Flora 269 (2020) 151639

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Highlighted Student Research

Phytotoxic effects of *Salvia rosmarinus* essential oil on *Acacia saligna* seedling growth

Alfredo Maccioni[a,b,*], Andrea Santo[a,c], Danilo Falconieri[b], Alessandra Piras[d], Emmanuele Farris[e], Andrea Maxia[a,b,1], Gianluigi Bacchetta[a,c,1]

[a] Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy
[b] Co.S.Me.Se, Consorzio Interuniversitario per lo Studio dei Metaboliti Secondari, Cagliari, Italy
[c] Banca del Germoplasma della Sardegna (BG-SAR), Hortus Botanicus Karalitanus (HBK), University of Cagliari, Cagliari, Italy
[d] Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria, Monserrato CA, Italy
[e] Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy

ARTICLE INFO

Edited by Fei-Hai Yu

Keywords:
Allelopathy
Invasive alien species
Seedling mortality
Natural products
Rosmarinus officinalis
Bio-herbicide

ABSTRACT

Using essential oils (EOs) to suppress unwanted species at the seedling stage is a promising way to decrease the use of synthetic herbicides and thus protect native plant diversity. We verified the effects of rosemary (*Salvia rosmarinus*) EOs at different concentrations on the growth and mortality of seedlings of the invasive alien species *Acacia saligna* derived from seeds collected at four wild populations in Sardinia and Sicily (Italy), by monitoring their survival, dry weight, shoot and root length during their early development. Six different spray solutions were applied to *A. saligna* seedlings: one with only distilled water; one with a solution of distilled water, ethanol and Tween 20; three with a rosemary EO concentration of 3.9, 7.8 and 15.6 mL/L; and one with the commercial herbicide DICOTEX© RTU.

The seedling survival and growth decreased with the increase of EO concentrations; the patterns were similar for all populations. The highest rosemary EO concentration tested (15.6 mL/L) strongly inhibited shoot and root length, dry weight and survival of *A. saligna* seedlings and its effect was significantly different compared to the other EO and control treatments, except for the control with commercial herbicide, that was the more effective compared to all other treatments. Our results suggest that the rosemary EO concentration at 15.6 mL/L is a valid tool for the biological control of *A. saligna*. This treatment is promising because it could be applied primarily to reduce the seedlings' emergence/development and establishment while ensuring the conservation of native plant diversity and the preservation of the Mediterranean habitats.

1. Introduction

Biological invasions of alien species are causing significant problems worldwide, including environmental change and loss of economic value, as well as loss of biological diversity and functionality in natural and agricultural ecosystems (Ankita and Chabbi, 2012). Invasive alien species (hereafter IAS) are well known to decrease native species richness, to alter water and/or fire regimes, to change the soil nutrient status and to moderate geomorphological processes (Lake and Leishman, 2004). These impacts can be cumulative, reducing biodiversity and jeopardizing ecosystem services (van Wilgen et al., 2012).

The Mediterranean Basin, the area of the world that ranks third for floristic originality (Myers et al., 2000), is characterised by large islands with hundreds of unique species (endemic level from 9% in the Balearic to 17.6 % in Crete) but strongly affected by IAS, whose percentage ranges from 6.7 % in Crete to 17.4 % in Sardinia (Médail, 2017). Compared to the mainland, islands are particularly prone to biological invasions of IAS, especially in the Mediterranean Basin (Médail, 2017). It has been demonstrated that, in the past decade, Mediterranean islands and coasts have become more vulnerable to biological invasions than mainland and inland areas (Vilà et al., 2007; Podda et al., 2010; Puddu et al., 2016; Roma-Marzio et al., 2016).

In order to reduce the risks of new species' introduction and to control the existing IAS to mitigate their impacts, several management strategies have been adopted. Legislative measures on IAS (van Wilgen et al., 2012) and their management – in particular through eradication

* Corresponding author at: Department of Life and Environmental Sciences, University of Cagliari, viale S. Ignazio da Laconi 13, 09123, Cagliari, Italy.
E-mail addresses: alfredomaccioni87@gmail.com (A. Maccioni), andreasanto85@gmail.com (A. Santo), danilo.falconieri@tiscali.it (D. Falconieri), apiras@unica.it (A. Piras), emfa@uniss.it (E. Farris), a.maxia@unica.it (A. Maxia), bacchet@unica.it (G. Bacchetta).

[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.flora.2020.151639
Received 4 March 2020; Received in revised form 29 May 2020; Accepted 3 June 2020
Available online 08 June 2020
0367-2530/

from the invaded habitats, control of the invaded areas and the use of herbicides (Acunto et al., 2017) – are the most effective strategies. The European Union, with Regulation 2014/1143/EU and subsequent implementations (Commission Implementing Regulation 2016/1141/EU; 2017/1263/EU; 2019/1262/EU), drew up an IAS list ('blacklist'), which has led to restrictions on keeping, importing, selling, breeding and growing IAS. *Acacia saligna* (Labill.) H.L. Wendl. (golden wreath wattle) was included in the blacklist on 15 August 2019 with Regulation 2019/1262/EU of 25 July 2019.

The negative effects of *Acacia* invasion on indigenous plant species and habitats, particularly on species richness, community structure, soil bulk density and water availability (Werner et al., 2010), have been widely documented (Le Maitre et al., 2011; Correia et al., 2014; Meloni et al., 2015). *Acacia* Mill. species show several reproductive traits that may contribute to their invasiveness, such as the ability to self-pollinate, a strong ability to perform vegetative reproduction, and the production of a large number of long-lived and highly viable seeds (Morris et al., 2011; Correia et al., 2014; Meloni et al., 2015). Furthermore, *Acacia* species introduce novel mechanisms (Callaway and Aschehoug, 2000) that interfere with the ecophysiological processes of indigenous species such as nutrient and water uptake, enzyme functions, seed germination, seedling growth, photosynthesis and respiration (Lorenzo et al., 2011). The invasiveness of *Acacia* species depends on their ability to compete with indigenous species for light, water and nutrients; in addition, invasiveness is often caused by the absence of pathogens and predators in the invaded habitats (Morris et al., 2011).

In the past 50 years, the continued use of commercial products such as synthetic agrochemical compounds (e.g., 2,4-D and 2,4,5-T) has caused resistance of IAS to herbicides as well as negative impacts on human health and the environment due to herbicide persistence in ecosystems (Macıas et al., 1999). Thus, natural products, e.g., essential oils (EOs), could become an alternative solution for the selective biological management of IAS and other weeds (Singh et al., 2003; van Wilgen et al., 2012; Maas et al., 2013; Scognamiglio et al., 2013; Maccioni et al., 2019). Moreover, EOs are biodegradable, and thus more sustainable and provide safer food products when applied in agriculture, alleviating the existing concerns regarding the effects on human health (Batish et al., 2006a; Saleem et al., 2013; Saad and Abdelgaleil, 2014).

EOs from different vascular plant families (e.g., Asteraceae, Lamiaceae and Myrtaceae) have different phytotoxic properties (e.g., herbicidal, insecticidal and pesticidal properties: see Singh et al., 2006, 2009; Taban et al., 2013). Several studies have already documented that EOs are involved in phytotoxic interactions, suppressing seed germination and/or negatively influencing root and shoot growth of other plant species (Li et al., 2014; Araniti et al., 2016).

Rosmarinus officinalis L. (rosemary), recently placed in the genus *Salvia* L. (Drew et al., 2017), one of the most common and characteristic components of the Mediterranean dwarf scrub vegetation (also named garrigue), is characterised by EOs with high concentrations of some monoterpenes, such as α-pinene, limonene, 1,8-cineole, borneol and camphor, which are known to inhibit seed germination, growth and survival of seedlings of many plant species (De Martino et al., 2012; Maccioni et al., 2019), because its phytotoxic effect (Angelini et al., 2003; Atak et al., 2016). Even if some studies have already investigated the composition of EOs of *S. rosmarinus* Spenn. (Atak et al., 2016) and their effects on the inhibition of IAS and weed growth (Singh et al., 2003; Batish et al., 2006b; Kekeç et al., 2012), nothing is known on the interaction between the EO of *S. rosmarinus* and *A. saligna* seedling growth and mortality.

As is apparent from a significant number of studies, actions to eradicate and control IAS have mainly been based on a single method, with chemical (synthetic herbicides) and mechanical methods being highly prevalent (Brunel et al., 2013; Willis, 2017). Therefore, there is an urgent need to go beyond the traditional methods to control IAS, in particular their eradication from and/or control in the invaded habitats and the use of biological/chemical herbicides against adult plants (Qasem, 2010; Acunto et al., 2017). From this perspective, using EOs to suppress unwanted species at the seedling stage seems a novel and promising approach. With the aim of testing a protocol that could be used and replicated in several Mediterranean areas, *S. rosmarinus* has been chosen as a to investigate its effects on *A. saligna* seedlings and to evaluate the possible use of this EO as a potential solution for the biological control of this IAS. Specifically, we aimed at (1) isolating and characterizing EOs from wild rosemary; (2) determining if the survival rate and growth (dry weight, root and shoot length) of *A. saligna* seedlings differ under different concentrations of the *S. rosmarinus* EO and if they differ between the EO treatments and the controls; (3) verifying if there are significant differences in the survival and growth between *A. saligna* seedlings exposed to different concentrations of the *S. rosmarinus* EO and those exposed to a commercial herbicide; and (4) verifying if there are significant differences, for all the response variables (survival, dry weight, root and shoot length), determined by the origin of *A. saligna* seeds (Sicily vs. Sardinia, and two populations at each island).

2. Material and methods

2.1. Plant material and essential oil

Salvia rosmarinus is native to the Mediterranean biogeographic region, where it can be found in coastal garrigues, scrublands, rocky cliffs and sunny inland dwarf vegetation, from sea level up to 2000 m of elevation. It is very widespread and abundant in Sardinia, including the coastal areas where *A. saligna* mostly grows. Furthermore, *S. rosmarinus* can be cultivated easily, and its anthesis occurs twice a year – although in some locations it blooms throughout the whole year – so the production of its EO is high in relation to its biomass. As a consequence of these factors, as well as the chemical composition of the EO, *S. rosmarinus* has been chosen as the target species. This study has been developed using the EO of this common species with the aim of proposing a protocol that could be used and replicated in several Mediterranean areas.

The flowering aerial parts from 10 sample individuals of *S. rosmarinus* were collected from a population in northern Sardinia (Su Canale, Monti, Gallura subregion, 40°50′ N – 09°23′ E). Then, the plant material was air-dried at 40 °C with forced ventilation for two days in an oven (FP 115, BINDER) at the Laboratory of Plant Biology and Pharmaceutical Botany of the University of Cagliari, Sardinia, Italy. The EO was obtained from the dried plant material by steam distillation for three hours. The rosemary EO was obtained by mixing the material collected from the 10 sampled individuals and stored at 4 °C in the dark until the chemical analyses took place.

2.2. GC-FID and GC–MS analyses

Gas chromatography analyses of the rosemary EOs were performed using a gas chromatograph (Agilent 7890A, Palo Alto, CA, USA) equipped with a 30 m × 0.25 mm i.d. with a 0.25 μm stationary film thickness HP-5 capillary column (Agilent J&W) and a flame ionization detector (FID). The temperature program used was raised from 60 to 246 °C at a rate of 3 °C min^{-1} and then held at 246 °C for 20 min (total analysis time: 82 min). The other operating conditions were as follows: carrier gas, helium (purity ≥ 99.9999 % – Air Liquide Italy); flow rate, 1.0 mL min^{-1}; injector temperature, 250 °C; detector temperature, 300 °C. Injection of 1 μL of diluted sample (1:100 in hexane, w/w) was performed with a 1:20 split ratio using an auto-sampler (Agilent, Model 7683B).

GC–MS analyses were carried out using a gas chromatograph (Agilent 6890 N) equipped with a 30 m × 0.25 mm i.d. with a 0.25 μm stationary film thickness HP-5 ms capillary column (Agilent J&W), coupled with a mass selective detector with an electron ionization device (EI) and a quadrupole analyser (Agilent 5973). The temperature program and the chromatographic operating conditions (except for the detector) were the same as those used for GC. The MS conditions were as follows: MS transfer line temperature, 240 °C; EI ion source temperature, 200 °C with ionization energy of 70 eV; quadrupole temperature, 150 °C; scan rate, 3.2 scan s^{-1} at *m/z* scan range, (30–480). The MSD ChemStation software was used to handle and process chromatograms and mass spectra (Agilent, rev. E.01.00.237). The compounds of the samples were identified by comparing mass spectra fragmentation patterns with those of a computer library (Adams, 2007), and linear retention indices (RI) were based on a series of C_8-C_{26} n-alkanes homologous to those reported in the literature (Adams, 2007). Quantification of constituents was calculated by integration of GC-FID peak areas without using the response correction factors.

2.3. Seed lot details

The genus *Acacia* belongs to Fabaceae (nom. alter. Leguminosae) and subfamily Caesalpinioideae DC. (Richardson et al., 2011), with ca. 1380 species native to tropical areas of the Americas, Africa, Asia and Australia. This genus encompasses some of the most important IAS in the world (Griffin et al., 2011). At least a third of them have been moved by humans to areas outside their natural range (Griffin et al., 2011), and 23 are confirmed as invasive in Mediterranean ecosystems (Pysek et al., 2004; Werner et al., 2010; Del Vecchio et al., 2013), where in fact no native *Acacia* species are found.

Acacia saligna is an evergreen fast-growing species native to southwestern Australia (Orchard and Wilson, 2001). In the last decades, this species has been widely used for stabilizing shifting sandy dunes, rehabilitating sandy mining areas and protecting barren rocks along roadsides, and it has been planted extensively in many regions of the world, thus becoming highly invasive in the Mediterranean Basin (Meloni et al., 2011; Crisóstomo et al., 2013; Del Vecchio et al., 2013). This species has no preference for any particular soil, and it can grow in a wide range of ecological conditions, including the high pH values (Musil, 1993) and sub-humid soils in semi-arid and arid Mediterranean climatic areas, especially on barren slopes, sand dunes (Midgely and Turnbull, 2003) and abandoned lands (Kutiel et al., 2004).

Seeds were collected from four *A. saligna* populations in Sardinia and Sicily (Table S1), the two largest islands of the Mediterranean Basin, which are representative of the Tyrrhenian islands biodiversity hotspot (Médail and Quézel, 1999) strongly affected by IAS (Médail, 2017). Moreover, the *A. saligna* populations from the two islands are genetically different (Jiménez et al., 2007; Lawson Handley et al., 2011).

Random samplings were done on populations of about 30–50 adult individuals, and the seeds were collected from 22 individuals in each population, which were about 10 m apart from each other. The seeds were collected in the period of natural dispersal, after which they were extracted from the fruits by hand using laboratory tweezers and then stored under controlled conditions (20 °C and 40 % relative humidity) for two weeks before the experiment.

2.4. Effect of essential oil spray on seedling development

Six different solutions were applied on early seedlings by spraying. To obtain the minimum number of seedlings required to start the experiment, 150 seeds from each population were incubated in light (12 h per day) at a constant temperature of 15 °C, in a growth chamber (MLR-351, S-ANYO) at the Sardinian Germplasm Bank of the Hortus Botanicus Karalitanus of the University of Cagliari, Sardinia, Italy. To facilitate germination, seeds were scarified using sandpaper no. 80 for 15 min. All seeds were sown under a laminar flow hood (KB, Faster) in 90 mm diameter plastic Petri dish on 1% agar substrate, which provided a solid, non-sterile medium for germination. After 16 days from germination, two seedlings were sown in polyethylene pots (70 mm × 70 mm × 90 mm), but only one seedling per pot was kept for the experiment. Prior to use, all pots were disinfected by immersion in a solution of NaClO (860 mM) for two hours and then were washed with distilled water for ten minutes. All pots were filled with a substrate, constituted by peat (70 %) and perlite 4 − 5 mm (30 %), sterilized at 90 °C for five hours in an oven (FP 115, BINDER). For each of the four tested populations, six replicates of ten seedlings per condition were inserted in a phytotron (Percival PGC-6HID) at a constant temperature of 20 °C, with 12 h of irradiance per day.

For the total duration of the experiment ten seedlings for each tested condition (for each of the four investigated populations) were sprayed with distilled water (Control 1), while further ten seedlings were sprayed only with the solution of distilled water, ethanol and Tween 20 (Control 2). Tween 20 was used for its surface activity to better dissolve the EO in water. Ten seedlings of each population were sprayed with a rosemary EO solution at each of three different concentrations (3.9, 7.8, 15.6 mL/L) one day/week, from a distance of 200 mm. The EO solution was prepared by mixing the established volumes of the EO with 10 mL/L of ethanol and 3 mL/L of Tween 20 ($C_{58}H_{114}O_{26}$, Polysorbate 20, Alfa Aesar). Ten seedlings of each population were also sprayed only with the commercial herbicide DICOTEX© RTU (Control 3). Prior to the experiment starting (36 days after the seed germination), a preliminary test was carried out to evaluate the effect of Control 2 on seedling development and to verify the absence of inhibitory activity of this compound on seedling growth.

All shoots of each seedling were equally exposed to the solution. Every week, each seedling of each treatment received 1.3 g of solution by spraying. All the seedlings were irrigated by osmosis with distilled water once a week for the total duration of the experiment, to maintain the substrate in the pots permanently moist, so that water availability was not a limiting factor for the seedling growth. The number of dead seedlings was annotated three days/week. After four weeks, at the end of the experiment, the shoot and root length for each survived seedling was measured by a digital caliper (960-D, USAG) and its dry weight (biomass) was measured after drying it in oven at 103 ± 1 °C for 17 h.

2.5. Data analysis

Seedling survival percentages were analysed applying the Kaplan-Meier method, followed by the Log Rank Test when $p < 0.001$. Shoot and root length and dry weight of seedlings were analysed by nested ANOVA with the factor “island” (Sardinia vs Sicily) nested with the factor “treatment” (C1-C2-C3-T1-T2-T3), and the factor “population” (two populations per each island) nested with the factor “island”. Treatment and island factors were considered fixed, while the population factor was considered random. ANOVAs were followed by the Tukey honestly significant difference (HSD) *post hoc* test when $p < 0.05$. All the statistical analyses were carried out using Minitab 19 Statistical Software.

3. Results

3.1. Phytochemical analysis

The rosemary EO was composed of a total of 25 compounds (99.8 % of the total composition), including α-pinene (21.5 %), bornyl acetate (16.8 %), borneol (10.2 %), camphor (9.7 %), camphene (7.5 %), 1,8-cineole (7.4 %), verbenone (4.4 %), limonene (4.3 %) and β-pinene (3.0 %) as the main constituents (Table S2). It was predominantly composed of oxygenated monoterpenes (51.8 %) and hydrocarbon monoterpenes (44.5 %), and a lower percentage of hydrocarbon sesquiterpenes (2.7 %) and other compounds (0.8 %) was present.

3.2. Seedling survival

Seedling survival was highly significantly affected by treatment (Tr)

Table 1
Kaplan – Meier method and Mantel – Cox Log-rank test testing the effects of population (Po), treatment (Tr) and island (Is) on *Acacia saligna* seedlings survival. Significant values are shown in bold.

	χ^2	DF	*p*
Treatment (Tr)	86.799	5	**< 0.001**
Population (Po)	1.451	3	0.694
Island (Is)	0.437	1	0.509

($p < 0.001$), but not by island and population ($p > 0.05$; Table 1).

The seedling survival from all populations decreased with the increase of EO concentrations (Fig. 1). At the end of the experiment, no more than 10 % of seedlings survived when treated with the commercial herbicide (C3; Fig. 1). Sic1 seedlings, with a 60 % of survival when treated using the highest rosemary EO concentration (15.6 mL/L), showed the highest resistance to the EO (Fig. 1c), compared to the other three populations (Sar1, Sar2 and Sic2), that showed a 70 % reduction of seedling survival (Fig. 1a, b, d).

3.3. Seedling dry weight

At the end of the experiment, the factor "treatment" (Tr) significantly influenced the seedling dry weight ($p < 0.01$; see Table 2); on the other hand, the "island" (Is) factor was not significant ($p > 0.05$; Table 2). The HSD post hoc test for all populations showed that the observed differences in seedlings' dry weight among treatments were statistically different ($p < 0.05$; Fig. 2); it highlighted that T1 = T2 = C1 = C2 < T3 < C3: this means that EO treatments at 3.9 mL/L (T1) and 7.8 mL/L (T2) were not significantly different ($p > 0.05$) compared to controls C1 and C2, while the treatment with EO concentration 15.6 mL/L (T3) significantly ($p < 0.05$) reduced seedlings' dry weight if compared to the other treatments (T1 and T2) and controls C1 and C2. The control C3 (commercial herbicide) had highly significant effects ($p < 0.001$) with respect to all other treatments (Fig. 2).

The EO increasing volume reduced the seedling biomass for all the populations (Fig. 2). In particular, Sar1 and Sic2 seedlings (Figs. 2a, d) had a significant reduction of their final biomass when nebulised with EO treatment T2 (7.8 mL/L), while Sar2 and Sic1 (Fig. 2b, c) showed a significant reduction only when nebulized with EO treatment T3 (15.6 mL/L). Sar1 and Sic1 seedlings had a drastic decrease of the final biomass when treated with C3 (Fig. 2a, c), whereas no seedlings of Sar2 and Sic2 survived until the end of the experiment if treated with C3 (Fig. 2b, d). Significant differences among of four population (Po) were highlighted ($p < 0.001$; Table 2).

Table 2
Nested ANOVA, testing the effects of treatment (Tr), island (Is) nested within treatment, and population (Po) nested within island on *Acacia saligna* seedling dry weight, shoot and root length. Significant values are shown in bold.

Effect		Dry weight		Shoot length		Root length	
	DF	F	*p*	F	*p*	F	*p*
Tr	5	5.44	**0.008**	5.31	**0.008**	4.00	**0.023**
Is (Tr)	6	0.63	0.704	0.45	0.832	0.51	0.791
Po (Tr x Is)	12	4.18	**< 0.001**	3.89	**< 0.001**	3.75	**< 0.001**
Error	216						

3.4. Shoot and root length

The factor "treatment" (Tr) was significantly effective on seedling shoot and root length reduction ($p < 0.05$; Table 2), whereas the "island" (Is) factor was not significant ($p > 0.05$; Table 2). The increasing EO volume reduced the seedling shoot and root length for all the populations (Fig. 3). Significant differences among of four population (Po) were highlighted ($p < 0.001$; Table 2).

The HSD post hoc test for all population showed that treatments were significantly different on shoot length (Fig. 3a, b, c, d): it showed that T1 = C1 = C2 < T2 = T3 < C3. This means that EO treatment T1 (3.9 mL/L) had no significant effects ($p > 0.05$) on *A. saligna* seedlings' shoot length when compared to controls C1 and C2; treatments T2 (7.8 mL/L) and T3 (15.6 mL/L) showed a significant effect ($p < 0.05$) when compared to the rosemary EO concentration T1 and controls C3. Interestingly, the T2 treatment was not statistically different ($p >$

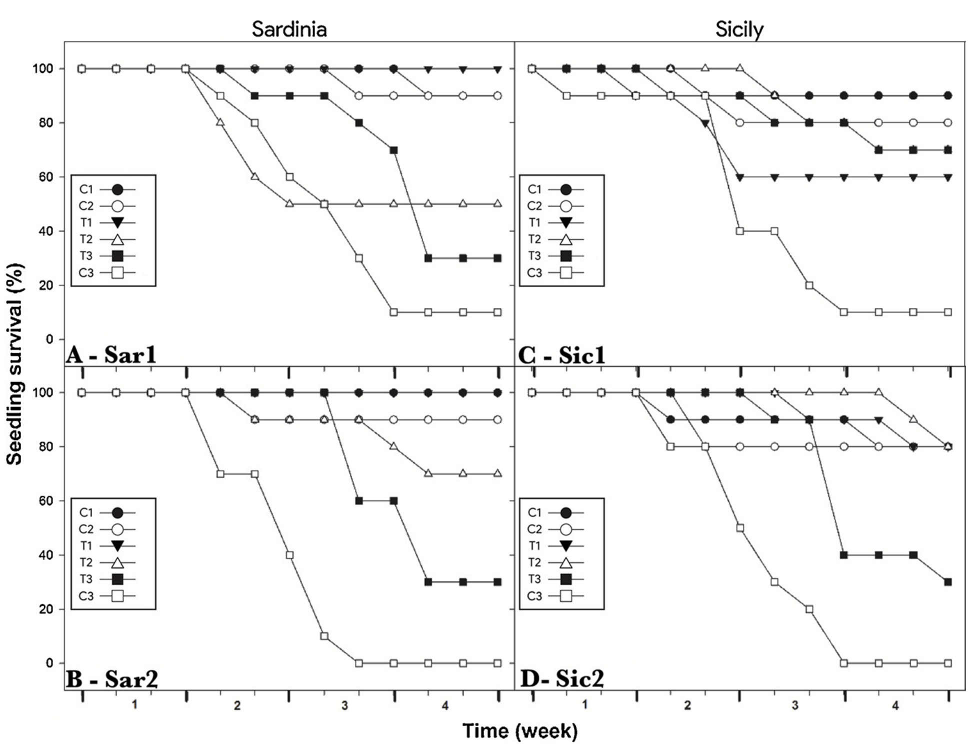

Fig. 1. Survival of *Acacia saligna* seedlings from Sardinia (Sar1 and Sar2) and Sicily (Sic1 and Sic2) for each treatment (C1: first control with distilled water; C2: second control 2 with ethanol and Tween 20; T1: 3.9 mL EO/L; T2: 7.8 mL EO/L; T3: 15.6 mL EO/L; C3: third control with commercial herbicide DICOTEX© RTU) during four weeks of *Salvia rosmarinus* essential oil nebulization. Means that do not share a letter are significantly different.

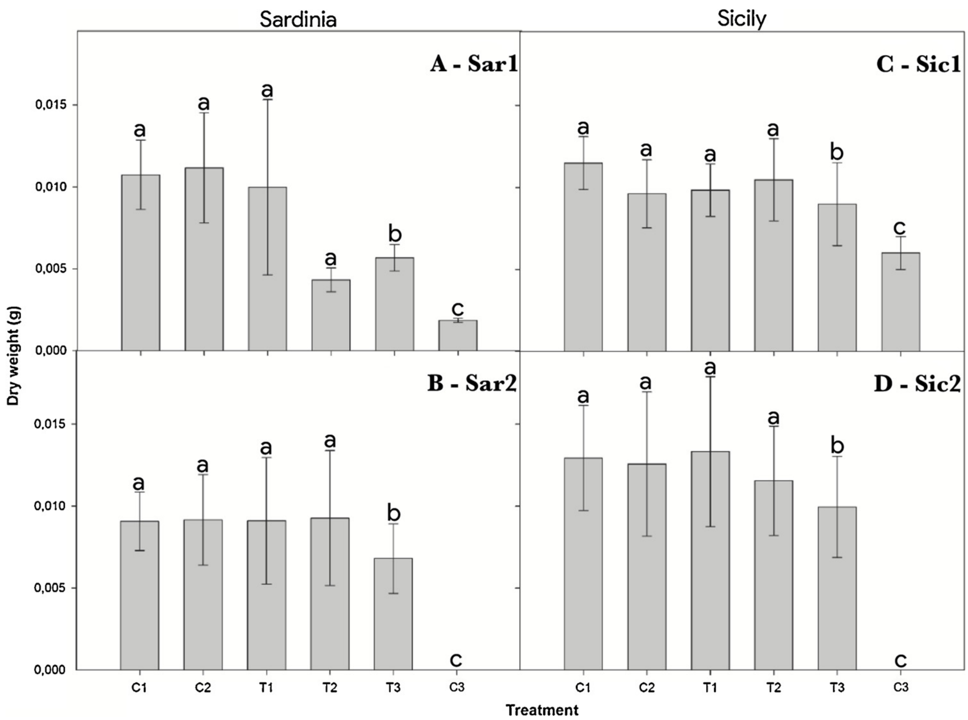

Fig. 2. Dry weight of *Acacia saligna* seedlings from Sardinia (Sar1 and Sar2) and Sicily (Sic1 and Sic2) for each treatment (C1: first control with distilled water; C2: second control with ethanol and Tween 20; T1: 3.9 mL EO/L; T2: 7.8 mL EO/L; T3: 15.6 mL EO/L; C3: third control with commercial herbicide DICOTEX© RTU) after four weeks of *Salvia rosmarinus* essential oil nebulization. For each treatment, data are the mean of the surviving seedlings four weeks after the beginning of the experiments. Means that do not share a letter are significantly different.

0.05) from treatment T3. The control with commercial herbicide (C3) had highly significant effects ($p < 0.001$) with respect to all other treatments (Fig. 3a, b, c, d).

Regarding root length, the overall patterns of the four populations are pretty similar, although there are some differences based on multiple comparisons (HSD post hoc test; Fig. 3e, f, g, h) showing that C1 = C2 = T1 < T2 = T3 < C3 (for more details see Fig. 3e, f, g, h). This means that T1 (3.9 mL/L) had no significant effects ($p > 0.05$) on *A. saligna* seedlings' root length when compared to controls (C1 and C2) and T2; the T2 treatment (7.8 mL/L) showed no significant effect ($p > 0.05$) when compared to controls (C1 and C2) and other EO treatments. The highest EO concentration tested at 15.6 mL/L (T3) was significantly different to C1, T1 and C3. The control with commercial herbicide (C3) had highly significant effect ($p < 0.001$) compared to the other treatments (Fig. 3e, f, g, h).

4. Discussion

Even though all five Mediterranean climate areas are biodiversity hotspots at the global level (Myers et al., 2000), they all suffer from invasions of alien plants, summing overall to > 1600 IAS (Arianoutsou et al., 2013). Vegetative propagation, large leaf size, summer flowering, a long flowering period and dispersal by wind or vertebrates were found to be common traits of invaders across the main Mediterranean islands (Lloret et al., 2005). Recently interesting allelopathic plants, as Labiatae family EOs rich in monoterpenes, are emerging as a valid IAS management alternative, in fact lots of researches proved that plants had a herbicidal activity (Benchaa et al., 2019). Eradication attempts are only occasional, performed on small islands and mainly conducted by means of manual and/or mechanical eradication (Brunel et al., 2013; Ruffino et al., 2014; Willis, 2017), but in these cases, careful monitoring for a long time after the eradication is still required to prevent germinations from the soil seed bank. An alternative solution can be the use of synthetic herbicides (Willis, 2017), but this method has the consequence of also impacting native plants, whose response after the treatment is often contrasting (Carlson and Gorchov, 2004). The use of EOs produced by native plants to restrict IAS, particularly during their early developmental stages (germination and seedling growth), therefore, seems an interesting and challenging option for the future (Qasem, 2010).

This study showed, by means of exposure to the *S. rosmarinus* EO, characterised by high concentrations of some monoterpenes, such as α-pinene, limonene, 1,8-cineole, borneol and camphor, that this complex, volatile compound has a potential bio-herbicidal effect on the growth and survival of *A. saligna* seedlings, as already proved on several other target species (Angelini et al., 2003; Atak et al., 2016). Previously, treating with rosemary fresh leaf leachate greatly reduced the root length of *Eleusine indica*, *Cynodon dactylon* and *Digitaria sanguinalis* (Chen et al., 2013), and the radicle length of *Echinochloa crus-galli*, *Capsicum annuum* and *Lactuca sativa* (Angelini et al., 2003). These studies are similar to the one carried out by us for the use of the rosemary EO, though the target IAS, the methods and the EO concentrations are different. Even if our study highlighted some variability among *A. saligna* populations in the response of the seedlings' root length to the rosemary EO (since *A. saligna* seedlings from Sar1 and Sic1 populations showed no variation in root length after EO application), as already observed by Angelini et al. (2003) for *Raphanus sativus*, *Portulaca oleracea* and *Chenopodium album*, one of the main results of this investigation is to have proven the null effect of the island of origin of the germinated seeds for all the response variables. Consequently, despite the validity of the recommendation to carefully evaluate different responses to the same treatment from geographically different IAS populations, maybe caused by local adaptation and/or genetic differences (Jiménez et al., 2007; Lawson Handley et al., 2011), which should be taken into account when planning management plans of IAS in nature, our findings strongly suggest that the proposed rosemary EO treatment against *Acacia saligna* seedlings could be effective at different geographical locations. In fact, the factor "treatment" was statistically significant ($p < 0.05$) on shoot and root length, dry weight and survival of *A. saligna* seedlings, and that the patterns of variation of the four response variables at the four populations were similar, although some differences were observed (Figs. 1–3).

The highest tested rosemary EO concentration at 15.6 mL/L (T3, Figs. 1–3) had a significant effect ($p < 0.05$) on survival and biomass of seedlings compared to the other treatments. Therefore, EO concentration at 15.6 mL/L has a potential bio-herbicidal effect on the

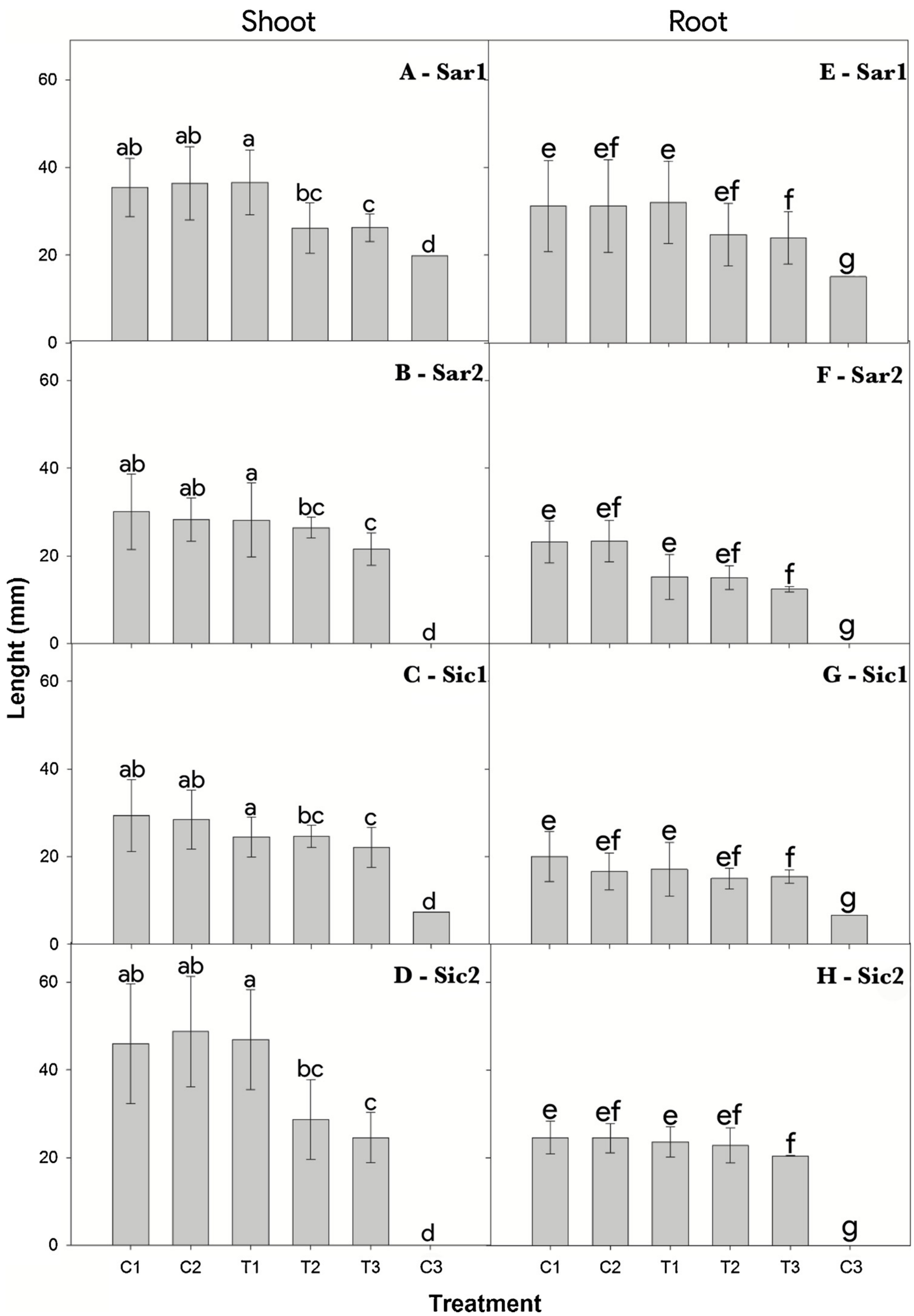

Fig. 3. Shoot and root lengths of *Acacia saligna* seedlings from Sardinia (Sar1 and Sar2) and Sicily (Sic1 and Sic2) for each treatment (C1: first control with distilled water; C2: second control with ethanol and Tween 20; T1: 3.9 mL EO/L; T2: 7.8 mL EO/L; T3: 15.6 mL EO/L; C3: third control with commercial herbicide DICOTEX© RTU) after four weeks of *Salvia rosmarinus* essential oil nebulization. For each treatment, data are the mean of the surviving seedlings four weeks after the beginning of the experiments. Means that do not share a letter are significantly different.

growth and survival of *A. saligna* seedlings. The other tested rosemary EO concentrations at 3.9 mL/L and 7.8 mL/L compared to controls (C1 and C2) were not statistically different ($p > 0.05$).

The treatment with commercial herbicide DICOTEX© RTU was more effective ($p < 0.001$) than other treatments (Figs. 1–3) indiscriminately on shoot and root length, dry weight and survival of *A. saligna* seedlings - the effects of the phenoxyalkanoic acid herbicides 2,4-D, introduced around 1950 and used worldwide, are well known to control the growth and survival of broad-leaved plants, as well as to cause environmental pollution (Tayeb et al., 2011). In light of the statistical analysis, the results here shown suggest that the rosemary EO concentration at 15.6 mL/L has effects on the growth and survival of *A. saligna* seedlings comparable to those caused by the commercial herbicide.

Furthermore, for some populations of *A. saligna* here studied (Sar2 and Sic2), we observed a different effect of rosemary EO on shoot growth in seedlings derived from the same population. A plausible explanation for this different effect on the two plant organs, when treated with secondary metabolites, was reported by Obroucheva (1999), who found that shoot growth is largely dependent on the elongation of each cell, which has already occurred in the embryo within the seed, while root growth requires both proliferation and elongation of cells. This explanation also suggests a different sensitivity and permeability of the two organs to the same EO concentration (Nishida et al., 2005; Taban et al., 2013).

Even if we did not deal with chlorosis and necrosis in our study, we observed chlorosis in some *A. saligna* seedlings. Data reported in the literature suggest that EOs and/or their constituents may inhibit plant growth also through electrolyte leakage (Maffei et al., 2001; Tworkoski, 2002), causing the disruption of the membrane's integrity and triggering the formation of reactive oxygen species (ROS) (Singh et al., 2009; Taban et al., 2013).

Our research highlighted that rosemary EO could successfully inhibit the emergence and growth of seedlings of *A. saligna*, which is a dangerous IAS for Mediterranean indigenous plant diversity. Our study demonstrates that natural compounds derived from EOs are an useful, eco-friendly alternative to synthetic herbicides. The EO concentrations used in this study were tested to identify the minimum quantity necessary to inhibit *A. saligna* seedling growth and survival, and our results suggest that rosemary EO at a concentration of 15.6 mL/L, compared to the controls, is a valid solution for the biological contrast of this IAS. This could help reduce the use of synthetic herbicides, thus mitigating environmental pollution and reducing the resistance of this IAS to herbicides.

Being conducted in a laboratory under controlled conditions, this study represents only a first step for evaluating the possible phytotoxic effect of rosemary EO on *A. saligna*. Further research is necessary to evaluate the effects of this EO on native plants and microbiological components of the soil when the EO is applied to seedlings in the field, although the amount of nebulised EO would be minimal. This would be possible at the local (islands) level and at the regional (whole Mediterranean Basin) level because the factor island turned out to be no statistically significant and the frequency and abundance of rosemary in natural and semi-natural environments around the whole basin.

5. Conclusions

Using EOs to suppress unwanted species at the seedling stage looks like a promising way to decrease the use of synthetic herbicides and thus protect native plant diversity.

Differences in seedling response (e.g., survival in time, dry weight and length) among our four populations of *A. saligna* highlighted low inter-population variability: the possible use of *S. rosmarinus* EO as a bio-herbicide against *A. saligna* seedlings should take into account some variability in response among populations, and, more importantly, the absence of effects derived by the different geographical origin of the seeds used in the experiment (no island effect).

Even if the application of the EO in the field would require careful tests, since the EOs might have negative side effects on native biodiversity or might get degraded before acting, this treatment is promising because it could be applied in a range of sites primarily to reduce seedlings' emergence/development and establishment while ensuring the conservation of native biodiversity and the preservation of Mediterranean habitats.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Alfredo Maccioni: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Supervision, Writing - original draft, Writing - review & editing. **Andrea Santo:** Methodology, Investigation, Data curation, Formal analysis, Software, Supervision, Writing - original draft, Writing - review & editing. **Danilo Falconieri:** Resources, Investigation, Formal analysis. **Alessandra Piras:** Resources, Investigation, Formal analysis, Writing - original draft. **Emmanuele Farris:** Validation, Writing - review & editing. **Andrea Maxia:** Conceptualization, Methodology, Resources, Project administration, Funding acquisition, Supervision, Validation, Writing - review & editing. **Gianluigi Bacchetta:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgements

The authors would like to thank Dr. Lina Podda for having collected seeds from Sardinia used in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.flora.2020.151639.

References

Acunto, S., Bacchetta, G., Bordigoni, A., Cadoni, N., Cinti, M.F., Duràn Navarro, M., Frau, F., Lentini, L., Liggi, M.G., Masala, V., Meloni, F., Pinna, R., Podda, L., Sanna, A., 2017. The LIFE+ project RES MARIS – recovering endangered habitats in the capo carbonara marine area, Sardinia (LIFE13 NAT/IT/000433): first results. Plant Sociol. 54, 85–95. https://doi.org/10.7338/pls2017541S1/11.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Carol stream, IL.

Angelini, L.G., Carpanese, G., Cioni, P.L., Morelli, I., Macchia, M., Flamini, G., 2003. Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J. Agr. Food Chem. 51, 6158–6164. https://doi.org/10.1021/jf0210728.

Ankita, G., Chabbi, M., 2012. Effect of allelopathic leaf extract of some selected weed flora of Ajmer district on seed germination of *Triticum aestivum* L. Sci. Res. Report. 2, 311–315.

Araniti, F., Sanchez-Moreiras, A.M., Graña, E., Reigosa, M.J., Abenavoli, M.R., 2016. Terpenoid trans-caryophyllene inhibits weed germination and induces plant water status alteration and oxidative damage in adult *Arabidopsis*. Plant Biol. 19, 79–89. https://doi.org/10.1111/plb.12471.

Arianoutsou, M., Delipetrou, P., Vilà, M., Dimitrakopoulos, P.G., Celesti-Grapow, L., Wardell-Johnson, G., Henderson, L., Fuentes, N., Ugarte-Mendes, E., Rundel, P.W., 2013. Comparative patterns of plant invasions in the Mediterranean Biome. PLoS One 8, 79174. https://doi.org/10.1371/journal.pone.0079174.

Atak, M., Mavi, K., Uremis, I., 2016. Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. Rom. Biotech. Lett. 21, 11149–11159.

Batish, D.R., Singh, H.P., Setia, N., Kaur, S., Kohli, R.K., 2006a. Chemical composition and phytotoxicity of volatile essential oil from intact and fallen leaves of *Eucalyptus citriodora*. Z. Naturforsch. C J. Biosci. 61, 465–471. https://doi.org/10.1515/znc-2006-7-801.

Batish, D.R., Singh, H.P., Setia, N., Kaura, S., Kohli, R.K., 2006b. Chemical composition and inhibitory activity of essential oil from decaying leaves of *Eucalyptus citriodora*. Z. Naturforsch. C J. Biosci. 61, 52–56. https://doi.org/10.1515/znc-2006-1-210.

Benchaa, S., Hazzit, M., Zermane, N., Abdelkrim, H., 2019. Chemical composition and herbicidal activity of essential oils from two Labiatae species from Algeria. J. essent. oil res. 31, 335–346. https://doi.org/10.1080/10412905.2019.1567400.

Brunel, S., Brundu, G., Fried, G., 2013. Eradication and control of invasive alien plants in the Mediterranean Basin: towards better coordination to enhance existing initiatives. Bul. OEPP/EPPO. 43, 290–308. https://doi.org/10.1111/epp.12041.

Callaway, R.M., Aschehoug, E.T., 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290, 521–523. https://doi.org/10.1126/science.290.5491.521.

Carlson, A.M., Gorchov, D.L., 2004. Effects of herbicide on the invasive biennial *Alliaria petiolata* (Garlic Mustard) and initial responses of native plants in a southwestern Ohio forest. Restor. Ecol. 12, 559–567. https://doi.org/10.1111/j.1061-2971.2004.00373.x.

Chen, F., Peng, S., Chen, B., Ni, G., Liao, H., 2013. Allelopathic potential and volatile compounds of *Rosmarinus officinalis* L. against weeds. Allelopathy J. 32, 57–66.

Correia, M., Castro, S., Ferrero, V., Crisóstomo, J.A., Rodríguez-Echeverría, S., 2014. Reproductive biology and success of invasive Australian acacias in Portugal. Bot. J. Linn. Soc. 174, 574–588. https://doi.org/10.1111/boj.12155.

Crisóstomo, J.A., Freitas, H., Rodríguez-Echeverría, S., 2013. Relative growth rates of three woody legumes: implications in the process of ecological invasion. Web Ecol. 7, 22–26. https://doi.org/10.5194/we-7-22-2007.
De Martino, L., Mancini, E., Marandino, A., de Almeida, L.F.R., De Feo, V., 2012. Chemistry and antigerminative activity of essential oils and monoterpenoids from Mediterranean plants. Curr. Bioact. 8, 13–49. https://doi.org/10.2174/157340712799828179.
Del Vecchio, S., Acosta, A., Stanisci, A., 2013. The impact of *Acacia saligna* invasion on Italian coastal dune EC habitats. C. R. Biol. 336, 364–369. https://doi.org/10.1016/j.crvi.2013.06.004.
Drew, B.T., Gonzalez-Gallegos, J.G., Xiang, C.L., Kriebel, R., Drummond, C.P., Walker, J.B., Sytsma, K.J., 2017. *Salvia* united: the greatest good for the greatest number. Taxon 66, 133–145. https://doi.org/10.12705/661.7.
Griffin, A.R., Midgley, S.J., Bush, D., Cunningham, P.J., Rinaudo, A.T., 2011. Global uses of Australian acacias: recent trends and future prospects. Divers. Distrib. 17, 837–847. https://doi.org/10.1111/j.1472-4642.2011.00814.x.
Jiménez, A., Pauchard, A., Cavieres, L.A., Marticorena, A., Bustamante, R.O., 2007. Do climatically similar regions contain similar alien floras? a comparison between the Mediterranean areas of central Chile and California. J. Biogeogr. 35, 614–624. https://doi.org/10.1111/j.1365-2699.2007.01799.x.
Kekeç, G., Mutlu, S., Alpsoy, L., Sakçali, M.S., Atici, Ö., 2012. Genotoxic effects of catmint (*Nepeta meyeri* Benth.) essential oils on some weed and crop plants. Toxicol. Ind. Health 29, 504–513. https://doi.org/10.1177/0748233712440135.
Kutiel, P.B., Cohen, O., Shoshany, M., 2004. Invasion rate of the alien species *Acacia saligna* within coastal sand dune habitats in Israel. Israel J. Plant Sci. 52, 115–124.
Lake, J.C., Leishman, M.R., 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. Biol. Conserv. 117, 215–226. https://doi.org/10.1016/S0006-3207(03)00294-5.
Lawson Handley, L.-J., Estoup, A., Evans, D.M., Thomas, C.E., Lombaert, E., Facon, B., Aebi, A., Roy, H.E., 2011. Ecological genetics of invasive alien species. BioControl. 56, 409–428. https://doi.org/10.1007/s10526-011-9386-2.
Le Maitre, D.C., Gaertner, M., Marchante, E.M., Ens, E.J., Holmes, P.M., Pauchard, A., O'Farrell, J.O., Rogers, A.M., Blanchard, R., Blignaut, J., Richardson, D.M., 2011. Impacts of introduced Australian acacias: implications for management and restoration. Divers. Distrib. 17, 1015–1029. https://doi.org/10.1111/j.1472-4642.2011.00816.x.
Li, Q., Yu, P., Chen, X., Li, G., Zhou, D., Zheng, W., 2014. Facilitative and inhibitory effect of litter on seedling emergence and early growth of six herbaceous species in an early successional old field ecosystem. The Scientific World Journal. 11. https://doi.org/10.1155/2014/101860.
Lloret, F., Médail, F., Brundu, G., Camarda, I., Moragues, E., Rita, J., Lambdon, P., Hulme, P.E., 2005. Species attributes and invasion success by alien plants on Mediterranean islands. J. Ecol. 93, 512–520. https://doi.org/10.1111/j.1365-2745.2005.00979.x.
Lorenzo, P., Palomera-Perez, A., Reigosa, M.J., Gonzalez, L., 2011. Allelopathic interference of invasive *Acacia dealbata* Link on the physiological parameters of native understory species. Plant Ecol. 212, 403–412. https://doi.org/10.1007/s11258-010-9831-9.
Maas, B., Clough, Y., Tscharntke, T., 2013. Bats and birds increase crop yield in tropical agroforestry landscapes. Ecol. Lett. 16, 1480–1487. https://doi.org/10.1111/ele.12194.
Maccioni, A., Santo, A., Falconieri, D., Piras, A., Manconi, M., Maxia, A., Bacchetta, G., 2019. Inhibitory effect of rosemary essential oil, loaded in liposomes, on seed germination of *Acacia saligna*, an invasive species in Mediterranean ecosystems. Botany. 97, 283–291. https://doi.org/10.1139/cjb-2018-0212.
Macıas, F.A., Galindo, J.C., Castellano, D., Velasco, R.F., 1999. Sesquiterpene lactones with potential use as natural herbicide models. 2. Guaianolides. J. Agr. Food Chem. 48, 5288–5296. https://doi.org/10.1021/jf0005364.
Maffei, M., Camusso, W., Sacco, S., 2001. Effect of *Mentha* × *piperita* essential oil and monoterpenes on cucumber root membrane potential. Phytochemistry 58, 703–707. https://doi.org/10.1016/S0031-9422(01)00313-2.
Médail, F., 2017. The specific vulnerability of plant biodiversity and vegetation on Mediterranean islands in the face of global change. Reg. Environ. Change 17, 1775–1790.
Médail, F., Quézel, P., 1999. Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities. Conserv. Biol. 13, 1510–1513. https://doi.org/10.1046/j.1523-1739.1999.98467.x.
Meloni, F., Dettori, C.A., Mascia, F., Podda, L., Bacchetta, G., 2011. Germination ecology of the invasive *Acacia saligna* (fabaceae) in sardinia: interpopulation variability and effects of temperature and salinity. In: Brunel, S., Uludag, A., Fernandez-Galiano, E., Brundu, G. (Eds.), Invasive Plants in the Mediterranean Type Regions of the World, pp, 374-385. Proceeding 2nd International Workshop, 2010-08-02/06. European Environment Agency. Trabzon, Turkey.
Meloni, F., Dettori, C.A., Mascia, F., Podda, L., Bacchetta, G., 2015. What does the germination ecophysiology of the invasive *Acacia saligna* (Labill.) Wendl. (Fabaceae) teach us for its management? Plant Biosyst. 149, 242–250. https://doi.org/10.1080/11263504.2013.797032.
Midgely, S.J., Turnbull, J.W., 2003. Domestication and use of Australian acacias: case studies of five important species. Aust. Syst. Bot. 16, 89–102. https://doi.org/10.1071/SB01038.
Morris, T.L., Esler, K.J., Barger, N.N., Shayne, M., Jacobs, S.M., Cramer, M.D., 2011. Ecophysiological traits associated with the competitive ability of invasive Australian acacias. Divers. Distrib. 17, 898–910. https://doi.org/10.1111/j.1472-4642.2011.00802.x.
Musil, C.F., 1993. Effect of invasive Australian acacias on the growth and nutrient chemistry of South African lowland fynbos. J. Appl. Ecol. 30, 361–372. https://doi.org/10.2307/2404637.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature. 403, 853–858. https://doi.org/10.1038/35002501.
Nishida, N., Tamotsu, S., Nagata, N., Saito, C., Sakai, A., 2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. J. Chem. Ecol. 31, 1187–1203. https://doi.org/10.1007/s10886-005-4256-y.
Obroucheva, N.V., 1999. Seed Germination: A Guide to the Early Stages. Backhuys Publishers, Leiden, The Netherlands.
Orchard, A.E., Wilson, A.J.G., 2001. Flora of Australia. 11A, Mimosaceae, *Acacia* part 1. Australian Biological Resources Study, Canberra, Australia.
Podda, L., Fraga, I., Arguimbau, P., Mayoral García-Berlanga, O., Mascia, F., Bacchetta, G., 2010. Comparación de la flora exótica vascular en sistemas de islas continentales: Cerdeña (Italia) y Baleares (España). An. Jardin Bot. Madrid. 67, 157–176. https://doi.org/10.3989/ajbm.2251.
Puddu, S., Podda, L., Mayoral, O., Delage, A., Hugot, L., Petit, Y., Bacchetta, G., 2016. Comparative analysis of the alien vascular flora of Sardinia and Corsica. Not. Bot. Horti Agrobot. Cluj-Na. 44, 337–346. https://doi.org/10.15835/nbha44210491.
Pysek, P., Richardson, D.M., Rejmanek, M., Webster, G.L., Williamson, M., Kirschner, J., 2004. Alien plants in checklist and floras: towards better communication between taxonomists and ecologists. Taxon 53, 131–143. https://doi.org/10.2307/4135498.
Qasem, J.R., 2010. Allelopathy importance, field application and potential role in pest management: a review. J. Agr. Sci. Tech. 4, 104–120.
Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Miller, J.T., Robertson, M.P., Rouget, M., Le Roux, J.J., Wilson, J.R.U., 2011. Human-mediated introductions of Australian acacias - a global experiment in biogeography. Divers. Distrib. 17, 771–787. https://doi.org/10.1111/j.1472-4642.2011.00824.x.
Roma-Marzio, F., Bedini, G., Müller, J.V., Peruzzi, L., 2016. A critical checklist of the woody flora of Tuscany (Italy). Phytotaxa 287, 001–135. https://doi.org/10.11646/phytotaxa.287.1.1.
Ruffino, L., Krebs, E., Passetti, A., Aboucaya, A., Affre, L., Fourcy, D., Lorvelec, O., Barcelo, A., Berville, L., Bigeard, N., Brousset, L., De Méringo, H., Gillet, P., Le Quilliec, P., Limouzin, Y., Médail, F., Meunier, J.-Y., Pascal, M., Pascal, M., Ponel, P., Rifflet, F., Santelli, C., Buisson, E., Vidal, E., 2014. Eradications as scientific experiments: progress in simultaneous eradications of two major invasive taxa from a Mediterranean island. Pest Manag. Sci. 71, 189–198. https://doi.org/10.1002/ps.3786.
Saad, M.M.G., Abdelgaleil, S.A.M., 2014. Allelopathic potential of essential oils isolated from aromatic plants on *Silybum marianum* L. Glo. Adv. Res. J. Agric. Sci. 3, 289–297. http://garj.org/garjas/index.htm.
Saleem, K., Perveen, S., Sarwar, N., Latif, F., Akhtar, K.P., Arshad, H.M.I., 2013. Identification of phenolics in mango leaves extract and their allelopathic effect on canary grass and wheat. Pak. J. Bot. 45, 1527–1535.
Scognamiglio, M., D'Ambrosca, B., Esposito, A., Pacifico, S., Monaco, P., Fiorentino, A., 2013. Plant growth inhibitors: allelopathic role or phytotoxic effects? Focus on Mediterranean Biomes. Phytochem. Rev. 12, 803–830. https://doi.org/10.1007/s11101-013-9281-9.
Singh, H.P., Batish, D.R., Kohli, R.K., 2003. Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. Crit. Rev. Plant Sci. 22, 239–311. https://doi.org/10.1080/713610858.
Singh, H.P., Batish, D.R., Kaur, S., Arora, K., Kohli, R.K., 2006. Pinene inhibits growth and induces oxidative stress in roots. Ann. Bot. 98, 1261–1269. https://doi.org/10.1093/aob/mcl213.
Singh, H.P., Kaur, S., Mittal, S., Batish, D.R., Kohli, R.K., 2009. Essential oil from *Artemisia scoparia* inhibit plant growth by generating ROS and causing oxidative damage. J. Chem. Ecol. 35, 154–162. https://doi.org/10.1007/s10886-009-9595-7.
Taban, A., Saharkhiza, M.J., Hadian, J., 2013. Allelopathic potential of essential oils from four *Satureja* spp. Biol. Agric. Hortic. 29, 244–257. https://doi.org/10.1080/01448765.2013.830275.
Tayeb, W., Chaieb, I., Hammami, M., 2011. Environmental fate and effects of 2,4-dichlorophenoxyacetic herbicide. Herbicides: Properties, Crop Protection and Environmental Hazards (Environmental Science, Engineering and Technology) UK Ed. Edition Editor: Karl D. Piotrowski, pp. 245–262.
Tworkoski, T., 2002. Herbicide effects of essential oils. Weed Sci. 50, 25–431. https://doi.org/10.1614/0043-1745(2002)050[0425:HEOEO]2.0.CO;2.
van Wilgen, B.W., Forsyth, G.G., Le Maitre, D.C., Wannenburgh, A., Kotzé, J.D.F., van den Berg, E., Henderson, L., 2012. An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. Biol. Conserv. 148, 28–38. https://doi.org/10.1016/j.biocon.2011.12.035.
Vilà, M., Corbin, J.D., Dukes, J.S., Pino, J., Smith, S.D., 2007. Linking plant invasions to global environmental change. Terrestrial Ecosystems in a Changing World. Springer, Berlin.
Werner, C., Ulrich, Z., Wolfram, B., Maguas, C., 2010. High competitiveness of a resource demanding invasive *Acacia* under low resource supply. Plant Ecol. 206, 83–96. https://doi.org/10.1007/s11258-009-9625-0.
Willis, K.J. (Ed.), 2017. State of the World's Plants 2017. Report. Royal Botanic Gardens, Kew.