Seed Dormancy in Cereal Weed *Adonis flammea* Jacq. (Ranunculaceae)

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ABSTRACT

In some European countries, *Adonis flammea* is very rare and endangered primarily due to increasing fertilizer and herbicide use, more efficient seed cleaning, and strong soil acidification. The aims of this study were to determine the requirements for embryo growth, dormancy break, and seed germination to characterize the type of seed dormancy (*via* effects of GA_3) and to evaluate the effects of light on germination ability. Germination responses of *A. flammea* were tested in light and dark conditions at three alternating temperatures (15/5°C, 20/10°C, 25/15°C) after stratification. Embryo growth was determined by measuring its lengths. Fresh seeds did not germinate during one month of incubation in either light or darkness over a range of temperatures. Seeds of *A. flammea* had underdeveloped embryos that must grow from about 0.2 to 1.5 mm in length before radicle emergence (germination); thus they had Morphological Dormancy (MD). In addition to MD, the seeds also had Physiological Dormancy (PD) at maturity in mid-July; moreover, the embryos grew during warm stratification, and the seeds needed a subsequent period of cold stratification to germinate. Thus, MD had been broken, but PD prevented germination. Therefore, the seeds have morpho-physiological dormancy. Germination was promoted by 12 weeks of warm stratification followed by 12 weeks of cold stratification. In conclusion, good conservation management in arable land for *A. flammea* involves annual cultivation, ideally in mid-summer (warm stratification) without subsequent disturbance until the following summer.

Keywords: Embryo length, Gibberellic acid, Morpho-physiological seed dormancy.

INTRODUCTION

Cereal weeds with critically small and highly fragmented populations are acknowledged to be among the most vulnerable groups in national floras (Cerovský, 1999; Baessler and Klotz, 2006; Fried *et al.*, 2009; Storkey *et al.*, 2012). A number of management changes, which impact different stages of annual plants life cycle, have been implicated in the regression of cereal weed populations and favoured other more ruderal species. These include the use of mineral fertilizers instead of organic manure, increased plant density and shading by the crop canopy, decreased crop diversity, increasing herbicide use, more efficient seed cleaning before sowing, change in soil preparation steps, plowing depth and changed crop rotation systems (Kleijn and van der Voort, 1997; Storkey *et al.*, 2012; Ball, 1992).

Adonis flammea Jacq. is an annual plant within the Ranunculaceae family. In Poland, flowering usually occurs in early June and seeds (achenes) are ripe from mid-June to August. Generally, the seed weighs 7.49±0.56 mg and measure 4.5×3.3 mm (unpublished data). Its main distribution area is Western Asia and Southern and Central Europe (Tutin *et al.,* 1964). This species occurs as a typical weed of arable fields,

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especially on the margins of cereal fields and also in the fallow lands.

Adonis flammea is now the most heavily regressing plant all over Europe (Kleijn and van der Voort, 1997; Pyšek *et al.*, 2005; Pinke *et al.*, 2008; Storkey *et al.*, 2012; Solé-Senana *et al.*, 2014). Because it is considered to be a threatened species, it is included in most red data books of its range countries.

Adonis belongs to the Ranunculaceae, a family reported to have Morphological Dormancy (MD) or Morpho-Physiological Dormancy (MPD), with rudimentary or linear embryos (Martin, 1946; Baskin and Baskin, 2004, 2014; Finch-Savage and Leubner-Metzger, 2006). Most Ranunculaceae species have an embryo that has not completed growth at the moment of seed dispersal. This underdeveloped embryo causes a delay in germination, which has been termed Morphological Dormancy (MD) (Nikolaeva, 2001; Baskin and Baskin, 2004). Very often, dormancy in these species is controlled by an additional Physiological Dormancy (PD), preventing germination at times unfavorable for seedling establishment. Seeds with both an underdeveloped embryo and physiological dormancy are referred to as Morpho-Physiologically Dormant (MPD) (Baskin and Baskin, 2004). However, the type of dormancy and the conditions that promote embryo growth and germination vary among species within this family. So far, morphological dormancy and four types of MPD have been reported in Ranunculaceae species (Forbis and Diggle, 2001; Baskin and Baskin, 2014). In *Delphinium tricorne* seeds with deep complex MPD, dormancy loss and embryo growth occur during cold stratification and GA_3 cannot replace the requirement for cold stratification (Baskin and Baskin, 2014). Recently, intermediate complex morpho-physiological dormancy that is broken by cold stratification has also been reported within the Ranunculaceae (Herranz *et al.*, 2010). There are few reports on embryo growth, morphology, and germination in *Adonis* spp. MPD dormancy

has been reported in *A. vernalis* (Poluyanova and Lyubarskii, 2008), *A. annua* (Godefroid, *et al.,* 2010) and *A. distorta* (Frattaroli *et al.*, 2013). Lee *et al*. (2011) observed that in *A. amurensis* seeds from Korean Peninsula warm followed by a cold temperature sequence was essential for embryo growth.

Since *A. flammea* reproduces sexually and its distribution and population persistence depend on successful seed production, seed germination, and seedling establishment. The germination requirements of *A. flammea* have not been studied so far. Germination requirements of rare species are often unknown, particularly of those whose material is difficult to obtain (Cerabolini *et al.*, 2004). Preservation and rescue of rare and threatened wild plants require considerable research and adequate knowledge about germination ecology, including seed dormancy pattern and germination preferences (Godefroid *et al.,* 2010; Kadis *et al.,* 2010; Lee *et al.,* 2011; Storkey *et al.,* 2012; Frattaroli *et al.,* 2013; Torra *et al*., 2015, 2016).

The present work aimed to test the hypothesis that *A. flammea* has some level of morpho-physiological dormancy (Baskin and Baskin, 2014), because it belongs to the Ranunculaceae, characterised by underdeveloped embryo that must grow before seeds can germinate. The primary aim of this study was to determine if the seeds of *A. flammea* had MD or MPD and, if so, to assess its level. The specific objectives of the present study were to determine: (i) If freshly matured seeds were dormant, (ii) The effects of temperature and light conditions, as well as GA_3, on breaking of dormancy and embryo growth, and (iii) The effect of warm stratification preceding the cold stratification on germination.

MATERIALS AND METHODS

Plant Material and Source of Seeds

Sample of 86 seeds of *A. flammea* was taken on the 10th July, 2010, from a single

natural population in arable fields in the vicinity of the village Burzenin (51° 47' N, 18° 83' E), 80 km south-west of Łódź, central Poland. Due to low availability of wild seed, seed for experimentation was derived from garden-grown offspring. Thus, seeds (2 weeks after harvest) were sown for cultivation at one site. This site was located in an experimental garden in the campus of University of Łódź, Faculty of Biology and Environmental Protection. During cultivation, the site was watered when needed, and weeds were removed manually. The cultivation site was visited regularly until the summer of 2014. The seeds were produced in insufficient number per year, that's why they were collected for three growing seasons on 16 July 2012, 27 July 2013 and on 11 July 2014 for germination experiments. Seed material was stored under dry condition in paper bags at room temperatures (22–24°C, 50−60% Relative Humidity (RH) for 10–14 days before studies were initiated.

Seeds Treatment and Germination

The seeds were incubated in 12 hours of light (10–20 µmol m^{-2} s^{-1}, 400–700 nm cool white fluorescent light each day) or in darkness (i.e. continuous darkness achieved by wrapping dishes in aluminum foil) at 12/12 hours daily alternating temperature regimes of 15/5, 20/10, and 25/15°C and then examined for germination. Each treatment consisted of two replications of 25 seeds each. The seeds were incubated in 9 cm (diameter) glass Petri dishes on filter paper moistened with distilled water or solutions of Gibberellic Acid (GA$_3$). Petri dishes were sealed with parafilm to minimize water loses. Germination counts for dark-treated seeds were made under soft green light. Seeds were considered germinated when radicle protrusion was visible. Germination percentages were determined on the basis of the number of viable seeds. The tetrazolium assay were used to determine the number of viable

dormant i.e., tetrazolium positive, and dead (tetrazolium negative) seed remaining after germination (Grabe, 1970) .

The alternating temperature regimes simulated mean maximum and mean minimum monthly temperatures during the growing season in central Poland: 25/15°C corresponds to summer, 20/10°C to early autumn and late spring, and 15/5°C to late autumn and early spring (Institute of Meteorology and Water Management 1961– 2000). The lowest temperature (5°C) was chosen because it was within the effective temperature range for cold stratification, and it was used to simulate the winter environmental conditions in the MPD studies (Baskin and Baskin, 2014).

Experiment 1: Requirements for Dormancy Break

The seeds collected in 2012 were used to investigate requirements for dormancy break and embryo growth. This germination experiment was performed using fresh seeds (2 weeks after harvest).

Seeds of some species require cold stratification at 5°C to break dormancy (Baskin and Baskin, 2014). Thus, seeds were stratified in light at 5°C for 12 weeks and then incubated in light and in darkness at 12/12 hours daily alternating temperature regimes of 15/5, 20/10, and 25/15°C for 2 weeks and then examined for germination. After two weeks, the seeds were no longer germinating.

Since the seeds of *A. flammea* are dispersed in summer, they require warm stratification prior to the cold. Thus, another subsample from the same seed lot was given 12 weeks warm stratification (25/15°C) in light and then cold stratification in light at 5°C for 12 weeks. Following the warm plus cold stratification period, seeds were incubated in light and in darkness at 15/5, 20/10, and 25/15°C for 2 weeks. Following the incubation period, the seeds of all treatments were examined for germination.

In addition, the results were compared to those produced when freshly (non-treated)

matured seeds were incubated for 20 weeks at 15/5, 20/10, and 25/15°C, both in darkness and in light. Fresh seeds were examined every 2 weeks, and seedlings were counted and removed from the Petri dishes. Water was added to dishes in the controls as needed.

Experiment 2: Embryo Growth Following Cold or Warm Stratification of Seeds

The assay was carried out with seeds collected on 22nd August, 2013. Twenty five freshly matured seeds were placed on two sheets of filter paper moistened with distilled water in a 9 cm (diameter) glass Petri dish in continuous darkness at room temperature (~24°C) for 24 hours. Embryos were excised using a razor blade, and their lengths measured under a dissecting microscope equipped with a micrometer. Embryo growth was also monitored in 25 seeds during warm (25/15°C) and during cold stratification (5°C) in light. Then, the embryos were excised from the seeds and their lengths were measured following 2, 4, 6, 8, 10, and 12 weeks of warm and of cold stratification. If a seed had germinated in the dish, embryo length was assumed to be equal to the critical embryo length (Baskin *et al.*, 2001), i.e., when embryos had grown enough to start splitting the seed coat.

Experiment 3: Effect of Gibberellic Acid (GA$_3$) on Germination and Embryo Growth

The influence of GA$_3$ on seed dormancy breaking was tested. The effect of two GA$_3$ concentrations on germination and embryo growth was investigated in seeds of *A. flammea* collected in 2014 and stored for 2 weeks. Next, the seeds were placed on two sheets of Whatman No. 1 filter paper in each of 9 cm (diameter) glass Petri dishes. The paper was moistened with either distilled water (control) or a solution of 100 or 1,000 mg L^{-1} of GA$_3$ dissolved in distilled water. Two replications of 25 seeds were used for

each treatment. The seeds from all 6 dishes were checked for germination after incubation in light for 8 and 12 weeks. Embryo growth was determined by measuring their lengths. Initial embryo lengths for all three variants (water, 100 and 1,000 mg L^{-1} GA$_3$) were determined in embryos dissected from other respective 25-seeds lots kept in moist filter paper at room temperature (~24°C) for 24 hours. The dishes were sealed with parafilm in order to avoid loss of water, and then incubated in light at 25/15°C. This thermo-period was chosen because it was different enough from the conditions required for cold stratification (Stokes, 1965), but within the range of temperatures used in similar studies (Hidayati *et al.*, 2000).

Data Analysis

Germination data were transformed to germination percentages based on the number of viable seeds. Means and standard deviations were calculated for germination percentages and embryo lengths. Means were compared by Analyses Of Variance (ANOVA) followed by Tukey's multiple comparison test (P= 0.05) (36) on arcsine-transformed data but non-transformed data are shown in figure1. A three-way ANOVA was used to test the effects and interactions of seed condition (non-treated, cold stratified and warm plus cold stratified), light condition (two levels), and thermo-period (two levels) on germination percentages, and a two-way ANOVA to test the interaction effect between GA$_3$ concentration (two levels) and length of incubation (two levels) on percentage seed germination and embryo growth.

RESULTS

A three-way ANOVA showed that germination was significantly affected by seed condition (P< 0.01), light condition (P< 0.01), thermo-period (P< 0.01) and their
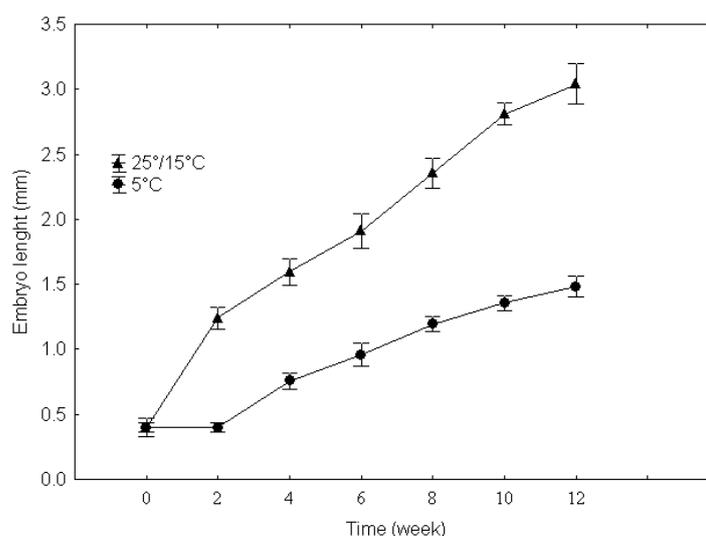
Figure 1. Embryo length (mm, mean±SD) in seeds of *Adonis flammea* during 2–12 weeks of warm (25/15°C) or cold (5°C) stratification.

interactions (P< 0.01) (Table 1). Light condition was the most important factor, with much more germination in light than in darkness. Freshly matured seeds of *A. flammea* collected in 2012 were dormant and, consequently, germinated neither in light nor in darkness at any thermo-period; moreover, none of them germinated in light after 20 weeks of incubation (Table 1). On the other hand, the seeds germinated to 73–79% in light at 15/5 and 20/10°C, and to 89% at 25/15°C, following 12 weeks of warm plus 12 weeks of cold stratification. Thus the seeds have deep simple MorphoPhysiological Dormancy (MPD). A few seeds (1–3%) germinated at the three thermoperiods following 12 weeks of warm plus 12 weeks of cold stratification in darkness. The seeds given only 12 weeks of cold stratification germinated to 12–31% at the three thermo-periods in light, while none germinated in darkness (Table 2).

A two-way ANOVA showed that the seed germination and embryo growth were significantly affected by GA_3 concentration (P< 0.01), length of incubation (P< 0.01), and their interaction (P< 0.01) (Table 3). Embryos in the freshly matured seeds were 0.42 mm (SD= 0.04, n= 25) long, 11% of the length of the seed (Figure 1). After 12 weeks of incubation at 5°C, the mean embryo length increased to 1.47 mm (SD= 0.12, n= 25). The seeds incubated at 25/15°C for 12 weeks showed a mean embryo length of 3.04 mm (SD= 0.07, n= 25). The average

Table. 1. Three-way ANOVA of effects of Seed condition (S), Light condition (L), Thermo-period (T), and their interactions on seed germination of *Adonis flammea*.

Factor	*df*	*F* value	*P* value
Seed condition (S)[a]	3	77.45	< 0.01
Light condition (L)[b]	1	21.03	< 0.01
Thermoperiod (T)[c]	2	28.34	< 0.01
S×L	3	34.27	< 0.01
S×T	3	18.04	< 0.01
L×T	2	14.43	< 0.01
S×L×T	6	62.67	< 0.01

[a] Non-treated (fresh), cold stratified and warm plus cold stratified; [b] Light *vs.* Darkness, [c] 15/5; 20/10, 25/15°C.

Table. 2. Effects of a cold stratification (5°C) period only or a warm stratification (25/15°C) plus cold stratification (5°C) period in light conditions on germination percentages (mean±SD) of *Adonis flammea* seeds incubated in light and darkness at different temperatures.[a]

Incubation temperatures (°C)	Cold stratification	Warm+cold stratification	Fresh
In light			
15/5	12 ± 1^{Bb}	73 ± 6^{Ba}	0 ± 0^{Ac}
20/10	27 ± 3^{Ab}	79 ± 8^{Ba}	0 ± 0^{Ac}
25/15	31 ± 3^{Ab}	89 ± 7^{Aa}	0 ± 0^{Ac}
In darkness			
15/5	0 ± 0^{Aa}	3 ± 0^{Aa}	0 ± 0^{Aa}
20/10	0 ± 0^{Aa}	1 ± 0^{Aa}	0 ± 0^{Aa}
25/15	0 ± 0^{Aa}	2 ± 0^{Aa}	0 ± 0^{Aa}

[a] Germination percentages of seeds followed by different uppercase letters within columns or different lowercase letters within rows are significantly different (Tukey's test, P= 0.05).

Table. 3. Results of two-way ANOVA showing the effects and interaction of GA_3 concentration (GA_3) and Length of incubation (L) on seed germination and embryo growth of *Adonis flammea* seeds.

Dependent variable	Factor	*df*	*F* value	*P* value
Germination	GA_3 concentration $(GA_3)^a$	1	16.87	< 0.01
	Incubation Length $(L)^b$	1	4.34	< 0.01
	$GA_3 \times L$	1	3.98	< 0.01
Embryo growth	GA_3 concentration $(GA_3)^a$	1	12.35	< 0.01
	Incubation Length $(L)^b$	1	3.07	< 0.01
	$GA_3 \times L$	1	11.64	< 0.01

[a] 100 or 1,000 mg L^{-1} of GA_3, [b] 8 or 12 weeks.

length of embryos was 3.46 (SD= 0.07, n= 25), when they had grown enough to start splitting the seed coat. Gibberellic acid had significant effect on embryo growth. The seeds in 100 mg L^{-1} GA_3 showed an embryo size ranging from 2.47 to 3.25 mm after incubation at 5 and 25/15°C for 12 weeks. In contrast, the seeds incubated at the same temperature regimes for the same period of time in 1,000 mg L^{-1} GA_3 had an embryo length of 2.62–3.38 mm (Table 4).

No seeds germinated after 8 weeks of incubation at 5°C in distilled water (control) or in the solutions of 100 or 1,000 mg L^{-1} GA_3. While, during 12 weeks of incubation at 5°C, 4% of seeds germinated in distilled water and 14.61% in the 100, mg L^{-1} GA_3 solutions, respectively. The seeds did not germinate during 8–12 weeks of incubation at 25/15 °C in distilled water (control) or in

solutions of 100 or 1,000 mg L^{-1} GA_3 (Table 4).

DISCUSSION

Fresh seeds of *A. flammea* have small, underdeveloped embryo at the time of dispersal in summer. Therefore, seeds have Morphological Dormancy (MD), embryo must elongate to a critical length before the radicle emergence. Since embryo growth and radicle emergence were not completed at suitable conditions (e.g. temperature and light/dark) in about 30 days, seeds also had Physiological component of Dormancy (PD) (Baskin and Baskin, 2014). Although the embryos grew during warm stratification, seeds of this species needed a subsequent period of cold stratification to germinate (Table 1, 3). Thus, in order to germinate

Table. 4. Effects of various concentrations of Gibberellic Acid (GA_3) on germination and embryo growth (mean±SD) of *Adonis flammea*. Seeds were collected in 2011.[d]

Time (Weeks)	Germination (%)			Embryo length (mm)		
	GA_3 concentration (mg L^{-1})					
	0	100	1000	0	100	1000
	Incubated at 5°C					
0	0^{Aa}	0^{Aa}	0^{Aa}	0.42 ± 0.02^{Aa}	0.42 ± 0.02^{Aa}	0.42 ± 0.02^{Aa}
8	0^{Aa}	0^{Aa}	0^{Aa}	0.98 ± 0.03^{Ba}	2.22 ± 0.03^{Bb}	2.27 ± 0.05^{Bb}
12	4 ± 1^{Ba}	14 ± 1^{Bb}	61 ± 5^{Bc}	1.47 ± 0.04^{Ca}	2.47 ± 0.05^{Cb}	2.62 ± 0.06^{Cc}
	Incubated at 25°/15°C					
0	0^{Aa}	0^{Aa}	0^{Aa}	0.42 ± 0.02^{Aa}	0.42 ± 0.02^{Aa}	0.42 ± 0.02^{Aa}
8	0^{Aa}	0^{Aa}	0^{Aa}	2.15 ± 0.03^{Ba}	2.31 ± 0.03^{Bb}	2.47 ± 0.05^{Bc}
12	0^{Aa}	0^{Aa}	0^{Aa}	3.04 ± 0.04^{Ca}	3.25 ± 0.05^{Cb}	3.38 ± 0.06^{Cc}

[d] Mean with different uppercase superscript letters within columns or with different lowercase superscript letters within rows are significantly different (Tukey's test, P= 0.05).

seeds require a dormancy-breaking pretreatment and they have both morphological and physiological dormancy, i.e. have Morpho-Physiological Dormancy (MPD).

Eight types of MPD have been distinguished based on warm and/or cold stratification requirements to break Physiological Dormancy (PD), temperature requirements for embryo growth and responses to Gibberellic Acid (GA_3) (Nikolaeva, 2001). The eight types of MPD are divided into two categories, namely, simple and complex, differing in temperatures required for embryo growth. In the simple MPD relatively high temperatures (≥ 15°C) are required, in the complex MPD relatively low (0–10°C). There are five types of simple MPD and three of complex MPD generally differing in the level of physiological dormancy: non-deep, intermediate, and deep (Baskin and Baskin, 2014). GA_3 has been used in attempts to promote germination of seeds with MPD, and its effects vary with the type of MPD. For the seeds of *A. flammea* that require warm plus cold stratification for germination, GA_3 replaced the warm but not cold stratification requirement for dormancy break (Table 5). The requirement for long period of warm plus cold condition for embryo growth and dormancy break indicates that *A. vernalis* seeds have simple

MPD. The known types of simple MPD are non-deep, intermediate, deep, deep epicotyls, and deep double. As seeds require warm or cold stratification to break physiological or morphological dormancy, respectively, they have non-deep simple MPD. In intermediate and deep simple MPD, part of the PD of the embryo is broken by high (summer) temperatures, and the embryo grows within the seed in autumn. However, seeds do not germinate until they have received several months of cold stratification during winter and the epicotyl emerges in spring. GA_3 can substitute cold stratification in seeds with intermediate simple MPD in some species such as *Chaerophyllum tainturieri* and *Aralia mantshurica* (Baskin and Baskin, 2014). On the other hand, GA_3 did not promote germination of the seeds with deep simple MPD, e.g. *Fraxinus excelsior* (Wcislińska, 1977) and *Jeffersonia dubia* (Rhie *et al.*, 2014). In the seeds with deep simple epicotyls MPD, radicle growth and emergence occur in autumn, PD of the epicotyls is broken during winter, and cotyledons emerge in spring. In the seeds with deep simple double MPD, PD of the radicle is broken during winter, radicle growth and emergence occurs in spring, PD of the epicotyls is broken the following winter, and shoot growth occurs next spring (Nikolaeva, 2001; Baskin and Baskin,

2014). The facts that (1) GA$_3$ only partially overcame dormancy (Table 2), and (2) Warm plus cold stratification was required to break dormancy, provide evidence that the seeds of this species have deep simple MPD, i.e., C$_{1b}$B-C$_3$ in the Nikolaeva (2001) formula system of the kinds of seed dormancy.

This level of MPD also has been found in other species of Ranunculaceae. Temperature requirements for embryo growth and seed dormancy break in *A. flammea* are similar to those of Asian species *A. amurensis*. Seeds of *A. amurensis* require high temperatures (25/15°C) for embryo growth followed by cold stratification (5°C) to break physiological dormancy of the fully developed embryo. Further, GA$_3$ can substitute warm but not cold stratification in these seeds (Lee *et al.*, 2011). Thus, in *A. flammea* seeds, the temperature requirements for embryo growth and radicle emergence as well as effects of GA$_3$ seeds correspond well with those for *A. amurensis*. Description of seed dormancy in *A. vernalis* provided by Rouhi *et al.* (2013), allowed me to conclude that the seeds of this species also have deep simple MPD requiring high temperatures (25/15°C) for embryo growth followed by cold stratification. The deep simple MPD is frequent not only in Ranunculaceae (Poluyanova *et al.* 2008) but was also documented in several species, e.g. in the families of Apiaceae (Nikolaeva *et al.*, 1985), Araliaceae (Nikolaeva *et al.*, 1985), Aristolochiaceae (Adams *et al.*, 2005), Berberidaceae (Baskin and Baskin, 1989), Caprifoliaceae (Hidayati *et al.*, 2000), Liliaceae (Kondo *et al.*, 2006), Fumariaceae, Liliaceae, Oleaceae, and Taxaceae (Nikolaeva *et al.*, 1985).

Light was found to inhibit germination in some species, whereas darkness prevented germination of light-requiring buried seeds (Pons, 2000; Baskin and Baskin, 2014; Kołodziejek and Patykowski, 2015). Both mechanisms were proposed to facilitate the formation of a persistent soil seed bank (Pons, 2000). However, in other species, the

role of light remains unclear or seems to be negligible (Schwienbacher *et al.*, 2011). The present experiments indicated that in the case of *A. flammea,* germination was more effective in light than in darkness (Table 1). Promotion of germination by light conditions may have important consequences in the ecological perspective. *Adonis flammea* is an annual weed, occurring in crop fields. Therefore, a substantial part of the seeds from each cohort can be assumed to be buried. Grime *et al.* (1981) suggested that species with light-requiring seeds can form soil seed banks and germinate at a subsequent stage, after soil disturbance. In contrast, Torra *et al.* (2015) found that other arable Ranunculaceae species including *Consolida orientalis*, *C. pubescens*, *D. gracile*, *D. halteratum* ssp. *Verdunense,* and *Nigella gallica,* germinated better in complete darkness than in a light regime.

Adonis flammea is a species vulnerable to extinction due to habitat alterations. Knowledge of rare species life-cycle and reproductive traits is essential to identify limits to population growth and persistence, especially in threatened wild species (Bevill and Louda, 1999). Nevertheless, in the present study, dispersal phenology of *A. flammea* was not investigated because a large amount of seeds would have been necessary. Crawford *et al.* (2007) also suggested that, when dealing with collections of rare and threatened species, seed quantity was often a limiting factor.

The ecological consequence of deep simple MPD in the seeds of *A. flammea* lie in the fact that these seeds are dormant at maturity in early summer and embryo growth is not initiated until late autumn, when daily minimum temperatures are within the range effective for cold stratification (Baskin and Baskin, 2014). In nature, the cold stratification requirement for dormancy break in the seeds of *A. flammea* occurs during winter, and the seeds germinate in early spring about 3 months before the canopy closure. Such a stimulatory effect of warm or cold

stratification pre-treatment on germination was also detected in other species with intermediate physiological dormancy i.e. in *Anemone nemorsa* (Mondoni *et al.*, 2009) and *Cardamine concatenate* (Baskin, and Baskin, 1995).

CONCLUSIONS

The fresh seeds of *Adonis flammea* Jacq. did not germinate during one month of incubation in either light or darkness over a range of temperatures. To germinate the seeds of this species required warm stratification followed by cold stratification, and presence of light. Ideal management in arable land for *A. flammea* involves annual cultivation, ideally in mid-summer (warm stratification) without subsequent disturbance until the following summer. The germination requirements found in this study will be useful for future ex situ conservation of *A. flammea*.

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خواب بذر در علف هرزغلات .Adonis flammea Jacq یا آلاله‌گان

ج. کلودزیجک

چکیده

در برخی کشورهای اروپایی، به علت مصرف روز افزون کود شیمیایی و علف کش ها و نیز پاک کردن موثر بذرها و اسیدی بودن شدید خاک، *Adonis flammea* بسیار کمیاب و در معرض انقراض است. هدف این پژوهش تعیین نیازهای رشد جنین، خواب شکنی بذر(dormancy break) و جوانه زنی بذر بود برای مشخص کردن نوع خواب بذر(از طریق اثرات GA₃) و ارزیابی اثرات نور روی توانایی جوانه زنی، واکنش های جوانه زنی *A. flammea* در شرایط نور و تاریکی و در سه درجه حرارت متناوب (۱۵°C و ۵)، (C° ۲۰ و 10) ، و (C° ۲۵ و۱۵) بعد از خواب شکنی (stratification) بررسی شد. رشد جوانه با اندازه گیری طول آن تعیین شد. بذر های تازه در طی یک ماه انکوباسیون و در حرارت های مختلف چه در نور و چه در تاریکی جوانه نزدند. بذر های .A

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_flammea_دارای جنین ناقص بودند که می بایست از ۰/۲ میلیمتر به ۱/۵ میلی متر رشد میکرد تا ریشه چه ظاهر شود(جوانه زنی).به این قرار، این بذر ها خواب موفولوژیک (MD) داشتند. افزون بر آن، بذر های مزبور در مرحله رسیدن در میانه ماه ژوئن خواب فیزیولوژیک (PD) هم داشتند. علاوه بر آن، جنین ها در طی خواب شکنی گرم هم رشد کردند و در پی آن نیازمند یک دوره خواب شکنی سرد بودند تا جوانه بزنند. بنا بر این، MD شکسته شده بود ولی PD از جوانه زنی جلوگیری میکرد. از این رو، این بذرها دارای خواب مورفو–فیزیولوژیک بودند. جوانه زنی این بذرها بعد از ۱۲ هفته خواب شکنی گرم و به دنبال آن ۱۲ هفته خواب شکنی سرد بهبود یافت. نتیجه گیری اینکه، مدیریت حفاظتی خوب برای _A. flammea_در اراضی کشت شده باید شامل کشت سالانه در میانه تابستان(خواب شکنی گرم) و بدون مداخله دیگر تا تابستان بعدی باشد.