorn, Werner & Beyschlag 2005; Werner *et al.* 2008). Our study demonstrates the existence of significant biogeographical differences in seed production and quality between native and invasive populations of *A. dealbata* and *A. longifolia* likely due to enemy release and increased resource availability in which could be a crucial mechanism to explain the invasion by this species.

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.f1kc3 (Correia *et al.* 2016).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Flowering, pod maturation and falling seeds phenology of the two studied *Acacia* species in Australia and Portugal.

Appendix S2. Climographs for studied populations in Australia and Portugal of *Acacia dealbata* and *A. longifolia* using climate data for the last 30 years.

Appendix S3. Estimated values and best model selected for each response variable for *A. dealbata*.