



Invasive *Teline monspessulana* and *Ulex europaeus* allelochemicals induce differential responses regarding the growth and physiological performance of two native Chilean tree species

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Abstract

Teline monspessulana and *Ulex europaeus* (Fabaceae) are highly invasive species distributed worldwide. In Chile, both species share a similar distribution area with the native species *Quillaja saponaria* and *Peumus boldus*. This research focused on determining the effects caused by the aqueous extracts of the aerial organs of both invaders on the initial growth and photosynthetic performance of the two native tree species. Experiments were established under controlled conditions simulating natural conditions where both invasive and native species coexist. Morphometric variables defining root and aerial development were evaluated. Photosynthetic performance was also evaluated by measuring chlorophyll fluorescence, pigment content, and non-structural carbohydrates. In *Q. saponaria* seedlings, the growth of aerial and root organs was affected, but the production of photoassimilates increased under allelochemical stress. No damage to the photosynthetic machinery was observed in *P. boldus*, but its initial growth was notably reduced, possibly compromising its survival when interacting with both invasive species. The results indicated that allelochemicals synthesized by the two invasive species differentially affected the morphophysiological performance of the two native species. In terms of carbon administration, our results indicate that *Q. saponaria* seedlings probably use the assimilated carbon for biomass and defensive compound production. In contrast, *P. boldus* seedling growth is more sensitive to allelochemical stress and is seriously compromised, maybe because its metabolism is mainly invested in defensive compounds to deal with invasive species.

Keywords Carbohydrates · Morphometry · *Peumus boldus* · Photosynthesis pigments · *Quillaja saponaria*

Abbreviations

W-Qs	<i>Q. saponaria</i> Seedlings irrigated with water
W-Pb	<i>P. boldus</i> Seedlings irrigated with water
ET-Qs	<i>Q. saponaria</i> Seedlings irrigated with <i>Teline monspessulana</i> aqueous extract
ET-Pb	<i>P. boldus</i> Seedlings irrigated with <i>Teline monspessulana</i> aqueous extract
EU-Qs	<i>Q. saponaria</i> Seedlings irrigated with <i>Ulex europaeus</i> aqueous extract
EU-Pb	<i>P. boldus</i> Seedlings irrigated with <i>Ulex europaeus</i> aqueous extract

Introduction

More than 70% of the plant population of Chile is developing in the Mediterranean forest area located in the central zone of the country, between 30° and 37°S latitude (Mcwethy et al. 2018). Although, these forests are currently dominated by *Acacia caven* (Mol.) Seigler & Ebinger and *Nothofagus*

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Blume trees, other native species such as *Prospopis chilensis* (Mol.) Stuntz, *Lithraea caustica* (Mol.) Hook & Arn, *Cryptocaya alba* (Mol.) Looser, *Quillaja saponaria* Molina, and *Peumus boldus* Molina, are also present. Deforestation due to changes in land use and forest fires are the main causes of species loss in the Chilean Mediterranean forest (Alaniz et al. 2022). Additionally, some native species suffer overexploitation due to their commercial use for the production of saponins (*Q. saponaria*) and boldine (*P. boldus*) or to their use for combustion as charcoal and firewood (Castro-Saavedra et al. 2016). This practice generates a greater frequency of open areas in forests, facilitating colonization by many invasive scrub species (Mcwethy et al. 2018).

Invasive Fabaceae species such as *A. dealbata* Link, *Ulex europaeus* L., and *Teline monspessulana* (L.) L.A.S. Johnson are characterized by their abundance and capacity to colonize deforested Chilean areas (Fuentes-Ramírez et al. 2011; García et al. 2014; Altamirano et al. 2016). According to the International Union for Conservation of Nature (Lowe et al. 2000), *U. europaeus* is considered to be among the 100 most harmful invasive species around the world. Foreign species have numerous mechanisms that make them successful in their non-native range. Special attention has been devoted to the allelopathic effect produced by these plants on native species. Fabaceae is recognized for having highly phytotoxic species, which mainly synthesize alkaloids and phenols (Wink 2020), like in *A. dealbata* (Aguilera et al. 2015a, b, c), *T. monspessulana* (Aguilera et al. 2023), and *U. europaeus* (Hornoy et al. 2012).

Although, the allelopathic potential of *A. dealbata* has been the most studied, recent studies have demonstrated the allelopathic potential of *T. monspessulana* and *U. europaeus*, mainly related to the presence of quinolizidine alkaloids and phenols compounds in both invasive species. The negative effects of aqueous extracts of *T. monspessulana* on the initial growth of the native Chilean species *Nothofagus obliqua* (Mirb.) Oerst. (Nothofagaceae) were recently described (Aguilera et al. 2023). Both *T. monspessulana* and *U. europaeus* also induce an inhibitory effect on the initial growth and polyphenol profile and concentration of *C. alba*, other Chilean native species (Rodríguez-Cerda et al. 2023).

The allelochemicals affect the integrity of the cell membrane (Hejl and Koster 2004), modify the water status and nutrient uptake (Soltys et al. 2012), alter the transport of electrons in the photosynthetic and respiratory chain (Hussain and Reigosa 2011), interfere with the hormonal balance of plants (Li et al. 2022), and alter protein synthesis and activity and gene expression (Aci et al. 2022). In addition, the compounds produced by the invasive species interfere with cell division and elongation (Anaya et al. 2002; Franche et al. 2009), altering the growth and development of recipient native plants. Growth cessation caused by allelopathic compounds affects plants along all life

stages of development from seed to mature plants, involving changes in seed germination, seedling growth, leaf area, dry matter, and production (Yarnia et al. 2009). Discharge of allelochemical components from plants into the environment occurs by exudation of volatile compounds or leaching of water-soluble toxins from aboveground parts responding to the action of rain (Gella et al. 2013). The inhibitory effect of plant extracts has been proportional to their concentration, with higher concentrations having a stronger inhibitory effect on growth (Ahmed et al. 2007).

Given the overlapping distribution of *U. europaeus* and *T. monspessulana* with the native species *Q. saponaria* and *P. boldus*, the objective of the present study was to investigate the impact of allelochemical compounds released by these two Fabaceae on the early growth and physiological parameters of both native species. Previous studies have indicated that *Q. saponaria* is sensitive to *A. dealbata* extracts, which reduce the length of the hypocotyl and radicle and induce damage at the root level (Aguilera et al. 2015b, c). Contrary, for *P. boldus*, as far as we know, no previous studies have focused on the effect of invasive species on its establishment. Furthermore, the allelopathic harmful potential of *U. europaeus* and *T. monspessulana* for the distribution limit of the Mediterranean forest has not been investigated. In this context, we hypothesize that the aqueous extracts of aerial organs of *T. monspessulana* and *U. europaeus* will induce negative effects on the physiological and biochemical performance and on the initial stages of growth of *Q. saponaria* and *P. boldus* seedlings. The results of this research will contribute to elucidating the effects of invasive species on the Chilean native flora and to the preparation of management and conservation plans for native species in their natural environment.

Materials and methods

Collection of plant material

Quillaja saponaria seeds were collected from adult trees of a native forest in Paine, Metropolitan Region, Chile. Due to the difficulty of inducing *P. boldus* germination under laboratory conditions, the assays were carried out with seedlings from a remnant of native forest located in the Hualpén Peninsula, Biobío Region, Chile. The aerial organs (stems, leaves, flowers, and pods) of 10 *U. europaeus* plants were collected in Lenga, Hualpén Peninsula, Biobío Region, Chile. The aerial organs of 10 *T. monspessulana* plants (stems, leaves, flowers, and pods) were collected on a hill located in the vicinity of the University of Concepción, Concepción campus, Biobío Region, Chile.

Collection and preparation of substrates

Substrates were collected at sites where *Q. saponaria* and *P. boldus* plants grow naturally in the Hualpén Peninsula, Biobío Region, Chile. The substrates were scooped at seven different points, to a depth of 5–7 cm below the canopy of each plant species, without exceeding the drip area. Each fraction consisted of approximately 1 kg of substrate, which were gathered in a pool. Under laboratory conditions, the substrates were separately sieved and deposited in plastic trays with alveoli regarding *Q. saponaria* and in 6 l plastic boxes regarding *P. boldus*.

Preparation of aqueous extracts of *T. monspessulana* and *U. europaeus*

The aerial organs (stems, leaves, flowers, and pods) of both *T. monspessulana* and *U. europaeus* were cut with pruning shears immediately after plant harvesting and left in aqueous maceration (360 g l^{-1} , aerial organs/distilled water) under agitation at 170 rpm at room temperature (approx. $15 \text{ }^\circ\text{C}$) and in the dark. After 24 h, the extracts were filtered through Whatman 1 filter paper, and 2% PMM (plant preservation medium, Plant Cell Technology, USA) was added. The preparation was refrigerated and left to stand in the dark at $4 \text{ }^\circ\text{C}$. The extracts were used to irrigate *Q. saponaria* and *P. boldus* seedlings.

Establishment of assay

Quillaja saponaria seedlings were cultivated in germinators consisting of 6 l plastic boxes with the bottom lined with filter paper and containing 30 ml of water and 2% PPM solution. When the seedlings reached 1–1.5 cm in length, they were planted in plastic trays with substrates (one tray with 50 alveoli per treatment) at the rate of one seedling per alveoli. *Peumus boldus* seedlings, collected from the field, were transplanted into 6 l plastic boxes (2 boxes with 5 seedlings per treatment) containing substrate.

During the first week of planting/transplanting, the seedlings of both species were irrigated with tap water. After this time, a plastic tray of 50 alveoli with *Q. saponaria* (W-Qs) seedlings and two 6 l plastic boxes with *P. boldus* (W-Pb) seedlings were only irrigated with water and used as controls. Additionally, one tray/two boxes of each species were irrigated with the aqueous extract of *U. europaeus* (EU-Qs and EU-Pb) and two others with the aqueous extract of *T. monspessulana* (ET-Qs and ET-Pb) at a rate of 10 ml per plant. Irrigation was carried out every three days for 90 days in the case of *Q. saponaria* and up to 180 days in the case of *P. boldus*.

Morphometric parameters

The stem and root length (cm), number of leaves and secondary root of seedlings of *Q. saponaria* ($n=20$) and *P. boldus* ($n=10$) from control and every treatment with invasive plant extracts were measured at the end of each assay. Due to the complex root system of *P. boldus*, the number of secondary roots (SR) was difficult to count and therefore a range of categorization was established as follows: numerous roots (> 40 SR), abundant roots (between 20–40 SR), few roots (< 20 SR) and no development of secondary roots (0). After the morphometric measurements, seedlings were wrapped with aluminum foil and placed in an oven at $60 \text{ }^\circ\text{C}$ until they reached a stable weight (around one week) and the biomass was evaluated as dry mass (g).

Chlorophyll fluorescence measurements

The transitory fluorescence of Chl *a* between light and dark adaptation was obtained using the Handy FluorCam (Photon Systems Instruments, Czech Republic) with the FluorCam 7 software (quenching protocol). The analysis was performed on leaves of native plants from each treatment and control ($n=5$ leaves per species and treatments) after 30 min of dark adaptation (W-Qs, EU-Qs, ET-Qs and W-Pb, EU-Pb, ET-Pb; $n=5$ for each treatment). The Kautsky effect (Stirbet 2011) was calculated to determine the Chl relaxation to excitation cycle. The following photosynthetic parameters associated with the Kautsky effect were calculated and used in the current study: F_0 —minimum fluorescence of PSII in dark-adapted state; F_m —maximum fluorescence of PSII in dark-adapted state; F_v/F_m —maximum PSII quantum yield in dark-adapted state, where $F_v = F_m - F_0$; $(F_m' - F')/F_m'$ —the quantum yield of PSII (ϕ_{PSII}) representing the number of electrons transferred per photon absorbed by PSII, where F_m' is the fluorescent signal when all PSII centers are closed in the light-adapted state and F' is the measurement of the light-adapted fluorescence signal; and NPQ—non-photochemical quenching calculated by $(F_m - F_m')/F_m'$ (Genty et al. 1990; Oxborough 2004).

Photosynthetic pigment contents

Fresh disks 10 mm in diameter ($n=10$ leaves for different plants and one disk per leaf) of native species from each treatment and control (W-Qs, EU-Qs, ET-Qs and W-Pb, EU-Pb, ET-Pb) were weighed and immersed in 80% acetone (v/v) for the extraction of photosynthetic pigments. The extracts were analyzed with a spectrophotometer (470, 667, and 664 nm of wavelength), and the contents of chlorophyll and carotenoids were calculated according to the equations proposed by Lichtenthaler and Wellburn (1983) and expressed as mg g^{-1} of fresh weight.

Non-structural carbohydrates

Total soluble sugar (TSS: combined glucose, fructose, and sucrose concentrations) and starch concentrations were evaluated by the method of Marquis et al. (1997) using 100 mg of fresh *Q. saponaria* leaf tissue. Total soluble sugars were extracted with a methanol/chloroform/water (12/5/3, v/v/v) solution, separated from nonpolar pigments and lipids with the addition of 2 ml chloroform and 1.5 ml deionized water (Dickson 1979). Starch from the insoluble fraction was hydrolyzed to glucose overnight using a sodium acetate buffer and amyloglucosidase (Sigma-Aldrich 10,115, USA) at 45 °C and then measured with a phenol–sulfuric acid reaction (Marquis et al. 1997). All samples were read in triplicates using 490 nm of absorbance (Chow and Landhäusser 2004) in a microplate reader (ELX800, BioTek, USA). The starch and soluble carbohydrate contents were expressed as mg mg⁻¹ of fresh weight.

Quantification of the stress tolerance

The ability to maintain growth in the presence of allelochemical stress conditions was quantified using the Stress Tolerance Index (STI) (Fernandez 1992; Negrão et al. 2017). Higher values of STI means higher tolerance to stress (Nazari and Pakniyat 2010).

Statistical analysis

The experiments were established according to a completely randomized design. The data were analyzed by one-way ANOVA and the means were compared by Tukey's test. Data that did not meet the assumptions of normality (Shapiro–Wilks test) and homogeneity (Levene test) were analyzed using the non-parametric Kruskal–Wallis test and the Mann–Whitney test for a posteriori comparison. Significant differences were considered when the *P*-value was ≤ 0.05.

Results

Effect of the invasive Fabaceae extracts on morphometric parameters of *Q. saponaria* and *P. boldus*

Aqueous extracts of *U. europaeus* and *T. monspessulana* significantly affected the early growth of *Q. saponaria* seedlings (Fig. 1a). The stem length was significantly reduced by irrigation with EU, but no differences were found between irrigation with water and with ET extract in *Q. saponaria* seedlings (Fig. 1b). In contrast, the radicular system, including root length and number of secondary roots, was reduced by 51.0% and 64.7%, respectively, by irrigation with

ET (Fig. 1c and d). Also, the number of leaves was more affected by ET, with a significant reduction in the numbers of leaves of *Q. saponaria* (Fig. 1E). Despite the differential effect of the two extracts (ET and EU) on the aerial and radicular structure and biomass of *Q. saponaria*, the total biomass, evaluated as dry mass, did not differ between treatments (Fig. 1f).

Aqueous extracts of *U. europaeus* and *T. monspessulana* significantly affected the initial growth of *P. boldus* seedlings (Fig. 2a). *T. monspessulana* extract and EU significantly reduced plant length by 35.6% and 57.1%, respectively, with significant differences between the two treatments (Fig. 2b). Although ET did not affect root length, EU significantly reduced (44.8%) this variable (Fig. 2c). The number of leaves also decreased significantly in both treatments, with significant differences between them (Fig. 2d). For example, EU caused a 2.5-fold reduction of the number of leaves of *P. boldus* seedlings, while ET caused a 1.2-fold reduction (Fig. 2d). The frequency of secondary roots formed under the effect of ET ranged from abundant to low, while the formation of secondary roots was infrequent under the effect of EU, and in at least two seedlings no secondary roots were formed (Fig. 2e). *Ulex europaeus* extract (EU) was the one that induced the greatest decrease (71.2%) in dry mass compared to control and ET, although the latter also significantly reduced this variable about 50% (Fig. 2f).

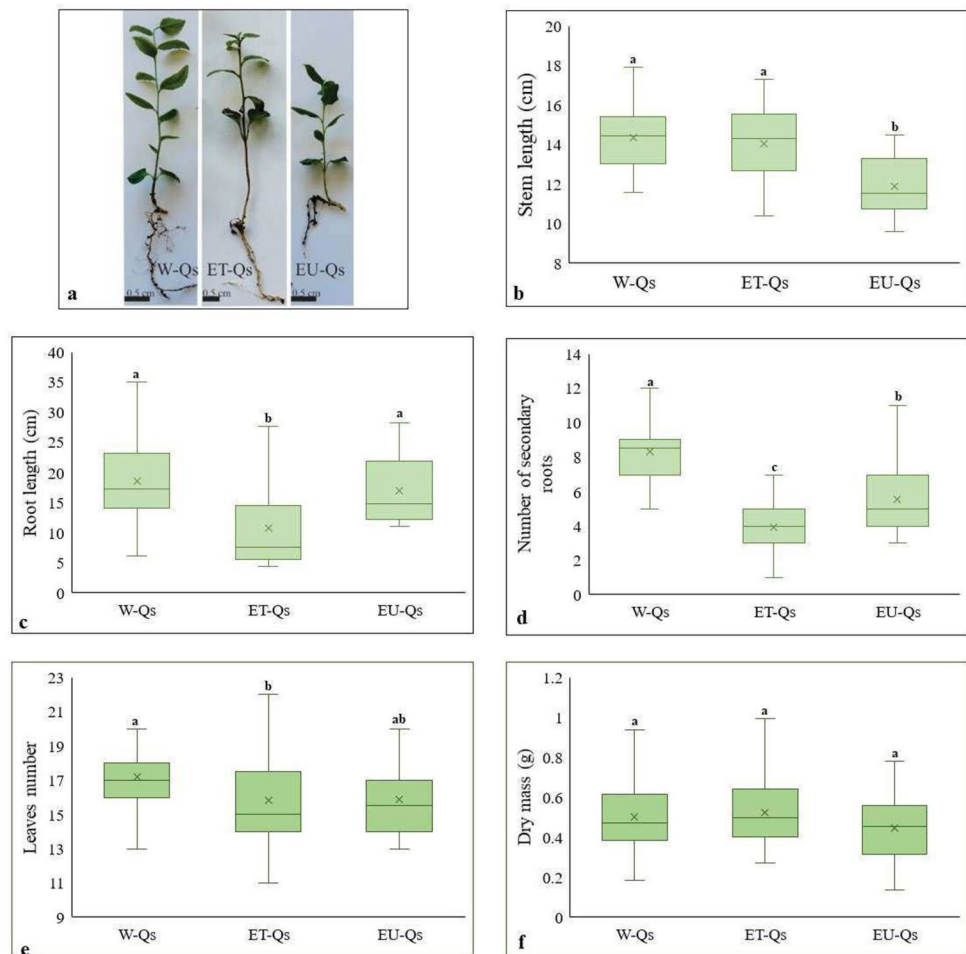
Chlorophyll a fluorescence and photosynthetic pigment contents

Fluorescence measurements indicated that neither Fv/Fm nor NPQ were significantly affected by invasive plant extracts in *Q. saponaria* (Table 1). In contrast, the quantum yield of PSII was significantly reduced by both EU-Qs and ET-Qs (Table 1). Total chlorophyll content tended to decrease with extract irrigation, although this decrease was significant only for EU-Qs (Fig. 3a). The carotenoid content of *Q. saponaria* increased significantly in the seedling leaves irrigated with EU and ET (Fig. 3b). For *P. boldus*, no parameter related to chlorophyll fluorescence (Fv/Fm, ϕ PSII and NPQ) or photosynthetic pigments (chlorophylls and carotenoids) showed variation after irrigation with ET or EU (Table 1; Fig. 3c and d).

Non-structural carbohydrate production in *Q. saponaria*

Due to the significant effect of both extracts (EU and ET) on the quantum yield of *Q. saponaria*, we decided to determine if the impact observed on light capture (Table 1) was extrapolated to the assimilatory phase; therefore, we evaluated carbohydrate production as a proxy of carbon assimilation in *Q. saponaria*. The total soluble sugars of leaves were

Fig. 1 Effect of irrigating *Quil-laja saponaria* seedlings with water (W-Qs), *Teline monspessulana* extract (ET-Qs) and *Ulex europaeus* extract (EU-Qs) on morphometric variables, **A** External appearance of the seedlings at the end of the assay, **B** Stem length, **C** Root length, **D** Number of secondary roots, **E** Leaf number, **F** Dry mass. Data are reported as the median. Different letters indicate significant differences between treatments at $P \leq 0.05$



significantly increased by ET and EU irrigations, increasing by 36.5% and 27.2% compared to water irrigation (Fig. 4a). Despite these variations, the starch levels of *Q. saponaria* were unchanged after ET and EU irrigation (Fig. 4b).

Stress tolerance index

The STI values for *Q. saponaria* irrigated with ET and EU extracts did not have significant differences between them ($P=0.229$) (Fig. 5). On contrast, the STI were lower for *P. boldus*, showing significant less tolerance to irrigation with *U. europaeus* than *T. monspessulana* extracts ($P < 0.01$) (Fig. 5).

Discussion

Allelopathy has been described as a complex phenomenon mediated by the release of certain chemical compounds, namely allelochemicals, which can induce stimulatory or inhibitory effects depending on the target species and the response studied (Espinosa-García 2007; Singh 2021). In

our study, the aqueous extract of *T. monspessulana* and *U. europaeus* induced differential effects on the morphometric, physiological, and biochemical parameters of the native *Q. saponaria* and *P. boldus* at the seedling level, i.e., at the stage of plant establishment which is a critical period of vegetative development.

Aqueous extract of invasive species differentially affected the development of *Q. saponaria* and *P. boldus*

The investigation revealed that allelochemicals extracts of *U. europaeus* and *T. monspessulana* induced a negative and differential effect on the aerial and radicular development of *Q. saponaria*, with EU-Qs mainly affecting stem length, while ET-Qs significantly reduced the number of roots and also induced leaf chlorosis. These differential responses are probably due to differences in the composition and concentration of active compounds released by *U. europaeus* and *T. monspessulana*, which can act separately or synergistically producing differential effects on the early growth of *Q. saponaria*, as previously described for *C. alba* (Rodríguez-Cerda

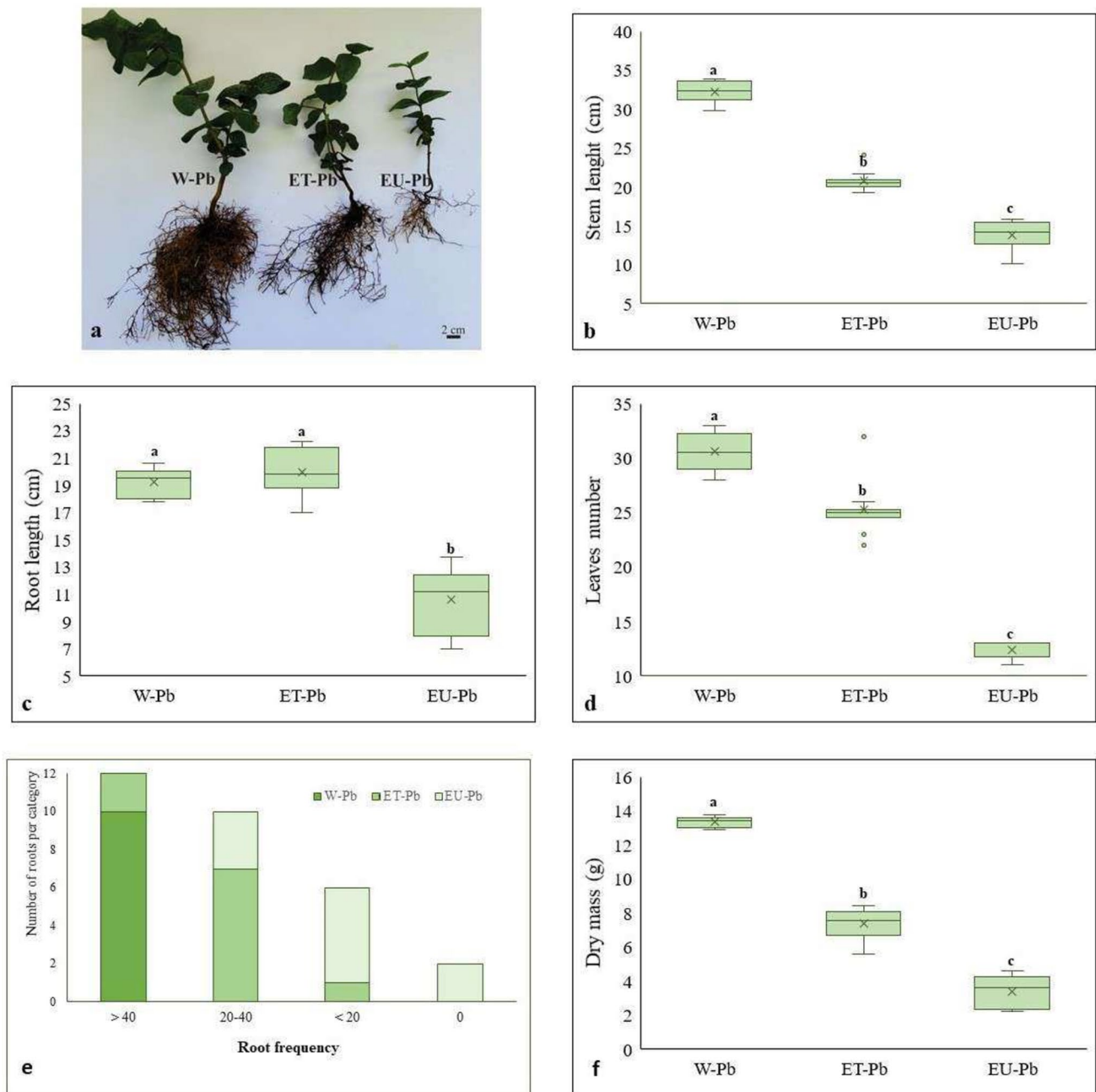


Fig. 2 Effect of irrigating *Peumus boldus* seedlings with water (W-Pb), *Teline monspessulana* extract (ET-Pb) and *Ulex europaeus* extract (EU-Pb) on morphometric variables, **A** External appearance of the seedlings at the end of the assay, **B** Stem length, **C** Root length,

D Leaf number, **E** Root frequency, **F** Dry mass. Data are reported as the median. Different letters indicate significant differences between treatments at $P \leq 0.05$

et al. 2023). Although both invaders affect the initial growth of *C. alba*, the damage induced by *T. monspessulana* occurs at the foliar level, while *U. europaeus* induces damage at the root level (Rodríguez-Cerda et al. 2023). Furthermore, *T. monspessulana* induced an increase in the phenolic and anthocyanin contents of *C. alba* leaves, while *U. europaeus* did not affect either the phenolic or anthocyanin contents (Rodríguez-Cerda et al. 2023). It has also been reported

that *T. monspessulana* induces initial growth retardation and histological damage on *N. obliqua* seedlings, related to the alkaloids and phenols detected in its aqueous extracts (Aguilera et al. 2023). Quinolizidine alkaloids have been reported in the aerial organs of *T. monspessulana* (Aguilera et al. 2023) and *U. europaeus* (Hornoy et al. 2012), which induce alteration of membrane permeability and protein synthesis (Latif et al. 2017). Specially, lupanine, reported

Table 1 Fluorescence analysis of *Quillaja saponaria* and *Peumus boldus* leaves irrigated with water (W), *Ulex europaeus* extract (EU) and *Teline monspessulana* extract (ET)

Plant species	Parameters	Treatments			P-values
		W	ET	EU	
<i>Q. saponaria</i>	Fv/Fm	0.84 ± 0.01	0.83 ± 0.01	0.83 ± 0.02	0.546
	Φ_{PSII}	0.71 ± 0.01 ^a	0.65 ± 0.02 ^b	0.66 ± 0.01 ^b	0.001
	NPQ	1.35 ± 0.1	1.72 ± 0.3	1.56 ± 0.3	0.161
<i>P. boldus</i>	Fv/Fm	0.8 ± 0.01	0.8 ± 0.01	0.8 ± 0.01	0.871
	Φ_{PSII}	0.6 ± 0.01	0.6 ± 0.01	0.6 ± 0.01	0.531
	NPQ	1.7 ± 0.06	1.6 ± 0.06	1.8 ± 0.06	0.600

The evaluated variables PSII maximal operating efficiency at saturating light (Φ_{PSII}), maximum PSII quantum yield in the dark-adapted state (F_v/F_m), non-photochemical quenching (NPQ) are represented by their mean and the standard error. Different letters in the same row, mean significant differences between treatments for P -value ≤ 0.05

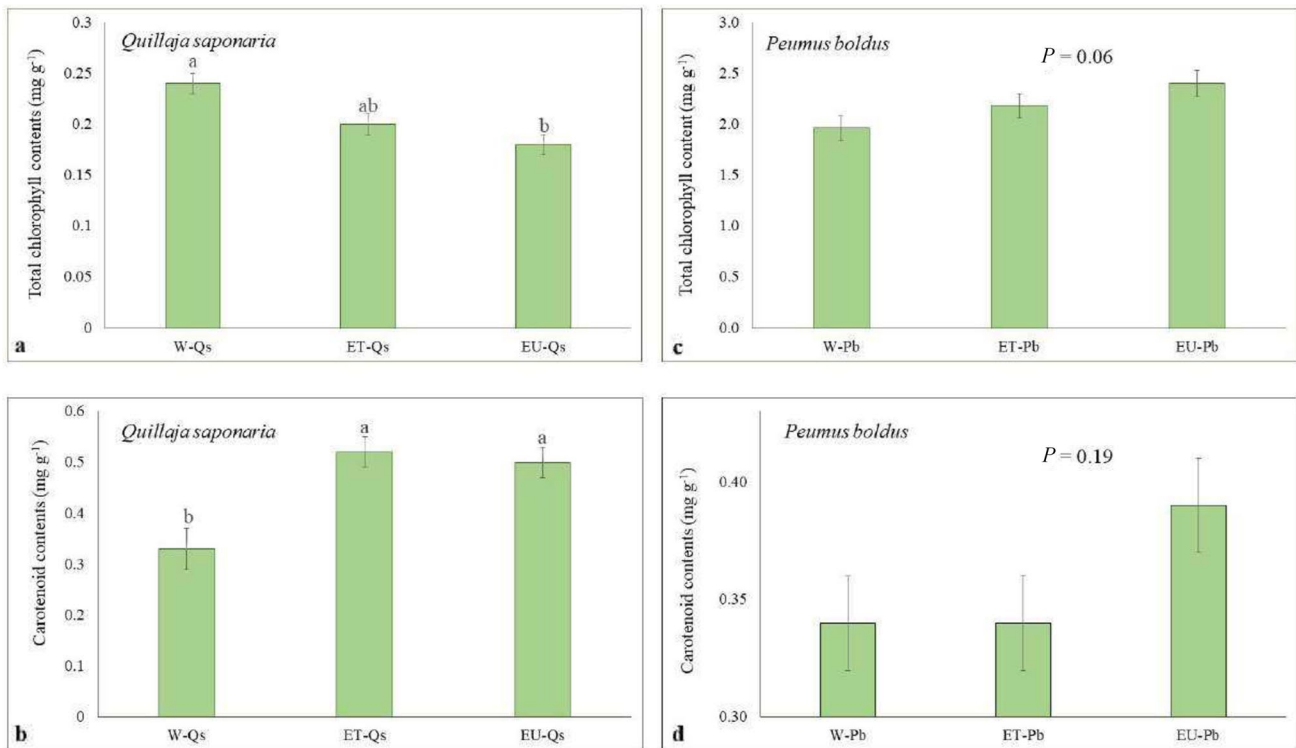


Fig. 3 Photosynthetic pigment contents of *Quillaja saponaria* and *Peumus boldus* seedlings irrigated with water (W-Qs), with the aqueous extract of *Teline monspessulana* (ET-Qs) and with the aqueous extract of *Ulex europaeus* (EU-Qs), **A** and **C** Total chlorophyll, **B** and

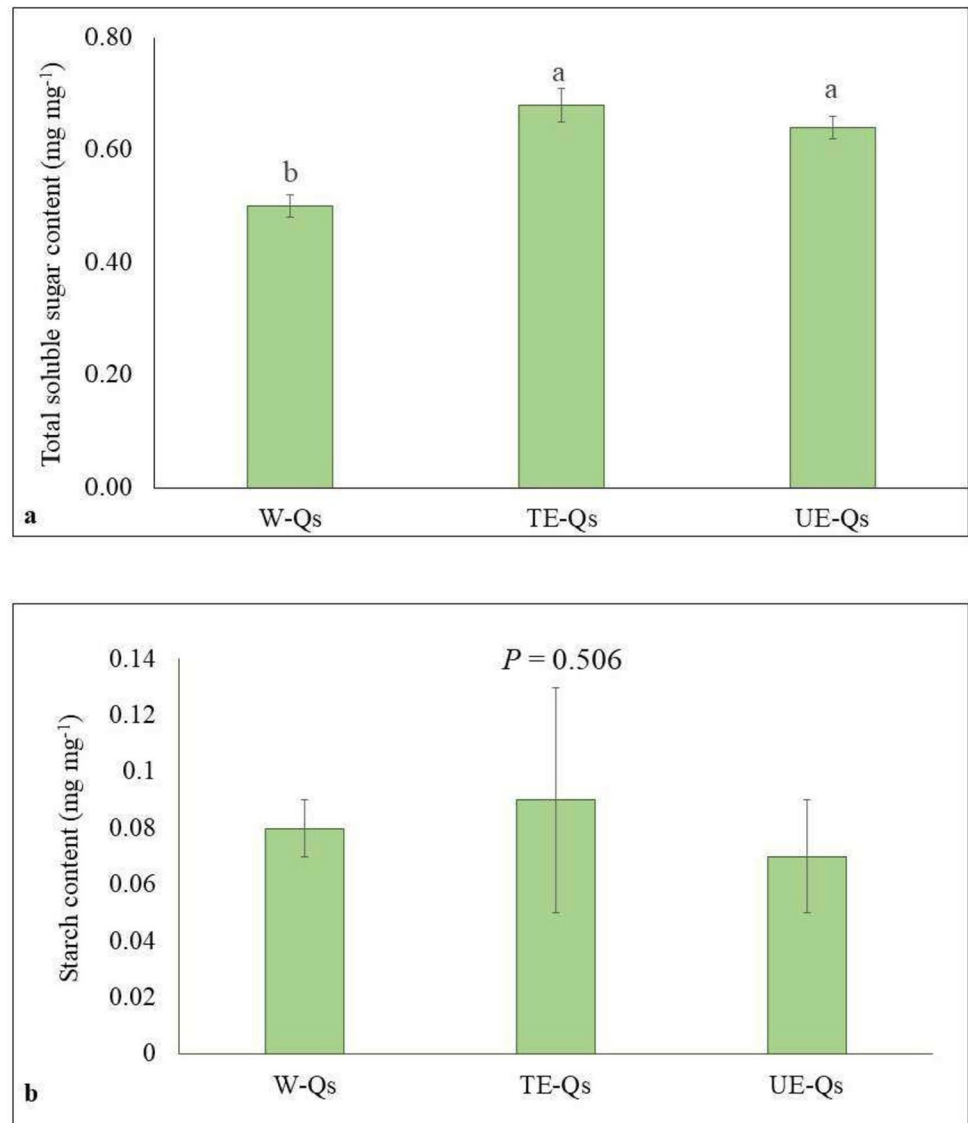
D Carotenoid contents. Data are reported as mean and standard deviation. Different letters indicate significant differences between treatments at $P \leq 0.05$

in both *T. monspessulana* (Aguilera et al. 2023) and *U. europaeus* (Hornoy et al. 2012) extracts reduces the germination of some plant species (Zamora et al. 2007), intercalating into DNA and inhibiting topoisomerase II (Bermúdez-Torres et al. 2009). Phenolic compounds, mostly phenolic acids and flavonoids, have been reported in *T. monspessulana* (Aguilera et al. 2023) and *U. europaeus* (Pardo-Muras et al. 2020) extracts. The phenols p-coumarin, quercetin, and vanillin acid, also described in both invasive species, have been

related to phytotoxic effects (Hierro and Callaway 2003, de las Heras et al. 2020).

The negative effect of allelopathy on growth is mainly due to disruption of normal functions regarding cell division, nutrient uptake, and vital enzyme activities (Cheng and Cheng 2015). Although other morphometric variables (number of secondary roots and leaves) were affected by the application of both extracts, ET-Qs did not affect aerial growth due to an elongation of the internodes, while EU-Qs

Fig. 4 Carbohydrate contents of *Quillaja saponaria* seedlings irrigated with water (W-Qs) and with aqueous extracts of *Teline monspessulana* (TE-Qs) and *Ulex europaeus* (EU-Qs), **A** Total soluble sugars, **B** Starch content. Data are reported as mean and standard deviation. Different letters indicate significant differences between treatments at $P \leq 0.05$



did not affect root length, contrary to what was reported for *C. alba* (Rodríguez-Cerda et al. 2023). Thus, although the effect of the two extracts resulted in phenotypically different *Q. saponaria* individuals, the total biomass was maintained, which can be interpreted as a change in the growth pattern rather than an effect on the growth per se in response to the allelochemical impact. In fact, the stress tolerance index (STI) used to assess stress tolerance showed us that *Q. saponaria* is more tolerant to allelochemical stress than *P. boldus*, because its capable of maintaining biomass under the influence of both extracts. A negative and more evident effect of irrigation with invasive extracts on growth was observed in *P. boldus* individuals. In this species, all the morphometric parameters evaluated were reduced by both extracts, although irrigation with *U. europaeus* was more damaging, being less tolerant under this irrigation regime (STI = 0.25 ± 0.02).

Although similarities have been reported in some alkaloids and phenols in both species, is known that the allelochemical effect is strongly concentration dependent and also influenced by the nature of its secondary metabolites (Singh 2021). These data indicate that *U. europaeus* may be more aggressive than *T. monspessulana*, significantly altering the development and establishment of native Chilean species. The current results and those reported for *C. alba* (Rodríguez-Cerda et al. 2023) can be interpreted as a species-specific response to allelopathic stress. More studies are needed, focusing on the chemical composition of extracts from different invasive species, and also evaluating their effect on germination and development of native species at early growth stages.

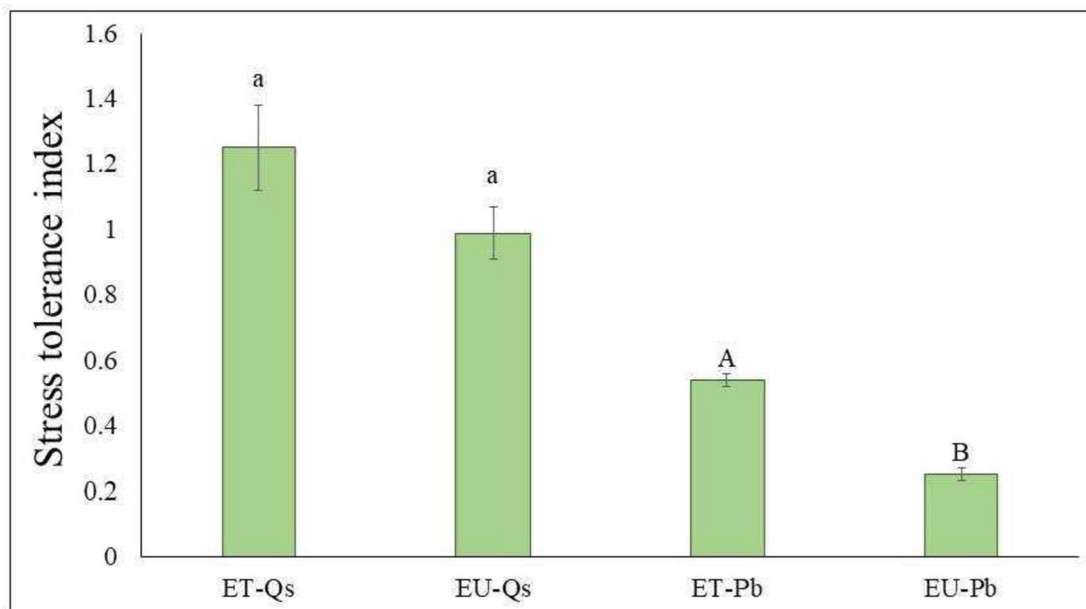


Fig. 5 Stress tolerance index for *Quillaja saponaria* and *Peumus boldus* seedlings irrigated with *Teline monspessulana* (ET-Qs and ET-Pb) and *Ulex europaeus* extracts (EU-Qs and EU-Pb). Data are reported as means and standard deviation. Different letters indicate

significant differences between treatments at $P \leq 0.05$. Lowercase letters indicate comparison between ET-Qs and EU-Qs and uppercase letters indicate comparison between ET-Pb and EU-Pb

Effects of aqueous extracts on photochemical system of Mediterranean species

Although photosynthesis measured by fluorescence of chlorophyll *a* has been reported to be sensitive to allelochemical stress (Huang et al. 2011; Bouhaouel et al. 2018), our Fv/Fm data were unchanged for both native species, indicating that there was no damage to the photosynthetic machinery due to the irrigation with extracts of invader species. The use of this parameter has been extensively applied to evaluate photoinhibitory damage by high and low temperatures and drought, among others (Maxwell and Johnson 2000). In contrast, the efficiency of photosystem II under light, here evaluated as quantum yield of PSII (Φ_{PSII}), was significantly reduced in *Q. saponaria* but not affected in *P. boldus*. Φ_{PSII} represents the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry (Maxwell and Johnson 2000). This parameter was reduced in *Q. saponaria* by irrigation with extracts of both invasive species, in agreement with a reduction in chlorophyll content and an increase in total carotenoids.

In plants, the photosynthetic efficiency is usually associated with an adequate level of chlorophyll pigments and any reduction in it can lead to changes/reduction on different fluorescence parameters (Torres Netto et al. 2005; Swoczyna et al. 2010, 2022). Experimental studies in algae have shown that the stress caused by allelochemicals can drastically reduce chlorophyll contents (Li et al. 2022).

Chlorophyll reduction was evident in *Q. saponaria* leaves, showing 35% and 65% chlorosis after irrigation with EU and ET, respectively (data not shown). The increase of carotenoids may be related to some mechanism expanding the antioxidant defense in response to the allelochemical effect exerted by the invasive plant to maintain homeostasis, as also reported for other species (Merhan 2017). Species such as wheat, cucumber, tomato, maize, and lettuce (El-khatib and Barakat 2016; Araniti et al. 2018; M'barek et al. 2019) have shown increased levels of carotenoids under allelochemical stress as a form of tolerance mechanism in response to oxidative stress. Consequently, the oxidative burst induced by allelochemical stress, in addition to increasing the carotenoid content, also induces another mechanism involving radical scavengers (Lu et al. 2003; Yin et al. 2010), including chlorophyll photoprotectors (Araniti et al. 2018; Mishra et al. 2023).

The significant effect of invasive extracts in *P. boldus* growth was not reflected on photosynthetic performance. The results of fluorescence measurements and chlorophyll content suggested no damage to the photosynthetic apparatus by extract irrigation. Although we observed a tendency to an increase in carotenoid content with EU irrigation, the lack of evaluation of other antioxidant defense compounds does not allow us to conclude if less inversion in growth was due to its use as a defense mechanism against the allelopathic stress.

Different mechanisms used by native species to deal with allelochemical stress

Despite the capacity to maintain its photosynthetic performance after irrigation with extracts of both invasive species, the biomass of *P. boldus* decreased significantly under the stress induced by *U. europaeus* and *T. monspessulana*. Any decrease in growth may indicate that the carbon gain by photosynthesis and consequent nitrogen assimilation is not being invested mainly in organ development, but may rather be used for the production of defensive compounds, considering that the two processes are competitive with each other (Su et al. 2021). In terms of carbon allocation, these results suggest that *Q. saponaria* seedlings can use the assimilated carbon for both biomass and defensive compound production. This ability enables the species to sustain its growth despite the presence of invasive species in its environment. In contrast, *P. boldus* seedling appear more sensitive to allelochemical stress and their growth is seriously compromised. Additionally, the unaltered pigments do not provide assurance of investment in defense mechanisms against invasive species. Further studies, evaluating changes in the antioxidant machinery and conducting a comprehensive characterization of photosynthetic and respiratory processes, would allow determining the level of resistance of native species to allelochemicals from invasive species, particularly in early development stages.

Conclusions

In some species, the identity of the target plants explains the variation of the allelopathic effect of the donor species (Barabasz-Krasny and Mozdzeń 2017; Zhang et al. 2021). In the present study, the pool of allelochemicals synthesized by the two invasive species differentially affected the morphophysiological performance of the two native species, a fact that can be interpreted as a species-specific response to allelopathic attack. *Quillaja saponaria* seedlings showed changes in some growth patterns, involving reductions of aerial and radicular organs. However, the capacity to increase photoassimilate production under allelochemical influence permitted it to maintain total biomass and to increase its antioxidant mechanisms, which may explain its higher tolerance against invasive species. In contrast, although no evident damage was observed in the photosynthetic machinery of *P. boldus*, at least in terms of light capture, the inability to increase carotenoids and the significant reduction in growth by the effect of allelochemicals suggest a detrimental effect on its early development stage and low tolerance to allelochemical impact, which could affect its survival in the presence of invasive species.

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Data availability Data available on request from the authors.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

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