

# Seed local adaptation and seedling plasticity account for *Gleditsia triacanthos* tree invasion across biomes

Pedro M. Tognetti<sup>1,\*</sup>, Noemí Mazia<sup>2</sup> and Gonzalo Ibáñez<sup>2</sup>

<sup>1</sup>IFEVA–CONICET and Facultad de Agronomía, Universidad de Buenos Aires, Argentina and <sup>2</sup>Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Argentina, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

\*For correspondence. E-mail [tognetti@agro.uba.ar](mailto:tognetti@agro.uba.ar)

Received: 13 March 2019 Returned for revision: 2 April 2019 Editorial decision: 30 April 2019 Accepted: 2 May 2019

- **Background and Aims** Phenotypic plasticity and local adaptation can contribute to the success of invasive species. While the former is an environmentally induced trait, the latter involves a selection process to filter the best genotype for a location. We examined the evidence for phenotypic plasticity and local adaptation for seed and seedling traits of the invasive tree *Gleditsia triacanthos*, with three origins distributed along an approx. 10° latitude gradient across three biomes.
- **Methods** In sub-tropical forests, dry woodlands and secondary temperate grasslands in Argentina, we harvested seeds from clusters of neighbouring trees (i.e. families) distributed within 15–20 km in each origin (biome). We manipulated the environmental conditions relevant to each biome, assuming that propagule availability did not represent an ecological barrier. In growth chambers, we evaluated seed imbibition and seed germination under different light, temperature and water potential. In a 2 year common garden, we evaluated the impact of resident vegetation removal on seedling survival and growth.
- **Key Results** Mean time to complete seed imbibition differed among origins; seeds from temperate grasslands reached full imbibition before seeds from dry woodlands and sub-tropical forests. Germination was always >70 %, but was differentially affected by water potential, and light quantity (dark–light) and quality (red–far red) among origins, suggesting local adaptation. In the common garden, vegetation removal rather than origin negatively affected seedling survival and enhanced seedling growth. Vegetation removal increased basal diameter, leaves per plant and spine number, and reduced the height:basal diameter ratio.
- **Conclusions** We conclude that local adaptation in seed germination traits and plastic changes in seedling allometry (e.g. height:diameter) may allow this tree to respond over the short and long term to changes in environmental conditions, and to contribute to shape *G. triacanthos* as a successful woody invader. Overall, our study revealed how local adaptation and plasticity can explain different aspects of tree invasion capacity across biomes.

**Key words:** *Gleditsia triacanthos*, tree invasion, regional study, sub-tropical forest, dry woodland, temperate grassland, seed germination, common garden, seedling establishment, seedling growth, variance components, Argentina.

## INTRODUCTION

Many invasive plants have a range of ecological tolerance that allows them to establish successfully in different environments (Richards *et al.*, 2006). Phenotypic plasticity and local adaptation may contribute to the distribution of widespread invasive plants (Drake *et al.*, 1990; Williamson, 1996; Agrawal, 2001; Sexton *et al.*, 2002; Parker *et al.*, 2003; Richards *et al.*, 2006; Zenni and Nuñez, 2013). Phenotypic plasticity is the ability of a genotype to generate different phenotypes according to the environment where it lives (Valladares *et al.*, 2006), and local adaptation is the genetically fixed advantage of a population under certain environmental conditions (Oduor *et al.*, 2016). For a successful tree invader, seed germination, seedling survival and early seedling growth are critical life stages, due to their sensitivity to environmental stressors (e.g. water, light and temperature; Cavender-Bares and Bazzaz, 2000; Donohue *et al.*, 2010). However, despite the importance of plasticity and

local adaptation in plant invasion, few works have simultaneously evaluated both seeds and seedlings from different populations of an invader species (see Geng *et al.*, 2007; Donohue *et al.*, 2010).

Phenotypic plasticity and local adaptation are central to community and invasion ecology. First, phenotypic plasticity may allow a species to colonize different environments despite a restricted genetic base, and thus could be advantageous under environments highly variable in space and time (Richards *et al.*, 2006). The fast responses of seedlings to sudden changes in biotic and abiotic conditions may enhance invader performance, whereas species lacking plasticity to maintain growth and survival in novel environments could fail as invaders (Sultan, 2000; Zenni and Nuñez, 2013; Oduor *et al.*, 2016). Although it is frequently assumed that phenotypic plasticity evolved as an adaptation to environmental heterogeneity, many responses could be attributed to passive plasticity (van Kleunen and Fischer, 2005). As passive and active plasticity may occur

simultaneously with opposite sign (negative and positive slope) and different magnitude (Forsman, 2015), it can be assumed that the final result represents a net balance between them. Secondly, local adaptation implies evolved traits that provide an advantage under specific conditions, regardless of the consequences of these traits for fitness in other habitats (Williamson, 1996; Kawecki and Ebert, 2004; Oduor et al., 2016). This implies that selective forces filtered the genotypes best adapted to local conditions (Sultan, 2000; Sexton et al., 2002; Parker et al., 2003; Oduor et al., 2016). However, the fact that woody species have more genetic diversity within rather than among populations, especially for outcrossing tree species, makes the process of local adaptation slow in comparison with that in herbaceous plants (Hamrick, 2004). In summary, phenotypic plasticity and local adaptation both may occur during invasion depending on the time elapsed since introduction and the number of introduction events. A long introduction time and a larger number of events may favour local adaptation (Roman and Darling, 2007; Donaldson et al., 2014). In contrast, high genetic interchange among populations through long-distance dispersal could weaken local adaptation (Hamrick et al., 1992; Oduor et al., 2016).

There exist complementary approaches to evaluate phenotypic plasticity and local adaptation. Ecologists frequently measure plant trait changes under contrasting environmental conditions, for example by manipulating variables of ecological interest such as water stress, light conditions and competition (Tsegay et al., 2005; Ramirez Valente et al., 2010; Rotundo et al., 2015; Mathiasen and Premoli, 2016) and constructing reaction norms (Via et al., 1995). The slope of the trait value across a set of environments indicates the level of plasticity of each genotype, and significant genotype  $\times$  environment interaction suggests local adaptation (Kawecki and Ebert, 2004; Hulme, 2008). Thus, local adaptation is reflected as the development of a specialized genotype for a specific range of environmental conditions. In addition, assigning phenotypic variance to different sources is a complementary way to understand phenotypic plasticity of a structured population (Ramirez-Valiente et al., 2010). Total phenotypic variability can be partitioned in a hierarchical manner, considering

populations, families within populations and individuals within families (Schnabel and Hamrick, 1990; Schnabel et al., 1991).

*Gleditsia triacanthos* L. is a leguminous tree native to US deciduous forests (Schnabel and Wendel, 1998) that invades different ecosystems in Africa (Nei et al., 2004), Australia (Csurhes and Kriticos, 1994), Europe (Ferus et al., 2013) and South America (Mazia et al., 2001; Marco et al., 2002; Tecco et al., 2012; Fernandez et al., 2017). This thorny tree was introduced into Argentina in around the 1850s to provide cattle shade, live fences and as ornamentals (Hudson, 1918; Fernandez et al., 2017). *Gleditsia triacanthos* has entomophile pollination, produces an indehiscent legume with >20 seeds per pod and its seeds are successfully dispersed by cattle and wildlife (Schnabel and Wendel, 1998; Marco and Paez, 2000; Fernandez et al., 2017). High seed production, great dispersal, rapid growth and a short juvenile period (Burton and Bazzaz, 1991; Marco and Paez, 2000) contribute to the high invasive potential of *G. triacanthos*. In Argentina, it has been reported to invade woodlands (Marco and Paez, 2000), temperate grasslands (Mazía et al., 2001; Ghersa et al., 2002; Chaneton et al., 2004) and sub-tropical forest ecosystems (Ferrerías and Galetto, 2010; Giorgis et al., 2011; Fernandez et al., 2017). According to a recent study developed in Argentina, *G. triacanthos* is excluded as an invader species in dry, cold and flooding environments (Fernandez et al., 2017).

Our aim was to examine phenotypic plasticity and local adaptation for seeds and seedlings of three populations of *G. triacanthos* from different origins in Argentina (i.e. sub-tropical forest, dry woodlands and temperate grasslands; Table 1), where this species is an aggressive invader (Fernandez et al., 2017). This study covers an approx. 10° latitudinal gradient with a wide range of climates, including temperature, seasonal precipitation regimes and light conditions (Table 1). First, in chamber experiments, we examined germination under different environmental conditions regarding water availability, temperature and light quantity (light–dark) and quality [red–far red (R/FR)], as these treatments comprise the conditions that seeds from different origins are exposed to during germination (Table 1). For instance, we exposed the seeds to hydric stress (–2 MPa), which is the frequent condition in Pampean

TABLE 1. Characterization of the three origins where seeds were collected and where *G. triacanthos* is a common invader

Origin	TG	DW	SF
Biome*	Temperate grassland	Dry woodland	Sub-tropical forest
Location	Carlos Casares (Buenos Aires)	Salsipuedes (Córdoba)	Valle de La Sala (Tucumán)
Latitude	35.53°S	31.09°S	26.78°S
Longitude	61.12°W	64.31°W	65.38°W
Altitude (m asl)	100	744	900–1000
Mean annual precipitation (mm)	1060	897	1031
Mean growing-season† precipitation (mm)	715.6	730.9	898
Mean annual temperature (°C)	16.1	17.3	19.5
Mean maximum temperature (°C)	22.6	24	25.8
Mean minimum temperature (°C)	10.4	14.2	11.3
Mean growing-season† temperature (°C)	20.6	21.5	23.7
Precipitation regime	Isohigro	Monsonic	Monsonic
Recent use history	Agriculture–cattle husbandry	Cattle husbandry	Cattle husbandry

Pods from three different families were collected, defined as groups of 15–20 neighbouring trees separated by 3–5 km from other families within each origin. In each origin, the spatial extent covered was around 15  $\times$  20 km. The common garden experiment was carried out in a temperate grassland in Buenos Aires (TG).

\*From Oyarzabal et al. (2018).

†The growing-season runs between October and April.

grassland after seed imbibition, and to conditions where water availability is non-limiting (0 MPa), which is frequent in subtropical forests (Table 1). While temperature and water potential may modify germination rates (Baskin and Baskin, 1998), light conditions, frequently associated with common disturbances in grasslands and forests, may modify the rate and shape of seedling development (Casal et al., 1991; Baskin and Baskin, 1998). Secondly, in a 2 year common garden field experiment, we evaluated the impact of resident vegetation on seedling survival and early growth. Small soil disturbances by burrow mammals in grasslands and canopy disturbances creating small and large gaps in forests both generate changes in light quantity and quality (Mazía et al., 2010, 2019). Overall, through performing germination trials and field experiments, we experimentally manipulated the environmental conditions, simulating an invasion window under conditions relevant to each biome, when propagule availability does not represent an ecological barrier (Mazía et al., 2001; Shea and Chesson, 2002; Donaldson et al., 2014).

We hypothesized that the requirements for seed germination vary in accordance with the prevailing abiotic conditions of each region (Donohue et al., 2010). If local adaptation was important (Table 1), we predicted that seeds from dry woodlands and subtropical forests find better conditions for germination under high temperature and water potential. In contrast, seeds from temperate grassland enhance germination under drier conditions. If plasticity was important, we predicted that germination is enhanced under light and a high R/FR ratio independently of the origin, as *G. triacanthos* is an early successional species that colonizes disturbed environments and seedlings do not recruit in shaded environments (Mazía et al., 2001). In a common garden, we hypothesized plastic responses in seedling growth and survival with the removal of resident vegetation, independently of the origin. We predicted that seedlings growth is higher without resident vegetation, independently of origin because this species is an early successional tree adapted to grow in disturbed environments (Schnabel and Wendel, 1998).

## MATERIALS AND METHODS

### Seed origin and collection

We collected *G. triacanthos* seeds from three different origins in Argentina, which correspond to different biomes in terms of vegetation physiognomy, climatic conditions and history of human use (Table 1). Carlos Casares, Buenos Aires Province, is a secondary temperate grassland, represented by a mosaic of native and exotic patches immersed within a matrix of agriculture (Tognetti et al., 2010). This origin shows iso-hygro precipitation, but its high evapotranspiration frequently determines negative water balance in summer. Salsipuedes, Córdoba Province, is a dry woodland, degraded by livestock and fragmentation (Zak et al., 2004). This origin shows the highest mean minimal temperature and summer precipitation, which generate warm and humid conditions during the growing season (Table 1). Finally, Valle de La Sala, Tucumán Province, is an open sub-tropical forest, characterized by a long history of livestock grazing (Fernandez et al., 2016, 2017). This origin shows the highest mean maximal and mean annual temperatures, that

combined with high precipitation (Table 1) results in humid and dense forests year-round (Oyarzabal et al., 2018). All these sites are highly invaded by woody plants, mainly *G. triacanthos* (Mazía et al., 2010; Tecco et al., 2012; Fernandez et al., 2017).

In each origin, seed collection followed a hierarchical sampling, with families nested within origins. Here, we considered a cluster of neighbouring trees ( $n = 15\text{--}20$ ) within a radius of 50 m as a family, and origin represents a group of families within a biome. To ensure the spatial representation of each origin, we harvested pods from 3–5 families distributed within a radius of 15–20 km. Families were separated by 3–5 km, probably being connected by dispersal and pollination and not representing independent genetic populations. *Gleditsia triacanthos* pods were collected in April–May 2009 (autumn) from the ground, to be sure seeds had completed the process of maturation. Given that pods are indehiscent, we were able to distinguish new vs. previous years pods by their state of degradation. In the laboratory, all pods from a family were treated as a group, thus seeds were extracted from pods and pooled at the family level. Seeds were air dried and kept at 5 °C until the start of experiments (i.e. <6 months later; Baskin and Baskin, 1998).

### Experimental design

We performed three experiments in growth chambers to evaluate seed germination traits (E1–E3) and one more experiment under field conditions to evaluate plant establishment and growth traits (E4). Some experiments did not account for the effect of families within origin (see below), although it was possible to test for differences among the origins of the plants.

**E1: seed imbibition.** To evaluate differences in seed imbibition, a prerequisite to start germination, we followed seed weight for 5 d (Baskin and Baskin, 1998). We placed ten seeds into each of five Petri dishes for each family and origin (temperate garden, one family; dry woodland, three families; sub-tropical forest, five families; temperate garden had only one family due to mislabelling). Seeds were scarified by manual abrasion with sandpaper, as it has produced the best results in previous assays (Mazía et al., 2013), and placed in fixed positions in each Petri dish to follow their individual seed weight. Petri dishes were placed in a growth chamber set at 20/30 °C in 12/12 h cycles, and were watered daily with 5 mL of deionized water. We weighed each seed daily to 0.1 mg (Mettler Toledo, Tec Instrumental, BA, Argentina), after drying with tissue paper.

**E2: seed germination and hypocotyl shape in response to light conditions.** Apart from germination, hypocotyl shape (i.e. the length:diameter ratio) has been related to adaptations to drought, herbivory or fire for wild species (Fisher, 2008) and to successful emergence under crusted soils for some cultivated species (Finch-Savage and Bassel, 2016). To evaluate seed germination and hypocotyl expansion in response to light, we exposed seeds to two contrasting light environments (following Casal et al., 1991; Batlla et al., 2000). These treatments simulated changes in light quantity (dark vs. light) and light quality (R vs. FR wavelength). First, under dark conditions, seeds were wrapped in a black polyethylene material receiving no light, while in light conditions, the seeds were exposed to the light of a fluorescent tube [photosynthetically active radiation

(PAR)  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Casal *et al.*, 1991; Batlla *et al.*, 2000; Ferreras *et al.*, 2015]. Secondly, to emulate changes in light quality received by reflection from other plants, we changed the R/FR ratio by enriching the light in R (660 nm, open gap) or FR wavelengths (730 nm, closed canopy). This replicated light signals in the absence or presence of competition, respectively (Batlla *et al.*, 2000). We placed 15 seeds into each of three Petri dishes for each family and origin (i.e. 45 seeds per family per treatment, split across three Petri dishes; temperate garden, five families; dry woodland, three families; sub-tropical forest, five families). In all cases, seeds were irradiated with their respective light treatments for 1 h (dark, light, R, FR), wrapped in black polyethylene and placed in a germination chamber (Cavadevices, Buenos Aires, Argentina) with alternating temperatures (20/30 °C in 12/12 h cycles; see Casal *et al.*, 1991). Initial counts were performed under green light (Burkart and Sanchez, 1969) and, after 15 d, we quantified the percentage of germination for each treatment.

*E3: seed germination in response to temperature and water potential.* To evaluate seed germination in response to different combinations of temperature and water potential, we utilized three temperature chambers (20, 25 and 30 °C; Cavadevices) and two water potentials (0 and -2 MPa; Baskin and Baskin, 1998). In each chamber, we placed three Petri dishes for each origin and water potential condition. Each Petri dish contained 15 scarified seeds on filter paper and was watered daily with 5 mL of a solution with a water potential of 0 (deionized) or -2.0 MPa. The reduction in water potential was achieved by adding 0.424 g of polyethylene glycol (PEG) per g of deionized water, following Hardegree and Emmerich (1990). To maintain the water potential of each treatment, we changed the filter paper daily before watering (Hardegree and Emmerich, 1990). The experiment lasted for 15 d. There was one chamber per temperature; thus temperature was not properly replicated. However, chamber effect is most likely to represent temperature, since chambers were specially designed to maximize control over other variables. This experiment did not consider families within origins.

*E4: seedling growth and survival in response to vegetation removal.* We established a field, common garden experiment to evaluate the effect of vegetation removal on the performance of *G. triacanthos* seedlings from three origins. The experiment was established within an old-field grassland in Carlos Casares (Buenos Aires Province, Table 1), which was cultivated for 50 years and then closed to agriculture in 2004. Vegetation resembled mid-successional old fields and was dominated by exotic annual and perennial grasses (e.g. *Lolium multiflorum*, *Sorghum halepensis* and *Festuca arundinacea*) and forbs (e.g. *Baccharis pingraea* and *Carduus acanthoides*; see Tognetti *et al.*, 2010). We established six blocks, each consisting of two  $3 \times 3$  m plots that were assigned to two vegetation removal levels: intact (-R) or vegetation removed (+R). Vegetation was manually removed at the beginning of the experiment (i.e. before transplanting seedlings), and subsequently every 3–4 months. Vegetation removal increased light penetration, measured as the proportion of PAR at soil level, from 11.5 to 89 % ( $F_{1,5} = 156.35$ ,  $P < 0.001$ ; light ceptometer: Cavadevices).

In December 2009, we planted 18 *G. triacanthos* seedlings per plot (3 origins  $\times$  3 families per origin  $\times$  2 individuals per

family), resulting in a total of 216 seedlings (basal diameter =  $1.79 \pm 0.21$  mm, height =  $9.98 \pm 2.7$  cm; mean  $\pm$  s.d.). Here, origin was a sub-plot within disturbance, and family was nested within origin, following a split-plot nested design (Schielzeth and Nakagawa, 2013). Seedlings were grown in a greenhouse by sowing scarified seeds in 90 cm<sup>3</sup> containers and growing them for 6 weeks before transplanting (Mazia *et al.*, 2013). Each seedling was labelled with a metal tag. During two complete growth seasons (January 2010 to March 2010, November 2010 to April 2011) we measured plant height, basal diameter and number of leaves. Plant height was measured with a standard measuring tape from the base to the insertion of the last fully expanded leaf. Basal diameter was measured with a calliper at 0.5 cm above the soil surface. In April 2011, we also counted the number of spines and measured the chlorophyll content for all living plants. Spines were counted in log-scale categories (0, no spines; 1, 1–10 spines per plant; 2, 10–100 spines per plant; 3, >100 spines per plant). Chlorophyll content was measured using a chlorophyll meter (SPAD 502 Plus Chlorophyll Meter, Konica Minolta, EU). SPAD values are strongly correlated with leaf N content (see Zakeri *et al.*, 2015).

#### Data analysis

Analyses were performed in R (R Core Team, 2017). We used linear mixed effects models [package ‘nlme’ (Pinheiro *et al.*, 2017) and ‘lme4’ (Bates *et al.*, 2015)] to evaluate seed imbibition and germination, plant growth and traits. This approach accounted for the nested nature of the experiments (i.e. families nested within origins), modelled variances to fulfil homoscedasticity assumptions (varIdent) and/or included a correlation structure (corAR1) to account for the lack of independence in measurements with time (imbibition and field experiment). We tested for the inclusion of these components using likelihood ratio tests (Zuur *et al.*, 2009). In all cases, we generated reaction norms for each origin in response to different environmental factors during germination (i.e. imbibition, water potential and light) or field conditions (i.e. vegetation removal); here, we considered origin and treatments as fixed effects. In parallel, we estimated variance components for all experiments (remIVCA function in the ‘VCA’ package; Erhardt and Schuetzenmeister, 2017). In these analyses, we considered ‘origin’ as a random factor. Therefore, these estimates were only considered indicative of the proportion of phenotypic variance assigned to blocks, origins, families and error, according to each experiment.

To evaluate seed imbibition, we modelled weight as a function of origin and days since the start of the experiment. After residual exploration, we included a quadratic term (Day<sup>2</sup>), which strongly improved model fit ( $\Delta\text{AIC} = 68$ ; likelihood ratio test:  $P < 0.001$ ) but excluded higher order interactions as they are difficult to interpret and may contain artefacts (i.e. Day and Day<sup>2</sup> correlation). To evaluate the effect of light conditions on seed germination, we performed two separate analyses comparing dark vs. light and R vs. FR treatments. Here, the model included light treatment, origin and their interaction. To evaluate the response to changes in water potential and temperature, we carried out a two-step analysis, given that temperature was not

replicated. First, we modelled germination as a function of water potential, origin and families nested within temperature, to test interactions (e.g. temperature  $\times$  water potential). Secondly, we ran a separate analysis within each temperature. For light and water potential data, germination percentage was arcsine transformed [i.e. arcsine [square root (germination/100)]] to improve the distribution of residuals and fulfil model assumptions.

To test if origins responded differentially to vegetation removal, we ran two separate analyses. First, we evaluated the temporal growth response of individual plants (height, basal diameter and leaf number). Here, we considered the factorial arrangement of origin, vegetation removal and time under a repeated measure model considering the consecutive measurements of each individual plant. We modelled variances by time and vegetation removal, and used the correlation structure to account for the consecutive readings on the same individual (Zuur *et al.*, 2009). Secondly, to focus on the change in traits in response to vegetation removal, we analysed the last sampling date (April 2011) under a factorial origin  $\times$  removal design. We also analysed relative growth rates (RGRs) for height, diameter and leaf number. The RGR was calculated as the natural logarithm of the final (April 2011) over initial (December 2009) values for each individual plant. Replication for the final measurements was still adequate, despite low survival in some groups (mean  $n = 1.47$  seedlings per treatment per family per block; range = 6–12 seedlings per treatment per family). Height, diameter, number of leaves and their RGRs were highly and positively correlated (i.e. Pearson's  $r = 0.75$ – $0.97$ ); to reduce spurious information, we present results for three of them (RGR<sub>height</sub>, diameter and number of leaves; see the Results).

To evaluate *G. triacanthos* seedling survival, we utilized two approaches. First, we estimated survival for each group employing the Kaplan–Meier method and using the ‘survival’ R-package (Therneau, 2015). This non-parametric method provides confidence intervals for the survival curves, allowing us to indirectly test differences among factors and treatments (Therneau and Grambsch, 2000). As it was not possible to test a factorial arrangement, we performed three separate comparisons: treatments (six levels), origins (three levels) and vegetation removal (two levels), adjusting  $P$ -values with a Bonferroni correction (Crawley, 2007). Secondly, to consider the fixed and random structure of the experimental design, we also performed a logistic regression for survival on the last sampling date using a binomial distribution and a logit link function under a generalized mixed effects model (see above, Crawley, 2007). Here, the origin and disturbance were fixed effects, and families nested within disturbance, nested within blocks were the random structure, thus permitting us to account for the fact that families were nested in origin, within each disturbance level in each block (split-plot nested design; Schielzeth and Nakagawa, 2013). Both Kaplan–Meier and logistic regression approaches produced consistent results.

## RESULTS

### Seed germination traits

Before imbibition, seed weight did not differ among origins ( $F_{2,6} = 1.53$ ,  $P = 0.29$ ; grey boxes in Fig. 1). During imbibition, the increase in seed weight differed among origins (Table

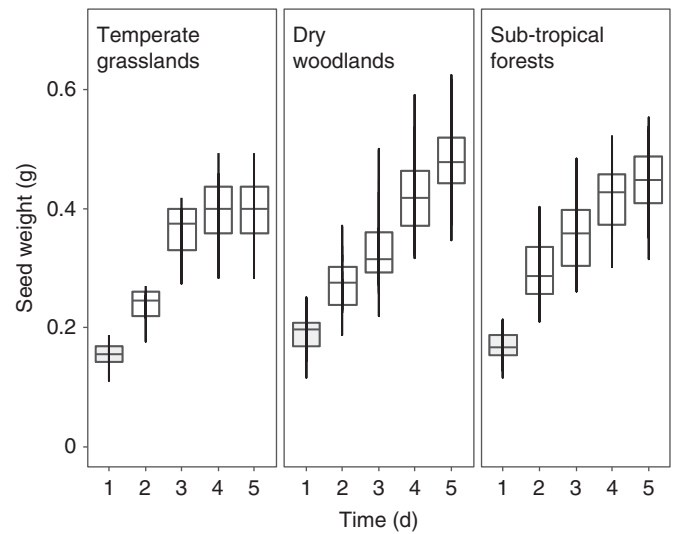


FIG. 1. Imbibition of seeds of *G. triacanthos* over time from temperate grasslands, dry woodlands and sub-tropical forests during 5 d in distilled water. Boxes are central quartiles with the median, and vertical lines indicate the 10–90 percentile range. Grey boxes indicate the initial seed weight.

2; Supplementary data Table S1a). Seeds from dry woodlands and sub-tropical forests increased their weight faster than seeds from temperate grasslands, whereas the seeds from this origin reached their maximum weight on days 4–5 and then stabilized (origin  $\times$  day 2 in Table 2; Fig. 1; Supplementary data Table S1a).

Water potential affected seed germination depending on seed origin and on temperature (water potential  $\times$  origin and water potential  $\times$  temperature interactions in Supplementary data Table S2), but in all cases seed germination was  $>70\%$  (near 90% for  $-2$  MPa; Supplementary data Table S1b). Considering each temperature separately, seed origin interacted with water potential (Table 3; Fig. 2A–C). In general, seeds from temperate grasslands consistently showed higher germination at  $-2$  MPa and lower germination at 0 MPa. In contrast, seeds from dry woodlands and sub-tropical forests changed in response to water potential across temperatures (Table 3; grey and black points in Fig. 2A–C). For example, the germination response of dry woodlands to increasing water potential (from  $-2$  to 0 MPa) changed from neutral to positive with the increase in temperature (Supplementary data Table S1b).

Light quantity (light vs. dark) and quality (R vs. FR) differentially affected germination rate and the shape of the hypocotyl (Table 4; Fig. 2D–G; Supplementary data Table S1c). First, neither origin nor R/FR light treatment affected the germination rate, although we detected that seed germination from sub-tropical forests showed a non-significant diminution under FR environment (Fig. 2E; Table 4). In addition, germination was higher for seeds from dry woodlands than for sub-tropical forests or temperate grasslands, independently of light quantity (Table 4; Fig. 2D). Secondly, the three origins differed in the initial shape of the seedling hypocotyl. Seeds from temperate grasslands produced shorter and wider hypocotyls independently of light quantity (Fig. 2F). In contrast, light quality (R/FR) differentially affected three origins (origin  $\times$  light; Table 4). While the hypocotyl shape of seedlings from

TABLE 2. ANOVA table with Wald-test results of the mixed effect models for the weight of seeds during imbibition for the three origins (temperate grasslands, dry woodlands and tropical forests) during 5 days. The model included a Day<sup>2</sup> term, as it improved model fit.

	d.f.	Imbibition	
		F	P
Origin	2,6	<b>8.97</b>	<b>0.015</b>
Day	1,425	<b>158.13</b>	<b>&lt;0.001</b>
Day <sup>2</sup>	1,425	<b>35.54</b>	<b>0.002</b>
O × D	2,425	<b>10.98</b>	<b>&lt;0.001</b>
O × D <sup>2</sup>	2,425	<b>11.62</b>	<b>&lt;0.001</b>
Family	$\widehat{\sigma}_f$		0.020 g
Residuals	$\widehat{\sigma}_e$		0.025 g

Last two rows indicate restricted maximum likelihood estimation for the standard deviation associated with the “families” within the three origins ( $\widehat{\sigma}_f$ ) and the residual standard error ( $\widehat{\sigma}_e$ ). Degrees of freedom are approximate and are detailed to indicate the correct model specification. Bold indicates significant terms in the model.

TABLE 3. ANOVA table with Wald test results of the mixed effects model for the effect of origin (temperate grasslands, dry woodlands and sub-tropical forest), water potential (0 and -2 MPa) and temperature (20, 25 and 30 °C) on *G. triacanthos* germination.

	d.f.	Germination	
		F	P
Origin	2,4	1.43	0.411
Water potential	1,41	<b>13.63</b>	<b>0.001</b>
Temperature	1,1	0.67	0.561
O × WP	2,41	<b>22.85</b>	<b>&lt;0.001</b>
O × T	2,41	0.26	0.789
WP × T	1,41	<b>4.56</b>	<b>0.038</b>

Even though temperatures were not truly replicated, differences between chambers probably represented temperature, since chambers were specially designed to maximize control over other variables (see Supplementary data Table S1 for separate temperature analyses). Degrees of freedom are approximate, and only detailed to indicate the correct model specification. Bold indicates significant terms in the model.

temperate grasslands did not change across light quality environments, seeds from dry woodlands increased and those from sub-tropical forests decreased hypocotyl shape with a lower R:FR ratio (higher FR; Fig. 2G).

#### Plant establishment and growth traits

Before establishment of the field experiment, plants differed in height and diameter among origins. Seedlings from temperate grasslands were shorter than those from dry woodlands and sub-tropical forests (height, TG<sup>a</sup> = 8.17 ± 0.34 cm; DW<sup>b</sup> = 10.12 ± 0.29 cm; SF<sup>c</sup> = 11.68 ± 0.32 cm; mean ± s.e.m.; letters indicate significant differences after Tukey test with  $\alpha = 0.05$ ), whereas the diameter of seedlings from sub-tropical forests and dry woodlands were wider than those from temperate grasslands

(diameter, TG<sup>a</sup> = 1.74 ± 0.25 mm; DW<sup>b</sup> = 1.85 ± 0.30 mm; SF<sup>ab</sup> = 1.79 ± 0.25 mm).

At the end of the experiment, vegetation removal rather than origin affected seedling traits (Table 5; Fig. 3; Supplementary data Table S1d), and there were no significant origin × vegetation removal interactions (all origin × removal  $P > 0.1$ ; Table 5). First, across vegetation removal treatments, the RGR for plant height was 25 % higher for temperate grasslands and dry woodlands with respect to sub-tropical forest seedlings (Fig. 3A; Table 5; Supplementary data Table S1). Secondly, vegetation removal increased final basal diameter by 4 mm (Fig. 3B) and reduced the height:diameter ratio by 11 % (Fig. 3C), resulting in more robust plants. Vegetation removal also increased leaves per plant by 32 leaves (+70 % with respect to controls) and spine number by one order of magnitude (Fig. 3D, E). Finally, neither vegetation removal nor origin affected chlorophyll levels (Table 5; Fig. 3F).

During the 2 years of the experiment, plant height increased differentially among origins (Supplementary data Table S3; Supplementary data Fig. S1). Plants from dry woodlands were taller and responded more positively to vegetation removal than plants from sub-tropical forests and temperate grasslands. However, basal diameter and the number of leaves responded positively to vegetation removal over time for plants of all origins (Supplementary data Table S3; Supplementary data Fig. S1a).

Both vegetation removal and origin affected plant survival (Kaplan-Meier:  $\chi^2_5 = 51.8$ ;  $P < 0.001$ , Fig. 4). Only approx. 41 % of plants from sub-tropical forests in plots with removed vegetation survived, in contrast to approx. 90 % for intact plots with plants from dry woodlands (Fig. 4). However, when vegetation removal and origin were analysed separately, vegetation removal ( $\chi^2_1 = 46.4$ ;  $P < 0.001$ ) was more important than origin ( $\chi^2_2 = 5.1$ ;  $P = 0.078$ ) in determining survival. On average, 45 % of plants survived in plots with removed vegetation [survival 95 % confidence interval (CI) = 38–53], against 77 % (survival 95% CI = 70–84%) in intact plots (Fig. 4). The generalized mixed effects model indicated that vegetation removal reduced final survival from 84 % to 62 %, independently of origin (likelihood ratio  $\chi^2$ : removal,  $\chi^2_1 = 12.67$ ,  $P < 0.001$ ; origin,  $\chi^2_2 = 1.08$ ,  $P = 0.57$ ; removal × origin,  $\chi^2_2 = 0.045$ ,  $P = 0.97$ ).

#### Variance components

For most traits, the standard deviation for families was smaller than the standard deviation for the error term (Tables 2, 4 and 5), indicating low differentiation of families within populations. Furthermore, the proportion of variance explained by origin was generally lower than that for residual error (Supplementary data Figure S2), indicating that plant variability within a family was higher than among origins.

## DISCUSSION

The role of local adaptation and plastic responses has been understudied, particularly among invasive trees (Lee, 2002; van

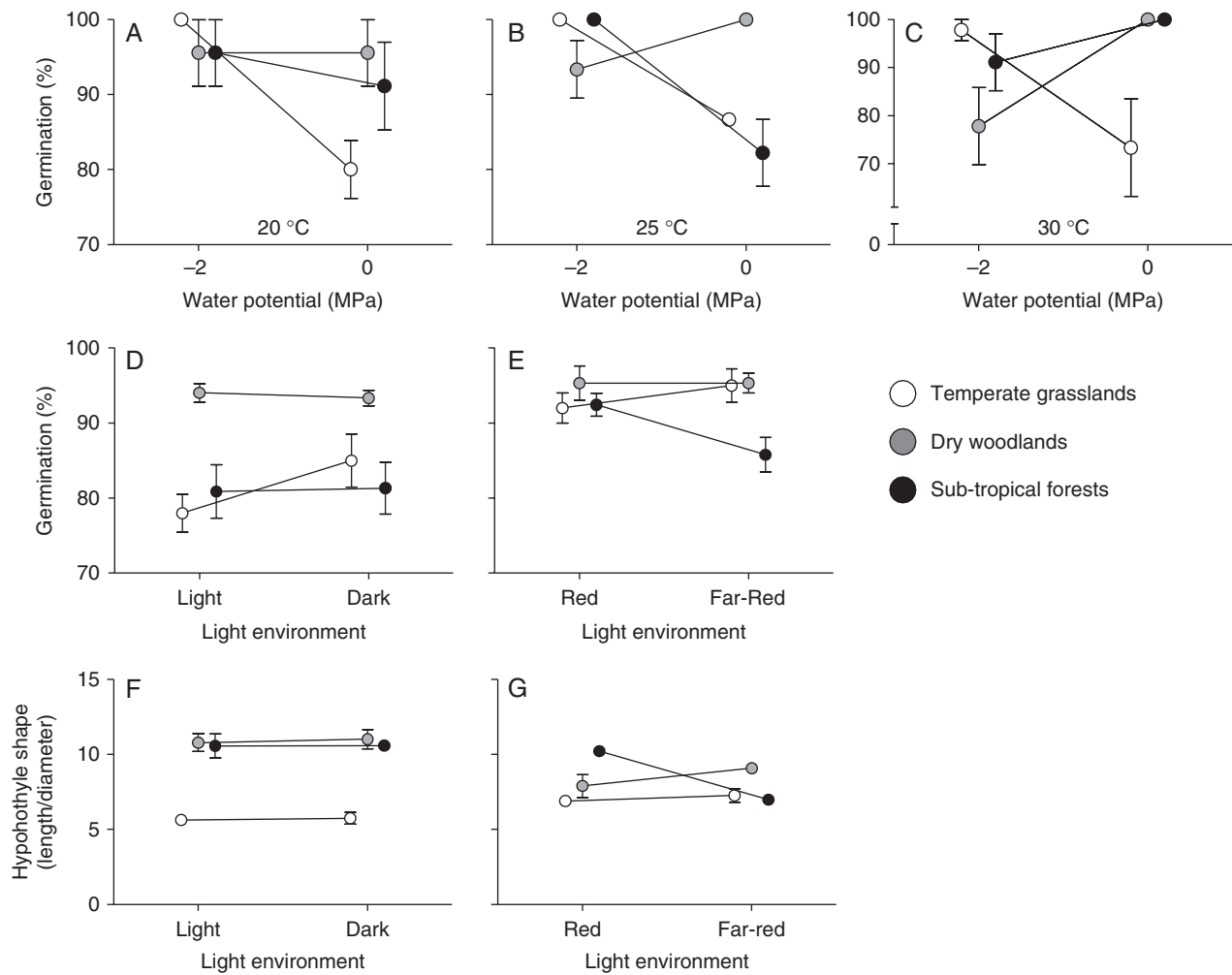


FIG. 2. Reaction norms to changes in water potential and temperature (A–C), and light conditions (D–G) on germination (A–E) and hypocotyl shape (F and G) of seeds from temperate grasslands, dry woodlands and sub-tropical forests. Vertical bars indicate  $\pm$  s.e.m.

TABLE 4. ANOVA table with Wald test results of the mixed effects models for the effect of light quantity (Light – Dark) or light quality (Red – Far Red) and the three origins (temperate grasslands, dry woodlands and subtropical forests) on the percentage of germination and the shape of the hypocotyl (length/diameter) of *G. triacanthos* seeds.

	Germination					Hypocotyl shape					
	Light–dark			R–FR		Light–dark			R–FR		
	d.f.	F	P	F	P	d.f.	F	P	d.f.	F	P
Light	1, 48	2.66	0.109	0.791	0.378	1, 363	0.09	0.753	1, 334	0.90	0.3434
Origin	2, 48	<b>7.90</b>	<b>0.001</b>	0.947	0.394	2, 20	<b>21.13</b>	<b>&lt;0.001</b>	2, 20	<b>6.78</b>	<b>0.0137</b>
O × L	2, 48	0.56	0.574	1.119	0.335	2, 363	0.11	0.891	2, 334	<b>63.08</b>	<b>&lt;0.0001</b>
Family	$\hat{\sigma}_f$	–	–	–	–		0.91 mm <sup>-1</sup> mm			1.22 mm <sup>-1</sup> mm	
Residuals	$\hat{\sigma}_e$	–	–	–	–		1.34 mm <sup>-1</sup> mm			1.90 mm <sup>-1</sup> mm	

The last two rows indicate restricted maximum likelihood estimation for the standard deviation associated with families within the three tree origins ( $\hat{\sigma}_f$ ) and the residual standard error ( $\hat{\sigma}_e$ ). Degrees of freedom are approximate and are detailed just to indicate the correct model specification. Bold indicates significant terms in the model.

Kleunen and Fischer, 2005; Facon et al., 2006; Oduor et al., 2016). Here, we found evidence of phenotypic plasticity and local adaptation for early establishment of *G. triacanthos*, an exotic tree invading different biomes worldwide. In germination

trials, we found signals of local adaptation in seed germination regarding changes in water potential and in hypocotyl shape in response to changes in light environment (Figs 1 and 2; Supplementary data Table S1). Even so, average germination

TABLE 5. ANOVA table with Wald test results of the mixed effect models for the effect of vegetation removal (intact/removed vegetation) and the three origins (temperate grasslands, dry woodlands and sub-tropical forests) on plant traits of *G. triacanthos* seedlings after 16 months (two growing seasons).

	d.f.	RGRh		Diameter		Shape (H/D)	
		F	P	F	P	F	P
Vegetation removal	1, 5	2.14	0.204	<b>10.55</b>	<b>0.022</b>	<b>13.11</b>	<b>0.015</b>
Origin	2, 20	<b>6.72</b>	<b>0.006</b>	0.33	0.722	0.10	0.901
O × R	2, 20	0.36	0.702	2.23	0.132	0.29	0.748
Family	$\hat{\sigma}_f$	0.18 ln (cm/cm)		0.43 mm		0.10 cm/mm	
Residuals	$\hat{\sigma}_e$	0.29 ln(cm/cm)		1.29 mm		1.53 cm/mm	
	d.f.	Leaves		Spines		SPAD	
		F	P	F	P	F	P
Vegetation removal	1, 5	<b>10.86</b>	<b>0.0216</b>	<b>10.14</b>	<b>0.024</b>	0.88	0.39
Origin	2, 20	0.40	0.672	1.72	0.204	1.62	0.221
O × R	2, 20	1.30	0.292	1.17	0.329	0.20	0.814
Family	$\hat{\sigma}_f$	19.98 counts		0.33 counts		3.34 units	
Residuals	$\hat{\sigma}_e$	25.39 counts		0.74 counts		5.83 units	

Measured traits were relative growth rate in height (RGRh), seedling diameter, shape (height/diameter), number of leaves, number of spines and chlorophyll level (SPAD). The last two rows in each section indicate restricted maximum likelihood estimation for the standard deviation associated with the families within origins ( $\hat{\sigma}_f$ ) and the residual standard error ( $\hat{\sigma}_e$ ). Degrees of freedom are approximate and are detailed just to indicate the correct model specification.

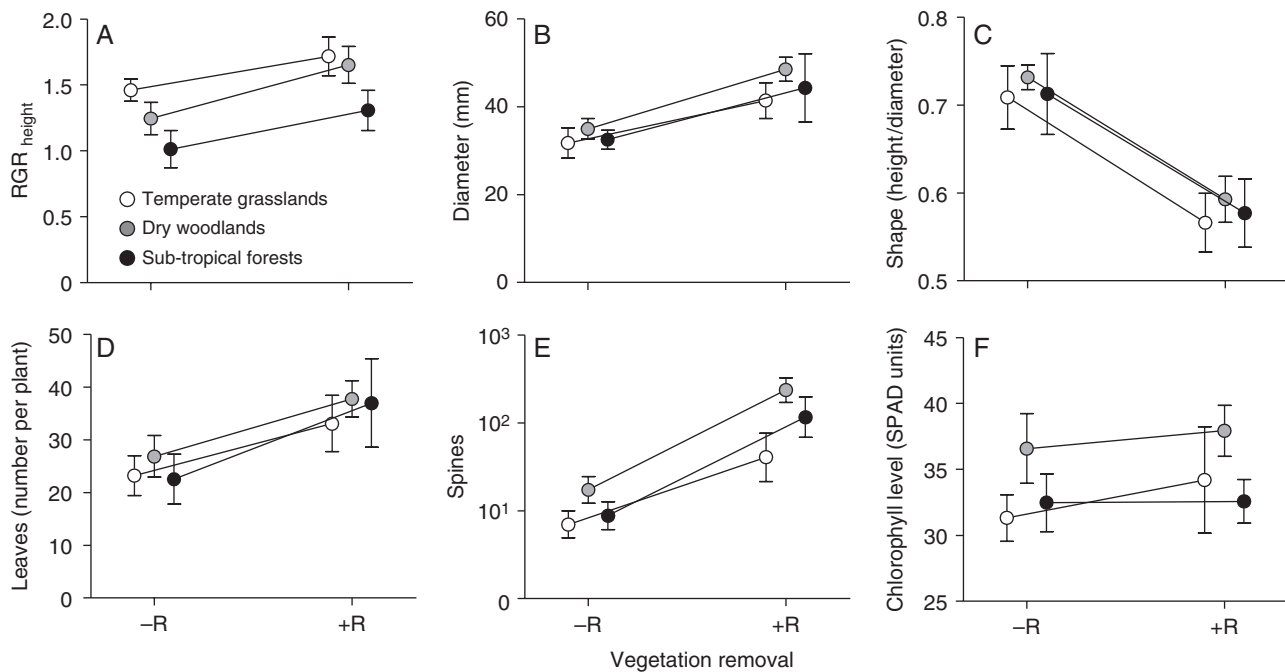


FIG. 3. Reaction norms in the common garden experiment for *G. triacanthos* seedlings growing in plots with intact (–R) or with resident vegetation removed (+R) after 16 months of growth under field conditions. (A) Relative growth rate in height [ $\ln(\text{cm}_{\text{final}} / \text{cm}_{\text{initial}})$ ]; (B) diameter (mm); (C) plant shape expressed as height / diameter ratio (cm/mm); (D) number of leaves (counts); (E) number of spines ( $\log_{10}$  scale); and (F) chlorophyll level in leaves (SPAD units). Seedlings from temperate grasslands, dry woodlands and subtropical forest were grown in a greenhouse before transplanting to the temperate grassland field site. Vertical bars indicate  $\pm$  s.e.m.

was high and independent of treatments and seed origin, suggesting that plastic responses could also play a role in explaining homeostasis (flat reaction norm; Forsman, 2015). In a common garden, vegetation removal increased seedling growth in diameter more than in height, whereas plants in intact plots grew less on average (Supplementary data Table S1). Such plastic changes in the height:diameter ratio with vegetation removal

highlight the ability of *G. triacanthos* to adjust their allometry according to changes in resource availability. Together, germination and common garden results highlight that phenotypic plasticity and local adaptation may allow *G. triacanthos* to respond over the short and long term to changes in environmental conditions and lastly contribute to shape this species as a successful woody invader.



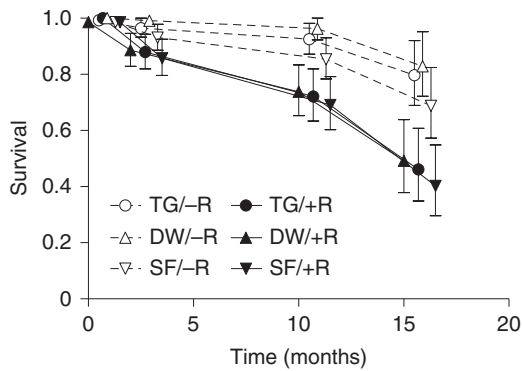


FIG. 4. Survival curves for the *G. triacanthos* seedlings (temperate grasslands, TG; dry woodlands, DW; sub-tropical forests, SF) and the two removed vegetation levels (–R/intact; +R/removed vegetation). Vertical bars are the Kaplan–Meier 95 % confidence intervals. Within a month, symbols were slightly displaced for clarity. See the Materials and Methods for further details.

#### Germination trials

Although all the experiments showed high levels of seed germination (>70 %; see Baker, 1965; Ferus *et al.*, 2013), *G. triacanthos* seeds exhibited distinctive germination patterns (Fig. 2). We found that seeds from temperate grasslands showed a consistent pattern of increasing germination with negative water potential (Fig. 2; Supplementary data Table S1). In contrast, seeds from dry woodlands and sub-tropical forests decreased germination under negative water potential. Interestingly, these opposite patterns could match prevailing environmental conditions in the origin (Table 1). Growing seasons in dry woodlands and sub-tropical forests are humid and warm (Table 1), as well as in the native range (Schnabel and Hamrick, 1990; Schnabel *et al.*, 1991; Fernandez *et al.*, 2017). Thus spring–summer precipitation allows successful seed imbibition and germination throughout the growing season (Tecco *et al.*, 2012; Fernandez *et al.*, 2017). In contrast, summers in temperate grasslands tend to be drier, and germination occurs in early summer. In this context, it is possible that seeds from temperate grassland had locally adapted to germinate under water stress condition and also had developed higher sensitivity to damage by imbibition, caused by rapid water uptake under high water potential (Powell and Matthews, 1979). This soil condition is rare during the period of seed germination of *G. triacanthos* in temperate grassland, and coincides with our records of scarce or null *G. triacanthos* invasion under oversaturated soil conditions (E. J. Chaneton, pers. obs.). The capacity to germinate at low water potential might have permitted *G. triacanthos* to succeed in the Pampa grasslands (Chaneton *et al.*, 2012). This finding suggests local adaptation of temperate grasslands seeds to achieve successful germination matching with abiotic conditions during the growth season. Similar results were found in seven populations of this woody species in the Danube basin (Ferus *et al.*, 2013). Besides these signals of local adaptation, our results also showed that the percentage seed germination of the three studied origins was relatively high under a wide range of experimental conditions (approx. 70 %; Supplementary data Table S1) that *G. triacanthos* might face in the early stages of germination (e.g. changes in water availability and light

conditions). Thus *G. triacanthos* germination might adjust to a general purpose genotype, defined as a species that thrives in a wide range of environmental conditions through phenotypic plasticity (Baker, 1965; Parker *et al.*, 2003).

We also found differences in hypocotyl shape in response to light environment among origins; seeds from temperate grasslands produced shorter and wider hypocotyls (Fig. 2; Table 4). Hypocotyl shape has been related to an adaptation to disturbances and harsh environments, thus allowing seedlings to maintain cotyledons close to the soil surface, preventing damage by fire, herbivory or drought (Fisher, 2008). Changes in hypocotyl shape may also facilitate successful emergence under crusted soils for some cultivated species (Finch-Savage and Bassel, 2016), thus enhancing seedling survival. Even though we cannot unequivocally assign these changes to any disturbance type, our results support the fact that the variations in morphological traits could be associated with environmental factors. For example, it is possible that the low length/diameter hypocotyl shape detected in the case of temperate grasslands could reduce early seedling death by drought. This abiotic stress is common in Pampean grasslands, and temporally coincides with *G. triacanthos* seedling emergence and early survival (Mazia *et al.*, 2001, 2010).

#### Common garden

In our experiment, phenotypic plasticity, rather than local adaptation, was evident for some seedling growth traits (Fig. 3). This result agrees with ecological theory, which states that the adaptative filter for successful germination and early establishment is stronger for seeds than for seedling survival and growth (Lee, 2002; Petit *et al.*, 2004). Traits related to dispersal or germination (Petit *et al.*, 2004; Donohue *et al.*, 2010) may be filtered out before traits related to growth or reproduction, which are usually more plastic (Lee, 2002). In fact, while barriers such as seed predation (Orrock *et al.*, 2006; Busch *et al.*, 2012) or extreme climatic events (i.e. dry years; Mazia *et al.*, 2010; Chaneton *et al.*, 2012) have dichotomous (live–dead) effects on early phases of establishment, filters such as the effect of resident vegetation (Chaneton *et al.*, 2012) can retard growth until a gap in the vegetation is opened (see Mazia *et al.*, 2001, 2013; Aranda *et al.*, 2015). This plastic response to disturbance mainly evidenced by changes in height:basal diameter ratio seems to be advantageous in any of the three ecosystems studied.

We found that seedling growth increased (e.g. basal diameter and leaf number) and the height:diameter ratio diminished when resident vegetation was removed (Figs 3 and 4). These results agree with previous evidences on invasive species traits in general, and with *G. triacanthos* in particular (Mazia *et al.*, 2013). In general, invasive species show positive responses to increased resource availability (Davis *et al.*, 2000; Facon *et al.*, 2006; Richards *et al.*, 2006). Such high capacity of resource capture before resident vegetation recovery generates different niche opportunities for invasive species (Davis *et al.*, 2000; Shea and Chesson, 2002). Interestingly, increasing leaf number and basal diameter suggest that seedlings in plots with vegetation removal could differentially accumulate reserves in the stem

(as starch; M. J. Aranda, pers. obs.) and deplete them during unfavourable periods or after fire or grazing. Contrarily, seedlings in intact plots showed a high height:diameter ratio (less robust seedlings), which could allow seedlings to persist under canopy light competition (Valladares *et al.*, 2006; Ramirez-Valiente *et al.*, 2010). The faster trees overcome the height of resident vegetation, the more benefits they obtain in terms of resource acquisition (Valladares *et al.*, 2006; Wakeling *et al.*, 2011; Aranda *et al.*, 2015). Finally, we found that the number of spines increased in plots with vegetation removal, which could be beneficial in repelling herbivores when the seedling is more apparent (Cooper and Owen-Smith, 1986). In summary, we found that *G. triacanthos* show responses in growth when resources increased. However, interestingly, this woody species also shows changes in allometry (height:diameter ratio); such plastic changes suggest a high capacity for successful establishment and high invader capacity under different and contrasting environmental conditions. In contrast to seedling growth results, vegetation removal diminished seedling survival (Fig. 4). Even so, a great proportion of seedlings survived independently of the applied treatment. Other studies have shown that differences in *G. triacanthos* survival may vary with climatic conditions, patch type and resident community (Mazia *et al.*, 2013, 2019).

We are aware that we did not include population genetic analysis or reciprocal transplanting that could help to discern between local adaptation and alternative causes (e.g. genetic drift and founder effects). However, considering that the process of local adaptation in woody plants is long (Premoli, 2003; Germino *et al.*, 2019), the high intrapopulation variability, probably originating from outcrossing fecundation and long-distance dispersal, could contribute to weaken the process of local adaptation (Schnabel and Hamrick, 1990; Schnabel *et al.*, 1991; Schnabel and Wendel, 1998; van Kleunen and Fischer, 2005; Oduor *et al.*, 2016). In agreement, our results showed weak signals of local adaptation and high individual variability, even within families and origin, which is expected for perennial, woody and predominantly outcrossing species (Hamrick *et al.*, 1992; Nybom, 2004). In addition, *G. triacanthos* populations were not isolated, but connected through animal and water dispersal (Schnabel and Wendel, 1998; Fernández *et al.*, 2017). This continuous genetic interchange could preclude the process of local adaptation and ecotype differentiation (Premoli, 2003; Hoffmann and Sgrò, 2011), but at the same time maintains the chance of invasion under a wide set of environmental and management scenarios (Hulme, 2008; Tecco *et al.*, 2015).

### Conclusions

Rapid local adaptation and phenotypic plasticity enable invasive plant species to become established in a broad range of novel habitats (Facon *et al.*, 2006; Oduor *et al.*, 2016). Although signs of local adaptations were weaker than plasticity, our study revealed that both mechanisms are plausible to explain the capacity of *G. triacanthos* to invade grassland, dry woodland and sub-tropical forest ecosystems. Interestingly, adaptation to local germination conditions and maintaining plastic responses to vegetation removal provide a versatile

strategy to invade different ecosystems. In particular, the capacity to germinate at low water potential might have promoted the success of *G. triacanthos* in the historically ‘tree-less’ Pampa grasslands (Chaneton *et al.*, 2012). Regional predictions for temperate grasslands, dry woodlands and sub-tropical forests suggest increases in summer precipitation and mean annual temperatures (Vera *et al.*, 2006; Barros *et al.*, 2015). Under global change scenarios (Hellmann *et al.*, 2008; Hoffman and Sgrò, 2011), high levels of phenotypic plasticity would allow *G. triacanthos* populations to respond to sudden changing environmental conditions better than locally adapted genotypes.

### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. **Figure S1**: height, diameter and number of leaves for the three *G. triacanthos* origins during two growth seasons under intact and removed vegetation. **Figure S2**: estimated percentage of the variance components including blocks, origins, families and error for different *G. triacanthos* traits in each of the chamber and field experiments. **Table S1**: estimates for the mixed effect models for imbibition, germination, hypocotyl shape and seedling traits of *G. triacanthos* from three origins. **Table S2**: ANOVA table with Wald test results of the mixed effect models for the effect of water potential and the three origins on the percentage of seed germination. **Table S3**: ANOVA table with Wald test results of the mixed effect model for the growth of *G. triacanthos* seedlings from three origins in response to vegetation removal levels during two growth seasons.

### FUNDING

This project was financed by Agencia Nacional de Promoción Científica y Tecnológica [PICT 2015-0166] and Universidad de Buenos Aires [UBACyT-2011/2014-20020100100615].

### ACKNOWLEDGEMENTS

We thank Luciano Fabi and Ezequiel Cirino for their help with germination trials and field experiments, Diego Batlla and Pedro Gundel for their suggestions on germination trials, and Fernando Biganzoli for advice on the statistical analyses. We specially thank the Handling Editor and two anonymous reviewers who provided constructive suggestions that improved the manuscript. Seeds were provided by Paula Tecco (Córdoba) and Héctor R. Grau (Tucuman). Doña Francisca Perrier de Magnin, the Pavia family and personnel from Estancia San Claudio have contributed to field experimentation for the last 40 years.

### LITERATURE CITED

- Agrawal AA. 2001. Phenotypic plasticity in the interactions and evolution of species. *Science* **294**: 321–326.
- Aranda MJ, Tognetti PM, Mazía CN. 2015. Grass competition surpasses the effect of defoliation on a woody plant invader. *Acta Oecologica* **68**: 37–42.

- Baker HG. 1965. Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL, eds. *The genetics of colonizing species*. New York: Academic Press, 147–168.
- Barros VR, Boninsegna JA, Camilloni IA, Chidiak M, Magrín GO, Rusticucci M. 2015. Climate change in Argentina: trends, projections, impacts and adaptation. *Wiley Interdisciplinary Reviews: Climate Change* 6: 151–169.
- Baskin CC, Baskin JM. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego: Academic Press.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.
- Batlla D, Kruk BC, Benech-Arnold RL. 2000. Very early detection of canopy presence by seeds through perception of subtle modifications in red:far red signals. *Functional Ecology* 14: 195–202.
- Burkart S, Sanchez RA. 1969. Interaction between an inhibitor present in the seeds of *Datura ferox* L. and light in the control of germination. *Botanical gazette* 130: 42–47.
- Burton PJ, Bazzaz FA. 1991. Tree seedling emergence on interactive temperature and moisture gradients and in patches of old-field vegetation. *American Journal of Botany* 78: 131–149.
- Busch M, Knight C, Mazía CN, Hodara K, Muschetto E, Chaneton EJ. 2012. Rodent seed predation on tree invader species in grassland habitats of the inland Pampa. *Ecological research* 27: 369–376.
- Casal JJ, Sánchez RA, Di Benedetto AH, de Miguel LC. 1991. Light promotion of seed germination in *Datura ferox* is mediated by a highly stable pool of phytochrome. *Photochemistry and Photobiology* 53: 249–254.
- Cavender-Bares J, Bazzaz FA. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* 124: 8–18.
- Chaneton EJ, Mazía CN, Machera M, Uchitel A, Ghera CM. 2004. Establishment of Honey Locust (*Gleditsia triacanthos*) in burned pampean grasslands. *Weed Technology* 18: 1325–1329.
- Chaneton EJ, Mazía N, Batista WB, Rolhauser AG, Ghera CM. 2012. Woody plant invasions in Pampa grasslands: a biogeographical and community assembly perspective. In: Myster R, ed. *Ecotones between forest and grassland*. New York: Springer, 115–144.
- Cooper SM, Owen-Smith N. 1986. Effects of plant spinescence on large mammalian herbivores. *Oecologia* 68: 446–455.
- Csurhes SM, Kriticos D. 1994. *Gleditsia triacanthos* L. (Caesalpinaceae), another thorny, exotic fodder tree gone wild. *Plant Protection Quarterly* 9: 101–105.
- Crawley MJ. 2007. *The R book*. Hoboken: John Wiley & Sons.
- Davis MA, Grime JP, Thompson K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88: 528–534.
- Donaldson JE, Hui C, Richardson DM, Robertson MP, Webber BL, Wilson JR. 2014. Invasion trajectory of alien trees: the role of introduction pathway and planting history. *Global Change Biology* 20: 1527–1537.
- Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. 2010. Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics* 41: 293–319.
- Drake JA. 1990. The mechanics of community assembly and succession. *Journal of Theoretical Biology* 147: 213–233.
- Erhardt S, Schuetzenmeister A. 2017. *Perform variance component analyses using R-Package VCA. User guides, package vignettes and other documentation*. [http://127.0.0.1:41324/help/library/VCA/doc/How\\_to\\_work\\_with\\_package\\_VCA.pdf](http://127.0.0.1:41324/help/library/VCA/doc/How_to_work_with_package_VCA.pdf).
- Facon B, Genton BJ, Shykoff J, Jarne P, Estoup A, David P. 2006. A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution* 21: 130–135.
- Fernández RD, Ceballos SJ, González Achem AL, Hidalgo MDV, Fernández HR. 2016. Quality and conservation of riparian forest in a mountain subtropical basin of Argentina. *International Journal of Ecology* 2016. 4842165. doi.org/10.1155/2016/4842165.
- Fernández RD, Ceballos SJ, Malizia A, Aragón R. 2017. *Gleditsia triacanthos* (Fabaceae) in Argentina: a review of its invasion. *Australian Journal of Botany* 65: 203–213.
- Ferreras AE, Galetto L. 2010. From seed production to seedling establishment: important steps in an invasive process. *Acta Oecologica* 36: 211–218.
- Ferus P, Barta M, Konôpková J, Turčeková S, Maňka P, Bibeň T. 2013. Diversity in honey locust (*Gleditsia triacanthos* L.) seed traits across Danube basin. *Folia Oecologica* 40: 163–169.
- Ferreras AE, Funes G, Galetto L. 2015. The role of seed germination in the invasion process of Honey locust (*Gleditsia triacanthos* L., Fabaceae): comparison with a native confamilial. *Plant Species Biology* 30: 126–136.
- Finch-Savage WE, Bassel GW. 2016. Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of experimental botany* 67: 567–591.
- Fisher JB. 2008. Anatomy of axis contraction in seedlings from a fire prone habitat. *American Journal of Botany* 95: 1337–1348.
- Forsman A. 2015. Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* 115: 276–284.
- Geng YP, Pan XY, Xu CY, et al. 2007. Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biological Invasions* 9: 245–256.
- Germino MJ, Moser AM, Sands AR. 2019. Adaptive variation, including local adaptation, requires decades to become evident in common gardens. *Ecological Applications* 29: e01842.
- Ghera CM, de la Fuente E, Suarez S, Leon RJ. 2002. Woody species invasion in the Rolling Pampa grasslands, Argentina. *Agriculture, Ecosystems & Environment* 88: 271–278.
- Giorgis MA, Cingolani AM, Chiarini F, et al. 2011. Composición florística del Bosque Chaqueño Serrano de la provincia de Córdoba, Argentina. *Kurtziana* 36: 9–43.
- Hamrick JL. 2004. Response of forest trees to global environmental changes. *Forest Ecology and Management* 197: 323–335.
- Hamrick JL, Godt MJW, Sherman-Broyles SL. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forest* 6: 95–124.
- Hardegree SP, Emmerich WE. 1990. Partitioning water potential and specific salt effects on seed germination of four grasses. *Annals of Botany* 66: 587–595.
- Hegarty T. 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant, Cell & Environment* 1: 101–119.
- Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS. 2008. Five potential consequences of climate change for invasive species. *Conservation Biology* 22: 534–543.
- Hoffmann AA, Sgrò CM. 2011. Climate change and evolutionary adaptation. *Nature* 470: 479–485.
- Hudson WH. 1918. *Far away and long ago: a history of my early life*. J.M. Dent.
- Hulme PE. 2008. Phenotypic plasticity and plant invasions: is it all Jack? *Functional Ecology* 22: 3–7.
- Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225–1241.
- van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166: 49–60.
- Lee CE. 2002. Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17: 386–391.
- Marco DE, Paéz SA. 2000. Invasion of *Gleditsia triacanthos* in *Lithraea ternifolia* montane forests of central Argentina. *Environmental Management* 26: 409–419.
- Marco DE, Paéz SA, Cannas SA. 2002. Species invasiveness in biological invasions: a modelling approach. *Biological Invasions* 4: 193–205.
- Mathiasen P, Premoli AC. 2016. Living on the edge: adaptive and plastic responses of the tree *Nothofagus pumilio* to a long-term transplant experiment predict rear-edge upward expansion. *Oecologia* 181: 607–619.
- Mazía N, Chaneton EJ, Ghera CM. 2019. Disturbance types, herbaceous composition, and rainfall season determine exotic tree invasion in novel grassland. *Biological Invasions* 21: 1351–1363.
- Mazía CN, Chaneton EJ, León RJC, Ghera CM. 2001. Limits to tree species establishment in pampean grassland and forest plant communities. *Oecologia* 128: 594–602.
- Mazía CN, Chaneton EJ, Machera M, Uchitel A, Feler MV, Ghera CM. 2010. Antagonistic effects of large- and small-scale disturbances on exotic tree invasion in a native tussock grassland relict. *Biological Invasions* 12: 3109–3122.
- Mazía CN, Tognetti PM, Cirino ED. 2013. Patch identity and the spatial heterogeneity of woody encroachment in exotic-dominated old-field grasslands. *Plant Ecology* 214: 267–277.
- Nei JL, Richardson DM, Rouget M, et al. 2004. A proposed classification of invasive alien plant species in South Africa: towards prioritizing species and areas for management action: working for water. *South African Journal of Science* 100: 53–64.

- Nybohm H. 2004.** Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.
- Oduor AMO, Leimu R, van Kleunen M. 2016.** Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. *Journal of Ecology* **104**: 957–968.
- Orrock JL, Levey DJ, Danielson BJ, Damschen EI. 2006.** Seed predation, not seed dispersal, explains the landscape-level abundance of an early-successional plant. *Journal of Ecology* **94**: 838–845.
- Oyarzabal M, Clavijo J, Oakley L, et al. 2018.** Unidades de vegetación de la Argentina. *Ecología Austral* **28**: 040–063.
- Premoli AC. 2003.** Isozyme polymorphisms provide evidence of clinal variation with elevation in *Nothofagus pumilio*. *Journal of Heredity* **94**: 218–226.
- Parker IM, Rodriguez J, Loik ME. 2003.** An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology* **17**: 59–72.
- Petit RJ, Bialozyt R, Garnier-Géré P, Hampe A. 2004.** Ecology and genetics of tree invasions: from recent introductions to Quaternary migrations. *Forest Ecology and Management* **197**: 117–137.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017.** *nlme: linear and nonlinear mixed effects models*. R package version 3.1–131. <https://CRAN.R-project.org/package=nlme>.
- Powell AA, Matthews S. 1979.** The influence of testa condition on the imbibition and vigour of pea seeds. *Journal of Experimental Botany* **30**: 193–197.
- Ramírez-Valiente JA, Sánchez-Gómez D, Aranda I, Valladares F. 2010.** Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiology* **30**: 618–627.
- R Core Team. 2017.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M. 2006.** Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* **9**: 981–993.
- Roman J, Darling JA. 2007.** Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* **22**: 454–464.
- Rotundo JL, Aguiar MR, Benech-Arnold R. 2015.** Understanding erratic seedling emergence in perennial grasses using physiological models and field experimentation. *Plant Ecology* **216**: 143–156.
- Schielzeth H, Nakagawa S. 2013.** Nested by design: model fitting and interpretation in a mixed model era. *Methods in Ecology and Evolution* **4**: 14–24.
- Schnabel A, Hamrick JL. 1990.** Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* **77**: 1060–1069.
- Schnabel A, Wendel JF. 1998.** Cladistic biogeography of *Gleditsia* (Leguminosae) based on *ndhF* and *rpl16* chloroplast gene sequences. *American Journal of Botany* **85**: 1753–1765.
- Schnabel A, Laushman RH, Hamrick JL. 1991.** Comparative genetic structure of two co-occurring tree species, *Maclura pomifera* (Moraceae) and *Gleditsia triacanthos* (Leguminosae). *Heredity* **67**: 357–364.
- Sexton JP, McKay JK, Sala A. 2002.** Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications* **12**: 1652–1660.
- Shea K, Chesson P. 2002.** Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* **17**: 170–176.
- Sultan SE. 2000.** Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537–542.
- Tecco PA, Ferreras AE, Gurvich DE, Funes G. 2012.** Similarities in recruitment but differences in persistence in two related native and invasive trees: relevance of regenerative and vegetative attributes. *Australian Journal of Botany* **60**: 368–377.
- Tecco PA, Pais-Bosch AI, Funes G, Marcora PI, Zeballos SR, Cabido M, Urcelay C. 2015.** Mountain invasions on the way: are there climatic constraints for the expansion of alien woody species along an elevation gradient in Argentina? *Journal of Plant Ecology* **9**: 380–392.
- Therneau TM. 2015.** *A package for survival analysis in S*. Version 2.38 <https://cran.r-project.org/web/packages/survival/index.html>
- Therneau TM, Grambsch PM. 2000.** *Modeling survival data: extending the Cox model*. New York: Springer.
- Tognetti PM, Chaneton EJ, Omacini M, Trebino HJ, León RJC. 2010.** Exotic vs. native plant dominance over 20 years of old-field succession on set-aside farmland in Argentina. *Biological Conservation* **143**: 2494–2503.
- Tsegay BA, Olsen JE, Junntilla O. 2005.** Effect of red and far-red light on inhibition of hypocotyl elongation in ecotypes of *Betula pendula*. *African Journal of Biotechnology* **4**: 50–56.
- Valladares F, Sanchez-Gomez D, Zavala MA. 2006.** Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* **94**: 1103–1116.
- Vera C, Silvestri G, Liebmann B, González P. 2006.** Climate change scenarios for seasonal precipitation in South America from IPCC-AR4 models. *Geophysical Research Letters* **33**: L13707. doi:10.1029/2006GL025759.
- Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995.** Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution* **10**: 212–217.
- Wakeling JL, Staver AC, Bond WJ. 2011.** Simply the best: the transition of savanna saplings to trees. *Oikos* **120**: 1448–1451.
- Williamson M. 1996.** *Biological invasions*. London: Chapman and Hall.
- Zak MR, Cabido M, Hodgson JG. 2004.** Do subtropical seasonal forests in the Gran Chaco, Argentina, have a future? *Biological Conservation* **120**: 589–598.
- Zakeri H, Schoenau J, Vandenberg A, Tayfeh Aligodarz M, Bueckert RA. 2015.** Indirect estimations of lentil leaf and plant N by SPAD chlorophyll meter. *International Journal of Agronomy* **2015**: 748074. doi.org/10.1155/2015/748074.
- Zenni RD, Nuñez MA. 2013.** The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos* **122**: 801–815.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009.** *Mixed effects models and extensions in ecology with R*. Berlin: Springer.