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Original Research Article

Seed Dormancy and Germination of *Heliotropium europaeum* L. (Boraginaceae), a Widespread Summer-annual Weed

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ABSTRACT

***Heliotropium europ*aeum L. (Boraginaceae) is a summer-annual weed of European origin. The aim of this study was to investigate the germination requirements of *H. europaeum* and to determine if seeds present some type of dormancy. Cold stratification and soaking in gibberellic acid (GA_3) were used as pre-sowing treatments for breaking dormancy in *H. europaeum* seeds. Untreated seeds showed an extremely high dormancy at all tested incubation temperatures (only ≤ 2% of them germinated after 8 weeks of incubation), and GA_3 and cold stratification drastically improved germination percentages (≥ 80%). The highest germination percentages of *H. europaeum* seeds soaked in GA_3 were achieved at the highest constant temperature (30ºC) and alternating temperatures (25/15ºC and 30/20ºC). Moreover, the lowest germination rates were observed at the two lowest constant temperatures (15ºC and 20ºC). Germination of seeds soaked in GA_3 increased as concentration of acid increased (from 10 to 1000 mg L^{-1}). Similarly, cold stratification increased germination at all tested stratification periods (from 7 to 360 days). Since embryos are fully developed and seed mass increased 52% after 24 hours of imbibition, showing that seed coat is water permeable, we conclude that *H. europaeum* seeds present physiological dormancy. Cold stratification and treatment with GA_3 were two effective methods for overcoming the physiological dormancy in seeds of *H. europaeum*. Knowledge of germination requirements may aid in developing tools and strategies for management of this weed.**

Keywords: Cold stratification, common heliotrope, gibberellic acid, physiological dormancy, seed imbibition

INTRODUCTION

Heliotropium europaeum L. (common heliotrope, European heliotrope), a summer-growing and herbaceous annual plant, is a member of the Boraginaceae family. The species of European origin is a widespread and common weed from the Mediterranean Basin to the Middle East, West Asia and Australia (Hunt *et al.* 2008, 2009). In the Mediterranean-type ecosystems of southtern Australia, common heliotrope was naturalised in the early 19th century (Hunt *et al.* 2009), and is now considered an economically important weed of both crops and pastures (Hunt *et al.* 2008, 2009). The species contains toxins (pyrrolizidine alkaloids) (Saeedi and Semnani 2009) and is toxic to livestock (Hunt *et al.* 2009). The plant successfully grows on road margins reaching heights near 60 cm (Juan and Talavera 2012). The vegetative and reproductive growth of *H. europaeum* is very fast and the weed senesces in autumn creating a persistent soil seed bank (Aliloo and Darabinejad 2013). Like many annual weeds (Martínez-Laborde *et al.* 2007), its short life cycle, its ability to prosper in highly disturbed environments, and the high number of small seeds produced per plant provides it with remarkable colonizing and invasive capacity.

As a plant species with an annual life cycle, *H. europaeum* completely depend on seed germination for regeneration and, therefore, information on seed germination requirements of the species is essential for increasing the success of control strategies of this weed. Germination behaviour is often described as adaptive in relation to habitat characteristics (Kos and Poschold 2010). Seed dormancy is a complex phenomenon by which seeds schedule their germination to coincide with favourable periods for seedling establishment (Bradford 2005). Once dormant seeds have received required signals from the environment dormancy is alleviated and germination can proceed (Ustarroz *et al.* 2016). Freshly matured seeds of many herbaceous weeds have physiological dormancy (Baskin and Baskin 2014). Weeds with physiological dormancy include summer annuals whore seeds require cold stratification to come out of dormancy (Kirmizi *et al.* 2011; Baskin and Baskin 2014). This type of seed dormancy is usually caused by a physiological inhibiting mechanism of the embryo that prevents radicle emergence (Baskin and Baskin 2014). *Heliotropium* species can differ in their germination

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behaviour. Thereby, while *H. subulatum* seeds have physiological dormancy (Baskin and Baskin 2014), seeds of Indian heliotrope (*H. indicum*), a common weed of rain-fed rice in many tropical counties, are not dormant and germinate at a wide range of temperatures (Chauhan and Johnson 2008). Information regarding *H. europaeum* requirements for germination is scarce. Hunt *et al.* (2009) examined common heliotrope's germination responses to temperature and water potential, and Aliloo and Darabinejad (2013) found that fresh seeds of *H. europaeum* had a high level of dormancy.

The aim of this study was to investigate the germination requirements of *H. europaeum* and to determine if seeds present some type of dormancy. The specific objectives were: (i) to determine water uptake during seed imbibition, (ii) to evaluate seed germination to different temperature regimes and (iii) to investigate the effects of pre-sowing treatments (gibberellic acid and cold stratification) for enhancing germination.

MATERIALS AND METHODS

Seed collection

Ripe seeds, belonging to 50 different individuals chosen at random, were collected in September 2014 from a wild growing population in the vicinity of the village of Soto del Real (40º45'N, 3º47'W, 919 m a.s.l.), northern Madrid Community, central Spain. Seeds were kept in paper bags, and then stored dry under laboratory conditions (at about 23ºC, under darkness, 30-35% relative humidity) for approximately 1 month until they were used in the germination tests. Visible damaged seeds were excluded from the experiments.

Water uptake during seed imbibition

To determine water uptake capacity during seed imbibition, three replicates of 100 seeds each were weighed using an analytical balance with an accuracy of 0.01 mg and then placed in glass Petri dishes on two discs of filter paper moistened with distilled water at room temperature (~23ºC). After each imbibition period (from 1 to 48 hours, see Figure 1), seeds were taken out of the Petri dishes, quickly surface-dried with filter paper, reweighed, and returned to the dishes. Percentage of water uptake (mean value ± standard error) was calculated as the amount of water taken up relative to initial seed mass.

General seed germination trials

Seeds were tested for germination at four different constant temperatures (15ºC, 20ºC, 25ºC, 30ºC) under a 16-h light photoperiod (light was provided by cool white fluorescent tubes with an irradiance of 35 μmol m^2 s^{-1}), and the alternate temperature regimes of 25/15ºC and 30/20ºC (the highest temperature for 16 h in light and the lowest one for 8 h in dark). In all trials, four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 mL distilled water) in 7-cm-diameter glass Petri dishes. Filter papers were rewetted regularly with distilled water as required. Dishes were checked three times a week over a total of an 8 weeks test period and germinated seeds were counted and removed. Seeds were considered germinated on emergence of the radicle from the seed coat. In all germination trials, seeds that had not germinated at the end of the incubation period were opened to determine whether they were empty and whether they contained an intact and healthy embryo.

Pre-sowing treatments

The pre-sowing treatments were:

1. Soaking in distilled water – Seeds were soaked in distilled water (three volumes of water for each volume of seeds) at room temperature (~23ºC) for 24 hours, and then tested for germination at all temperature regimes assayed (see Table 1). Non-soaked seeds were sown in the same conditions of temperature and light and they were used as a control seeds (dry control);
2. Soaking in GA_3 – In a first trial, seeds were soaked for 24 hours at room temperature (~23ºC) in an aqueous GA_3 solution (1000 mg L^{-1}; three volume of GA_3 solution for each volume of seeds), and then tested for germination at all temperature regimes (see Table 1). Non-soaked seeds were sown in the same conditions of temperature and light and they were used as a control seeds (dry control). In a second trial, seeds were soaked for

24 hours in GA_3 solutions (three volume of GA_3 solution for each volume of seeds) of different concentration (from 10 to 1000 mg L^{-1}), and then tested for germination at two alternating temperature regimes (25/15ºC and 30/20ºC, see Table 2). Seeds soaked in distilled water for 24 hours were sown in the same conditions of temperature and light and they were used as a control seeds (wet control);

3. Cold stratification – Seeds were cold stratified in moist vermiculite under darkness at 5ºC for different stratification periods (from 7 to 360 days, see Table 3), and then tested for germination at 30/20ºC. Non-stratified seeds were sown in the same conditions of temperature and light and they were used as a control seeds.

Table 1. Effect of different temperature regimes and pre-sowing treatments on the final germination percentages (mean ± standard error) and mean germination time (MGT, days ± standard error, values in brackets) of *Heliotropium europaeum* seeds.

Temperature	Germination (% ± SE) and MGT (days ± SE)		
(ºC)	Control	H_2O	GA_3
15	0 aA (NC)	1 ± 0.87 aA (NC)	40 ± 2.83 bA (19.75 ± 0.53 b)
20	1 ± 0.87 aA (NC)	1 ± 0.87 aA (NC)	59 ± 3.57 bAB (14.40 ± 0.20 b)
25	2 ± 1.00 aA (NC)	6 ± 1.00 aA (NC)	68 ± 4.00 bAB (8.72 ± 0.29 a)
30	1 ± 0.87 aA (NC)	6 ± 2.24 aA (NC)	72 ± 6.48 bB (7.27 ± 0.29 a)
25/15	2 ± 1.73 aA (NC)	4 ± 2.00 aA (NC)	72 ± 8.60 bB (8.80 ± 0.54 a)
30/20	1 ± 0.87 aA (NC)	7 ± 1.66 aA (NC)	80 ± 5.10 bB (8.15 ± 0.39 a)

Mean germination values followed by the same uppercase letter within a column or by lowercase letter within a row are not significantly different from each other ($p>0.05$). MGT values followed by the same letter are not significantly different from each other ($p>0.05$).
Control, untreated seeds (dry control); H_2O, soaking for 24 h in distilled water; GA_3, soaking for 24 hours in a gibberellic acid solution (1000 mg L^{-1}). NC = MGT was not calculated when final germination percentage was ≤ 10%.

Table 2. Effect of different GA_3 concentrations on the final germination percentages (mean ± standard error) and mean germination time (MGT, days ± standard error) of *Heliotropium europaeum* seeds.

Concentration of	Germination (% ± SE)		MGT (days ± SE)	
GA_3 (mg L^{-1})	25/15ºC	30/20ºC	25/15ºC	30/20ºC
0 (control)	4 ± 2.00 a	7 ± 1.66 a	NC	NC
10	15 ± 2.60 ab	19 ± 1.66 b	9.75 ± 0.92 a	8.02 ± 0.52 a
25	13 ± 1.66 ab	25 ± 1.66 b	9.12 ± 0.71 a	8.97 ± 0.86 a
50	35 ± 4.33 bc	32 ± 3.74 bc	8.97 ± 0.66 a	8.15 ± 0.50 a
100	34 ± 3.32 bc	32 ± 4.90 bc	8.90 ± 0.33 a	7.15 ± 0.73 a
250	48 ± 7.87 cd	64 ± 4.47 d	8.02 ± 0.61 a	8.37 ± 0.64 a
500	61 ± 3.57 cd	58 ± 4.12 cd	7.62 ± 0.29 a	7.22 ± 0.19 a
1000	72 ± 8.60 d	80 ± 5.10 d	8.80 ± 0.54 a	8.15 ± 0.39 a

Numbers in a column followed by the same letter are not significantly different from each other ($p>0.05$).
Results after 8 weeks of seed incubation at 25/15ºC or 30/20ºC. NC = MGT was not calculated when final germination percentage was ≤ 10%.Control = seeds soaked for 24 hours in distilled water (wet control).

Table 3. Effects of cold stratification on the final germination percentages (mean ± standard error) and mean germination time (MGT, days ± standard error) of *Heliotropium europaeum* seeds.

Cold-stratification period (days)	Germination (% ± SE)	MGT (days ± SE)
0 (control)	1 ± 0.87 a	NC
7	62 ± 5.38 b	15.35 ± 0.84 c
15	76 ± 6.32 b	9.90 ± 0.83 ab
30	75 ± 4.97 b	8.82 ± 0.48 a
60	68 ± 5.48 b	9.02 ± 0.28 a
75	65 ± 3.28 b	7.55 ± 0.06 a
100	72 ± 2.45 b	7.87 ± 0.22 a
120	70 ± 3.32 b	8.20 ± 0.43 a
180	84 ± 2.45 b	9.15 ± 0.27 a
360	70 ± 4.22 b	12.51 ± 0.42 bc

Numbers in a column followed by the same letter are not significantly different from each other ($p>0.05$).
Results after 8 weeks of seed incubation at 30/20ºC. Control = non-stratified seeds. NC = MGT was not calculated when final germination percentage was ≤ 10%.

Data analysis

For all experiments, final germination percentage (mean value ± standard error) was calculated. The final germination percentages were arcsine transformed and then subjected to ANOVA (untransformed data appear in the Tables) using the computing package SPSS (SPSS Inc., Chicago; USA). The transformed data were checked for normality and homogeneity of variances before ANOVA analysis. Before any statistical analysis, the number of empty seeds and non viable seeds in each replicate (ca. 5% of whole seeds sown) were always excluded when calculating the final germination percentage. Moreover, for seeds soaked in GA_3 and for stratified seeds, mean germination time (MGT, mean value in days ± standard error) was calculated. This parameter was determined according to the following formula: $MGT = \Sigma DN/\Sigma N$; where D is the number of days counted from the date of sowing and N is the number of seeds germinated on day D. MGT value was not calculated when germination percentage was less than 10%. Where ANOVA indicated a significant effect, a comparison of mean values was carried out through the least significant difference test (LSD) at 5% level of significance.

RESULTS

The mean fresh mass for a lot of 100 seeds was 70.13 ± 0.80 mg. There were significant differences ($p<0.001$) in mass of seeds before and after imbibition in distilled water. Thus, *H. europaeum* seeds imbibed water quickly and after 1 and 2 hours of imbibition in distilled water seed mass increase was 22.62 ± 1.78% and 30.56 ± 1.74%, respectively (Figure 1). By 24 and 48 hours seed mass increase was 52.05 ± 2.36% and 52.31 ± 1.45%, respectively (Figure 1). Therefore, after 1 hour of imbibition, heliotrope seeds reached 43% of the water uptake percentage reached after 48 hours. Thus, the seed coat of *H. europaeum* does not prevent imbibition of water.

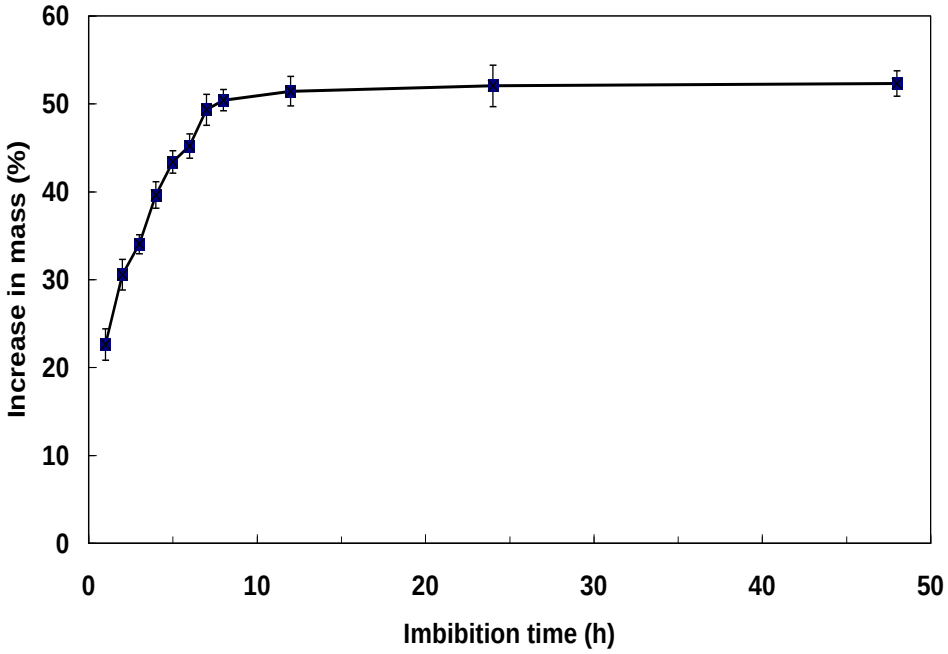

Figure 1. Mean (± SE) increase in mass of Heliotropium europaeum seeds incubated on filter paper moistened with distilled water at ~23ºC

The final germination percentages reached by untreated seeds of *H. europaeum* were less than or equal to 2% after 8 weeks of incubation in light at a wide range of constant (15ºC, 20ºC, 25ºC, 30ºC) and alternating temperatures (25/15ºC, 30/20ºC) (Table 1). For all temperature regimes, seed germination was not significantly ($p>0.05$) affected by soaking in distilled water for 24 hours (Table 1). However, germination percentages of seeds soaked in a GA_3 solution of 1000 mg L^{-1} ranged from 40 to 80% depending on incubation temperature. The highest improvement in germination (80%) was achieved at 30/20ºC. The germination of *H. europaeum* seeds was affected by temperature ($p<0.001$) and treatment ($p<0.001$) and the interaction of the two factors was not significant ($p=0.157$). For each incubation temperature used, soaking in GA_3 had a significant effect ($p<0.05$) on germination in comparison to control seeds and seeds soaked in distilled water (Table 1). The percentage of viable seeds (seeds with intact and healthy embryos) which did not germinate was ca. 95% of whole seeds sown. For seeds soaked in a GA_3 solution of 1000 mg L^{-1}, MGT values reached at the highest constant temperatures (25ºC and 30ºC) and alternating temperatures (25/15ºC and 30/20ºC) were significantly ($p<0.05$) lower than those reached at the lowest constant temperatures (15ºC and 20ºC) (Table 1).

Seed germination seeds ranged from 13 to 80% for seeds soaked in GA_3 depending on concentration of GA_3 and incubation temperature (Table 2). Germination percentage showed a positive correlation with concentration of GA_3 ($r^2=0.764$ and $p=0.004$ for 25/15ºC; $r^2=0.742$ and $p=0.006$ for 30/20ºC). Therefore, seed germination increased as concentration of GA_3 increased (Table 2). For 30/20ºC, GA_3 was significantly ($p<0.05$) effective when compared to control seeds (seeds non-soaked in GA_3) for all tested concentrations (Table 2). However, for 25/15ºC, GA_3 was only significantly ($p<0.05$) effective for concentrations from 50 to 1000 mg L^{-1} (Table 2). Besides, for each GA_3 concentration, germination of seeds was similar between both alternating temperature regimes used (25/15ºC and 30/20ºC) (Table 2). For both alternating temperature regimes, no significant differences ($p>0.05$) were found for the MGT values reached at the different concentrations of GA_3 (Table 2).

A short period of cold stratification (7 days) was highly effective in promoting germination (62%) (Table 3). Seeds cold stratified germinated from 62 to 84%, depending of stratification period (Table 3). All stratification periods increased significantly ($p<0.05$) the germination when compared to control seeds (non-stratified seeds). The highest germination percentage (84%) was achieved by stratified seeds for a 180-days

period. However, no significant differences ($p>0.05$) were found for germination percentages reached at the different stratification periods (Table 3). Seeds stratified at the lowest (7 days) and the highest (360 days) stratification period germinated more slowly than seeds stratified at the other periods (from 15 to 180 days). The fastest germination was recorded by seeds stratified for a 75-days period (Table 3).

DISCUSSION

Germination percentages achieved by *H. europaeum* seeds were extremely low at all tested incubation temperatures. Thus, only a very small fraction (equal to or less than 2%) could germinate without any pre-sowing treatment after 8 weeks of incubation in light at the different temperature regimes. Therefore, an extremely high percentage of freshly matured seeds of *H. europaeum* expressed strong dormancy. The percentage of viable seeds (seeds with intact and healthy embryos) which did not germinate was very high (≥ 95%). Moreover, the high viability of non-germinated seeds was confirmed by the high germination percentages (80-84%) that were achieved by treated seeds (seeds soaked in GA_3 and cold-stratified seeds).

Heliotropium europaeum seeds absorbed water quickly during the first 24 hours of imbibition in distilled water, indicating that their seed coat does not prevent absorption of water. Therefore, these seeds did not exhibit physical dormancy, according to classification system of Baskin and Baskin (2014), where physical dormancy is defined as the result of a water-impermeable layer in the seed or fruit. Besides, *H. europaeum* seeds have a well developed and differentiated embryo (i.e. cotyledons and radicle can be distinguished). Since embryos are fully developed and seeds of *H. europaeum* are permeable to water but require cold stratification for germination that would mean they have some type of physiological dormancy (see Baskin and Baskin 2014). Seeds of some *Heliotropium* species, as *H. subulatum* (Baskin and Baskin 2014), have also physiological dormancy; however, other species, as *H. indicum* (Chauhan and Johnson 2008), present non-dormant seeds. In our study, only 1-2%, of heliotrope seeds germinated without any treatment and, therefore, they expressed strong dormancy. This high level of dormancy at the fresh harvesting stage was also found by Aliloo and Darabinejad (2013) using heliotrope seeds collected in North West of Iran.

According to Baskin and Baskin (2014), there are three types of physiological dormancy: non-deep, intermediate, and deep. The duration of the cold stratification period required for breaking dormancy can indicate the type of physiological dormancy. A 12-weeks period of cold stratification is adequate to break dormancy in seeds of many species (Baskin and Baskin 2014). Non-deep physiological dormancy is broken by relatively short (1-8 weeks) periods of cold stratification and GA_3 can promote germination (Baskin and Baskin 2014). In our study, physiological dormancy of *H. europaeum* seeds was broken by a very short period of cold stratification (1 week) and the application of a GA_3 solution of 1000 mg L^{-1}. Gibberellic acid increased the final germination percentages for all tested incubation temperatures. Moreover, germination of seeds soaked in GA_3 increased as concentration of acid increased, and GA_3 was significantly effective for concentrations equal to or higher than 50 mg L^{-1}. Since GA_3 and cold stratification are highly effectives for promoting seed germination, *H. europaeum* seeds would be classified into the non-deep physiological dormancy type (Baskin and Baskin 2014). In contrast, Hunt (2006) found that germination of common heliotrope seeds did not require cold stratification.

The germination percentages of untreated seeds and seeds soaked in distilled water were equal to or lower than 7% depending on incubation temperature regimes. Therefore, most freshly matured seeds of *H. europaeum* are dormant and did not germinate. This fact could indicate that, in natural habitats, this species should have the capacity to form persistent soil seed banks. Physiological dormancy is an adaptive trait because it allows seed germination over time and space (Baskin and Baskin 2014). In the Mediterranean Basin, heliotrope seeds mature in late summer and, therefore, seeds would receive an enough period of cold stratification during winter season after dispersal to be able to germinate to high percentages. Therefore, the implication of a cold-stratification requirement to break dormancy in seeds of *H. europaeum* with non-deep physiological dormancy is clear: most of seeds will not germinate until late spring and early summer of the next year.

In our study, the highest germination percentages of *H. europaeum* seeds soaked in GA_3 were achieved at the highest constant temperature (30ºC) and alternating temperatures (25/15ºC and 30/20ºC). Moreover, the

lowest germination rates were observed at the two lowest constant temperatures (15ºC and 20ºC). These temperature requirements are in agreement with field data showed by Hunt *et al*. (2009), and they suggest an adaptation of germination in late spring and early summer. Thus, in the site where *H. europaeum* seeds were collected for carrying out this study, seeds of this species germinate in late spring and early summer (personal observation), when mean daily temperatures exceed 22ºC. Therefore, the species behaved as summer-annual weed.

CONCLUSIONS

We found that seed coat of *H. europaeum* was permeable to water and, therefore, seeds did not present physical dormancy. Cold stratification and applications of GA_3 stimulated seed germination (i.e. break dormancy). Based on obtained results we conclude that heliotrope seeds present non-deep physiological dormancy. In the natural habitat, cold stratification is an important part of the dormancy-breaking requirement of these seeds. The results of our study could help to develop effective management strategies for the control of common heliotrope and would help in predicting the potential of these species for spreading into new areas. To our knowledge, this is the first study which highlighted the existence of non-deep physiological dormancy in *H. europaeum* seeds.

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REFERENCES

Aliloo AA, and Darabinejad S (2013). Evaluation of different techniques for breaking seed dormancy of *Heliotropium europaeum* L. (Boraginaceae). Journal of Biological and Environmental Sciences, 7: 87-91.

Baskin CC, and Baskin JM (2014). Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination (2nd edition). Academic Press, San Diego, CA, USA.

Bradford KJ (2005). Threshold models applied to seed germination ecology. New Phytologist, 165: 338-341.

Chauhan BS, and Jonhson DE (2008). Seed germination ecology of purple-leaf button weed (*Borreria ocymoides*) and Indian heliotrope (*Heliotropium indicum*): two common weeds of rain-fed rice. Weed Science, 56: 670-675.

Hunt JR (2006). The Ecology of Common Heliotrope (*Heliotropium europaeum* L.) in a Mediterranean Dry-land Cropping System. The University of Melbourne, Melbourne, Australia.

Hunt JR, Cousens RD, and Knights SE (2008). The biology of Australian weeds 51: *Heliotropium europaeum* L. Plant Protection Quarterly, 23: 146-152.

Hunt JR, Cousens RD, and Knights SE (2009). *Heliotropium europaeum* only germinates following sufficient rainfall to allow reproduction. Journal of Arid Environments, 73: 602-610.

Juan R, and Talavera S (2012). *Heliotropium europaeum* L. In: Talavera S. *et al*., editors, Flora Iberica: Plantas Vasculares de la Península Ibérica e Islas Baleares. Vol. 11. Real Jardín Botánico, CSIC, Madrid, Spain. pp. 528-529.

Kirmizi S, Güleryüz G, and Arslan H (2011). Germination responses to Ga3 and short-time chilling of three endemic species: *Tripleurospermum pichleri*, *Cirsium leucopsis* and *Senecio olympicus* (Asteraceae). Plant Species Biology, 26: 51-57.

Kos M, and Poschold P (2010). Why wait? Trait and habitat correlates of variation in 10 germination speed among Kalahari annuals. Oecologia, 162: 549-559.

Martínez-Laborde JB, Pita-Villamil JM, and Pérez-García F (2007). Secondary dormancy in *Diplotaxis erucoides*: a possible adaptative strategy as an annual weed. Spanish Journal of Agricultural Research, 5: 402-406.

Saeedi M, and Semnani M (2009). Chemical composition and antimicrobial activity of the essential oil of *Heliotropium europaeum*. Chemistry of Natural Compounds, 45: 98-99.

Ustarroz D, Kruk BC, Satorre EH, and Ghersa CM (2016). Dormancy, germination and emergence of *Urochloa panicoides* regulated by temperature. Weed Research, 56: 59-68.