



REPRODUCTIVE TRAITS OF THE INVASIVE SPECIES *ACACIA DEALBATA* LINK. IN THE NORTHERN MEDITERRANEAN BASIN

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ABSTRACT - Among the many exotic plants introduced in the last two centuries in Northern Mediterranean Basin *Acacia dealbata* Link. is one of the most invasive. Despite its presence in the region since many years, it has not yet been investigated how the species has established itself and has formed small forests. In this study, we aimed to gather data on reproductive trait of the invasive species *A. dealbata* at the northern limit of the Mediterranean bioclimatic region. In six naturalized populations, fruit and seed set were estimated and seed germination was tested in laboratory. Two out of the six populations failed to set fruits, and the fruit set was always low as observed in other invaded areas of the world. Only three populations produced germinating seeds whose rates were comparable to other invasive areas. The seeds may constitute a long-lasting seed bank that may favour the colonization of the species in areas disturbed by external factors. In conclusion, the reproductive system may contribute only partially to the spread and invasiveness of the species in the Northern Mediterranean basin, while the plant to spread itself adopts also other vegetative strategies.

KEYWORDS: NW ITALY; ALIEN SPECIES; *ACACIA DEALBATA* LINK.; FRUIT AND SEED SETS; SEED GERMINATION

INTRODUCTION

Biological invasions are considered one of the main causes of the alteration of ecosystems, the introduction of disease vectors and the reduction in distribution and diversity of native species' populations (Vitousek et al., 1996; Walker & Steffen 1997; Fong & Chen, 2010). The introduction of invasive alien species in new regions of the world is mainly caused by human activity, which allows (accidentally or voluntarily) species to overcome their biogeographic barriers and to colonise new territories with favourable environmental conditions for their reproduction and with different competitors in the new ecological balances (Richardson et al., 2001). Voluntary introduction of alien plant species may be due to their use (e.g., for ornamental or landscape purposes), while accidental introduction may be because they are present as contaminants in commercial products. If naturalised, these species cause

alterations to the local flora and in the extreme cases a substitution of the native vegetation, causing damages not only to the environment but often also to the economy (Vilà et al., 2011). Appropriate risk-assessment programs and monitoring of invasive flora are therefore often necessary, as well as the application of procedures to check and contrast alien species. These procedures should be based on a deep knowledge of the invasive species and their capability to spread successfully in the new habitats. Reproductive traits are crucial for the establishment and the maintenance of populations in new areas (Bezeng et al., 2013; Correia et al., 2014) and, therefore, for the invasion process (Pyšek & Richardson, 2007). Thus, studies on reproductive biology of alien species are necessary for the assessment of invasiveness and for the definition of effective control measures in the future.

The silver wattle (*Acacia dealbata* Link.) is an evergreen tree native to south-eastern Australia (Orchard & Wilson, 2001), where it occurs in dry sclerophyll forests on acid soils. In the last 250 years, *A. dealbata* has been introduced worldwide in areas with a Mediterranean climate and nutrient-poor ecosystems (Stock et al., 1995). In 1790, the plant was introduced in Europe for aesthetic and decorative purposes and for beekeeping, starting from 1860 the plant became invasive in the south of France (Sheppard et al., 2006) and, at present, it is widely naturalized in southwest Europe (Tutin et al., 2001; Sheppard et al., 2006; Celesti-Grapow et al., 2010). In Mediterranean regions of the world, the plant is invasive along coastal areas in dunes, maquis and anthropogenic systems (edges of motorways, burned wood near agricultural areas), favoured by some of its biological characteristics (Lorenzo et al., 2010): the nitrogen-fixing symbiosis (May & Attiwill, 2003; Sheppard et al., 2006; Gallagher et al., 2011; Rodríguez-Echeverría et al., 2012), the high water-use efficiency (Rascher et al., 2011), and the allelopathy (Rice, 1984). However, the spread capability of *A. dealbata* is supposed to be linked to its reproductive success (Correia et al., 2014), and the invasiveness of the species in Europe is different from place to place (Lorenzo et al., 2010; González-Muñoz et al., 2012; Correia et al., 2014). In this study, we aimed to gather data on reproductive trait of the invasive species *A. dealbata* at the northern limit of the Mediterranean bioclimatic region. We selected several locations where the species spontaneously form groves in the region. In these populations, we verified the capability of the species to reproduce sexually analysing fruit and seed production, and seeds germination. The results of this study will give insights useful to understand what control measures might be effective to limit the invasiveness of this plant in the future.

MATERIALS AND METHODS

Study species

Acacia dealbata Link. blooms in Europe from December to April from year to year with weather conditions and it shows a long-lasting bloom (Stone et al., 2003). The flowers are displayed in large racemose inflorescences made up of numerous globose bright yellow flower heads of 20–32 individual flowers. Each flower does not have perianth but all stamens together create the showiness of each head attracting pollinators.

Pollinator assemblages vary on an annual, seasonal and geographic basis (Bernhardt, 1989). In their native range, *A. dealbata* is visited by a variety of flower foragers, but the most important pollinators are usually bees and wasps

(Apoidea), followed by flies, beetles and birds (Bernhardt, 1989; Stone et al., 2003). In other parts of the introduced range of *A. dealbata*, generalist pollinators are the most abundant visitors (Correia et al., 2014).

Although *A. dealbata* is predominantly self-incompatible, different degrees of self-compatibility may occur (Stone et al., 2003; Correia et al., 2014). However, selfed progeny of *A. dealbata* has a reduced growth and survival (Correia et al., 2014), which would erode the reproductive assurance benefits of selfing (Herlihy & Eckert, 2002; Gibson et al., 2011).

Study area and selected populations

Acacia dealbata is a pressing problem in the Northern Mediterranean basin, in particular in the NW Italy (Liguria region), strongly involved in trading activities of exotic plants in Europe since the XIX century (Mack, 2001). Moreover, nowadays the land use and climate change are favouring the spread of invasive alien species and their pressure on the rich local flora (Gritti et al., 2006). Six populations were chosen in Liguria region, along the Western Riviera between Ventimiglia and Genoa (Table 1 and Fig. 1). These six populations were chosen because they were evidently of natural origin (derived from a spontaneous spread) and with more than 50 individuals each. The study populations grow on calcareous soil from the sea to about 700 metres of altitude and they are all characterized by the same climatic conditions (Mesomediterranean climate).

Reproductive success

In January 2018, from four to ten racemose inflorescences (about 20 cm length, bearing from 28 to 208 flower heads) per population were randomly chosen on different individuals and permanently labelled with an identification number, for a total amount of 45 inflorescences and 3278 flower heads. In July 2018, they were collected before fruit dehiscence to prevent seed loss.

To estimate fruit set, we calculated the proportion of flower heads producing a fruit with mature seeds, giving that in most cases only one fruit develops from a flower head as reported in literature (Gibson, 2012). To estimate the number of seed per legume, seeds and aborted ovules were counted under a Leica M205 C stereomicroscope. Seeds were visually distinguished as: “filled” when they showed seed coat and cotyledons well formed, assuming standard dimensions; “aborted” when they showed a seed coat but non formed cotyledons, assuming reduced dimensions; “unfertilized ovules” when they did not show seed coat and cotyledons well formed, aborted at an early developmental stage. The number of seed per legume was transformed as $\text{Log}_e + 1$ to account for valid zero values. The seed set was calculated as filled seeds/total number of ovules (filled seeds + aborted seeds + unfertilized ovules).

Table 1. The study populations and their geographical references. For each population are reported: codes used in the study (Code); localities of the study populations (Locality); geographical references (Coordinates); dimension of the population expressed in square metres (Surface); number of specimen (Nsp).

Code	Locality	Coordinates	Surface	Nsp
Pop1	Soldano (IM)	43.8004°N 7.6473°E	1,000	50
Pop2	Piani d'Invrea, Varazze (SV)	44.3668°N 8.6000°E	10,000	>500
Pop3	Pian delle Monache, Pegli (GE)	44.4408°N 8.8021°E	2,000	50
Pop4	Villa Gavotti, Sestri Ponente (GE)	44.4324°N 8.8315°E	5,000	>100
Pop5	Maneggio di Cesino, Bolzaneto (GE)	44.5037°N 8.9044°E	1,000	50
Pop6	Torbella, Begato (GE)	44.4449°N 8.9079°E	1,000	50

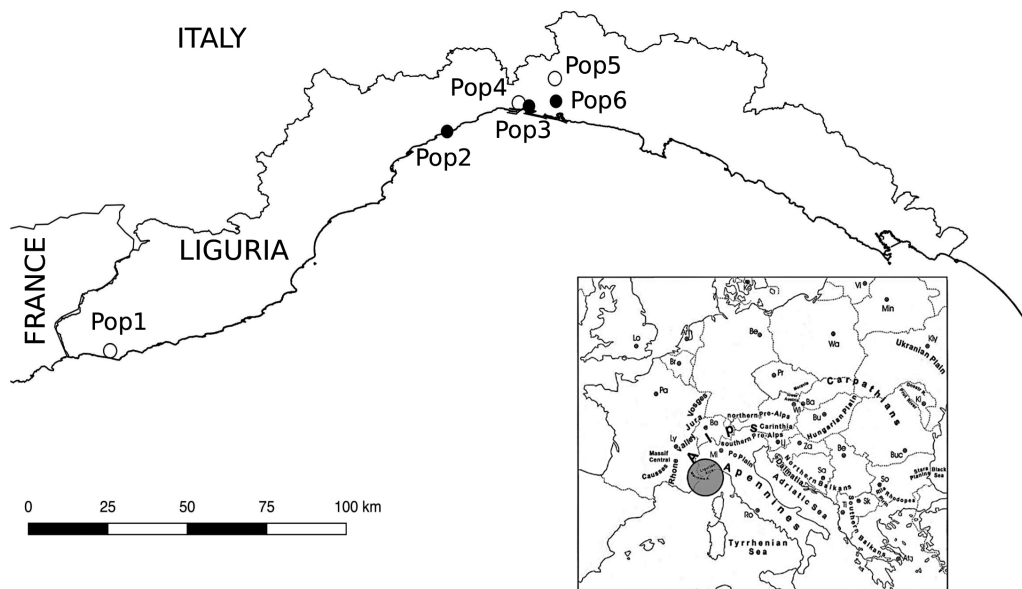


Figure 1. The study area and the geographical distribution of the six *A. dealbata* populations. The populations codes are those reported in Table 1.

Fruit set and seed set values may range from 1 (full success) to 0 (reproductive failure).

Germination test

The seeds, collected in July 2018 in the populations producing seeds (see Table 2), were subjected to visual inspection and only filled seeds were kept at room temperature for the following germination test (October 2018). A final amount of 610 seeds from three populations (Pop 2=300; Pop 4=260 and Pop 6=50) was used for the following tests. Seed weight (mg + sd) was obtained by weighting 380 seeds (Pop2= 140; Pop4= 200; Pop6= 40) randomly selected from each population. The germination test, performed in a temperature- and light-controlled climate chamber (HPP Peltier Memmert), was preceded by a pre-treatment to break the physical dormancy characterizing *Acacia* genus as reported in literature (Baskin & Baskin, 1998; Thompson et al., 2003; Yousif et al., 2019). A mechanical scarification

with sandpaper was performed and followed by a soak in hot water, according to the method successfully used by Piotto & Di Noi (2003) modified as follow. The mechanical scarification was carried out with a plastic cylinder (Ø 5 cm x 8 cm height), lined internally with sandpaper (FEPA P80), and agitated with an automatic shaker (New Brunswick scientific) at 500 r.p.m. for 5 min. Seeds were then soaked in hot water (100° C) and kept at room temperature for 14 hours. Finally, Seeds were sterilized (10 min. in a water solution of NaClO 2.5%, and three times rinsed in distilled water) and sown in sterile Petri dishes (Ø 9 cm) on 2 disks on filter paper Perfect2® (90 gr/m²) moistened with sterilized distilled water (Hartmann & Kester, 1983; Bacchetta et al., 2006). Seeds were incubated for 100 days at 25 ± 1° C under a 12 h photoperiod (white light 2700K) according to the results (G%= 100) of preliminary test performed by us in 2017 (personal data) and data from an Australian database (*A. dealbata* in <https://www.treeproject.org.au/order/seedling-database>).

Germination was checked three times a week and seeds were considered germinated when the healthy and white radicle had emerged through the integument. The total germination rate (G) was calculated at the end of the experiment (International Seed Testing Association, ISTA, 2019).

To test whether the populations differed significantly in weight per seed and in the total germination rate we applied the non-parametric Kruskal-Wallis test and Pairwise Wilcoxon Rank Sum Tests post hoc test using the R ‘stats’ package implemented in R (R Core Team 2018).

RESULTS

Reproductive success

Two out of the six populations failed to set fruits (i.e., Pop3 and Pop5) in the canopies of the specimens selected. A very low fruit set was detected in Pop1, Pop4 and Pop6 (0.02 sd 0.03, 0.11 sd 0.19 and 0.06 sd 0.05, respectively). The highest fruit set was recorded in Pop2 (0.25 sd 0.33, Table 2).

In most cases, we observed only one fruit per flower head, and only in very rare cases (0.5%) the development of two fruits per flower head was observed. We detected a mean fruit set of 0.07 sd 0.10 (Table 2).

In Pop1, where we detected the lowest fruit set and the few legumes collected had only aborted ovules (Table 2). The mean seed number per legume was 2.37 sd 2.74 ($\text{Log}_e+1 = 0.90$ sd 0.81 – Table 2). Taking into account populations developing seeds we detected a mean seed set of 0.57 sd 0.27, while seed set per population ranged from 0.27 sd 0.07 to 0.82 sd 0.12 (Table 2).

Germination test

The mean seed weight was significantly different among the three populations: significantly higher in Pop2 (0.01112 mg; sd 0.00111) than in Pop4 (p-value 0.030) and in Pop6 (p-value 0.008); intermediate in Pop4 (0.00921 mg; sd 0.00084) and significantly higher than Pop6 (p-value 0.006) and lowest in Pop6 (0.00427 mg; sd 0.00140). The germination rate showed a mean value of 50.75%, but it varied among populations (Fig. 2) with the significantly higher values recorded in Pop4 (76.92%; seeds = 260; p value 0.01) and in Pop2 (63.33%; seeds = 300; p value 0.04) compare to Pop6 (12.00%; seeds = 50).

Table 2. Fruit set and seed set recorded in the six populations. For each population are reported: number of ramets investigated (N); number flower heads investigated (Fl-h); number of fruits detected (Fr); fruit set values (Fr set); total ovules number detected (O); total number of seeds (S); seed set values (S set); number of ovules per fruit (O/Fr); number of seeds per fruit (S/Fr); seed per fruit transformed as Log_e+1 . In brackets standard deviation is reported.

	N	Fl-h	Fr	Fr set (sd)	O	S	S set	O/Fr (sd)	S/Fr (sd)	Log_e+1 (sd)
Pop1	8	377	7	0.02 (0.03)	12	0	0.00	1.61 (0.67)	0.00	0.00
Pop2	4	470	171	0.25 (0.33)	880	287	0.27 (0.07)	6.64 (2.27)	1.84 (0.84)	1.01 (0.28)
Pop3	10	577	0	0.00	0	0	0.00			
Pop4	6	467	45	0.11 (0.19)	350	279	0.82 (0.12)	7.53 (2.06)	6.31 (2.38)	1.94 (0.35)
Pop5	8	796	0	0.00	0	0	0.00			
Pop6	9	591	36	0.06 (0.05)	87	53	0.60 (0.26)	2.21 (0.46)	1.32 (0.66)	0.63 (0.44)
Total	45	3278	259	0.07 (0.10)	1329	619	0.57 (0.27)	4.50 (3.02)	2.37 (2.74)	0.90 (0.81)

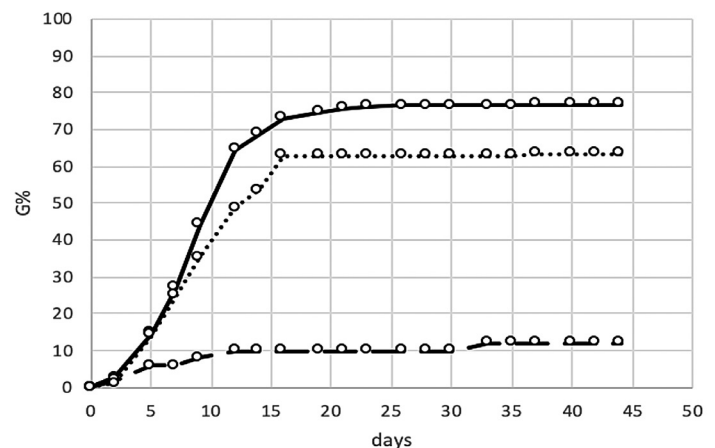


Figure 2. Germination percentages of the three *A. dealbata* populations producing seeds. Dotted line = Pop2, continuous line = Pop4, dashed line = Pop6.

DISCUSSION

Only four out of six studied populations yielded fruits and three out of six populations yielded seeds (Table 2). These results suggest that the reproductive system is not the only way for *A. dealbata* to spread itself at the northern limit of the Mediterranean bioclimatic region and other solutions are adopted, for example resprouting. Invasion by resprouting in *A. dealbata* has been already observed in Chile and other parts of Europe and it was suggested as one of the mechanism to facilitate the rapid colonization of disturbed environments (Marchante et al., 2008; Lorenzo et al., 2010; Fuentes-Ramirez et al., 2011).

The low number of fruits per flower head (usually one) detected in NW Italian populations of *A. dealbata* as well as the low production of fruit per inflorescence are in line with previous findings in the genus and specifically in *A. dealbata* (Tybirk, 1993; Kenrick, 2003; Gibson et al., 2011). In particular, our results are in line with fruit set detected for the species in invaded areas like S Africa and Portugal (roughly 0.15 and 0.01, respectively - Rodger & Johnson, 2013; Correia et al., 2014).

In general, the low fruit set detected in the invaded range of *A. dealbata* was explained by pollen limitation (Correia et al., 2014). Pollen limitation occurs when pollen quantity or quality is low or when pollination services is low (Totland et al., 1998). In Liguria *A. dealbata* blooms early (from December to February) when the number and diversity of insects available for pollination is low, probably resulting in pollen limitation. In such cases, according to literature the dimension of the population may favour pollinator attraction (Ågren, 1996) and cross-pollination (Brys et al., 2008). Even further investigations are necessary and this study gathered only preliminary data on the reproductive capacities of the species, the fruit set variability detected seems to be coincident with the population size. In fact, the relatively high production detected in Pop2 might be linked to the presence in the population of more than five hundreds plants (Table 1). On the contrary, the low fruit set recorded in Pop4 and Pop6 might depend to their smaller population size, eventually favouring pollen limitation and self-pollination. In facts, in a predominantly self-incompatible species like *A. dealbata*, the result might be a lower fruits production (Barret, 2011; Gibson et al., 2011; Rodger & Johnson, 2013; Correia et al., 2014).

The number of seeds per legume (Table 2) detected in NW Italian populations is in accordance with results of previous studies on *A. dealbata* in Australia and S Africa (Broadhurst & Young, 2006; Rodger & Johnson, 2013) and with what more generally observed in *Acacia* (Morgan et al., 2002). Moreover, we observed variable values of seed set among the populations (Table 2), in line with the results detected in

the native area of the species (Broadhurst & Young, 2006). The among populations variability in seed set may be linked to local variations in mating opportunities (Broadhurst & Young, 2006), pollen viability, resource limitation and self-pollen deposition in a predominantly incompatible species (Correia et al., 2014).

The percentage of seed germination detected in NW Italian populations is generally high (63-77%) and only Pop6 had a low germination (12%; Fig. 2) and its seed were the lighter too. This result is in line with the expectation that small and light seeds have lower growth and seedling survival than large and heavy ones (Armstrong & Westoby, 1993; Vera, 1997; Bonfil, 1998). However, the percentages of germination detected in this study are similar to those reported for other invasive areas (Rodger & Johnson, 2013; Correia et al., 2014). Seeds of *A. dealbata* as well as other *Acacia* species are characterized by a physical dormancy (Baskin & Baskin, 1998; Thompson et al., 2003) primarily broken by heat pulse like fire (Richardson & Kluge, 2008; Fuentes-Ramirez et al., 2011; Gibson et al., 2011). This dormancy may enable *A. dealbata* to colonize disturbed areas. In fact, in areas with strongly affected by fire disturbance (Martínez et al., 2009), altering the natural vegetation dynamic in Mediterranean fire-prone ecosystems, the species may be favoured in new areas colonization by its long-lasting seed bank (Richardson & Kluge, 2008).

CONCLUSIONS

The data gathered on reproductive trait of *A. dealbata* show that only some of the studied populations use the reproductive system to implement the invasiveness of the species in the Northern Mediterranean basin by creating a long-lasting seed bank. The other populations may adopt other strategies to increase their invasiveness (e.g., sprouts at root-crown and root levels) as frequently observed in other invaded areas of the world with a Mediterranean and sub-Mediterranean climate.

Long-lived seed banks and ability to resprout are key determinants of persistence; these traits are hugely influential ingredients of invasive success since they ensure persistence and effectively permanent occupancy of invaded sites (e.g. Richardson & Cowling, 1992; Gibson et al., 2011). On this base, it is so difficult to define what control measures might be effective to limit the invasiveness of this plant in the future (Lorenzo et al., 2009).

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