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JOURNAL OF PLANT PROTECTION RESEARCH Vol. 54, No. 2 (2014)

DOI: 10.2478/jppr-2014-0028

Dormancy breaking and seed germination of the annual weeds *Thlaspi arvense, Descurainia sophia* and *Malcolmia africana* (Brassicaceae)

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Received: October 16, 2013
Accepted: May 28, 2014

Abstract: In Iran, *Descurainia sophia*, *Malcolmia africana*, and *Thlaspi arvense* are abundantly found as importunate weeds in winter cereal. Understanding the timing of seed germination under natural conditions is crucial for learning how to manage these annual weeds. Therefore, this study was conducted to evaluate the effect of soil burial, dry storage, cold stratification, KNO_3, GA_3, and scarification on the seed dormancy and germination of these three species. Species had significantly different responses to the treatment. In *D. sophia*, seeds buried at a depth of 10 cm for 60 days (55%), and seeds dry stored at 20°C for 180 days (45%) showed the highest level of germination. In *M. africana*, the germination percentage reached 95% when seeds buried at a depth of 1 cm were soaked in a GA_3 concentration of 150 ppm. *T. arvense* had the lowest level of germination compared to the other species. The highest percentage of *T. arvense* germination was obtained in seeds treated with 150 ppm GA_3. Potassium nitrate partly increased germinability in seeds of *M. africana*, which initially were less dormant than those of *T. arvense* and *D. sophia.*

Key words: Brassicaceae, cold stratification, depth of burial, gibberellic acid, seed dormancy, seed germination

Introduction

Descurainia sophia (flixweed), *Malcolmia africana* (African mustard), and *Thlaspi arvense* (field pennycress) are three important annual weed species belonging to the Brassicaceae family (Hultén 1968; Best 1977; Stark 1987; Makarian *et al.* 2008). Although all these species have medicinal uses (Shahina 1994), they are documented as the principal weeds of winter cereal especially wheat in Iran (Shimi and Termeh 2004) as well as in many other countries. These weeds can cause severe crop yield reduction (Hultén 1968; Stark 1987; Makarian *et al.* 2008).

To cope with the unpredictable environments in which weeds grow, weeds have developed many characteristics related to seed morphology and physiology such as dormancy, vigor and storability (Benech-Arnold *et al.* 2000). A seed is regarded dormant when it does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors, which are otherwise favourable for seed germination (Baskin and Baskin 1998, 2004). Dormancy usually makes it difficult to predict the timing and extent of weed emergence in the field (Benech-Arnold *et al.* 2000). In addition, dormancy defines the range of temperature conditions required for seed germination. This situation increases the distribution of germination over time (Foley 2002; Taab and Andersson 2009).

The timing of seed germination and weed emergence are among the main factors determining the success of a weed in agricultural ecosystems (Cousens and Mortimer 1995), which are greatly related to environmental signals (Fenner 1991; Hilhorst and Toorop 1997). Dormancy in many species has a seasonal variation (Milberg and Andersson 1997) that is related to seasonal temperature changes (Atwater 1980; Hilhorst and Karssen 1992). In winter annuals, high summer temperatures promote loss of dormancy, while low temperatures during autumn and winter may cause the induction of dormancy. Furthermore, after-ripening alleviates physiological dormancy (Baskin and Baskin 1998) and increases the sensitivity of seeds to hormones, chemicals, and physical treatments that promote germination (Hilhorst and Karssen 1988; Derkx and Karssen 1993). Exogenous gibberellins (usually gibberellic acid GA_3 and GA_{4+7}) have also been shown to break the dormancy in many species (Karam and Al-Salem 2001). The optimum temperature for seed germination of *T. arvense* is approximately 25°C and constant temperatures of 18 to 20°C decrease germinability of *T. arvense* (Cross 1933). Wagenvoort and Van Opstal (1979) found that dry-stored *T. arvense* seeds do not germinate under constant temperature, with or without light, and the germination was less than 10% even at alternating temperatures. However, the germination rate increased up to 50% when the seeds were treated with a nitrogen

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fertilizer solution, and at alternating temperatures. Scarification of the seed coat of *T. arvense* was found to promote germination. Nitrate was also found to stimulate the germination of *T. arvense* in light (Saini *et al.* 1987; Milberg 1997) but not in darkness (Saini *et al.* 1987). A significant germination-increase was reported for seeds of *T. arvense* buried in soil over winter (Andersson *et al.* 1997). Seeds of *D. sophia* maintain deep dormancy in soil for several months which enables the species to build a large seed bank in the soil (Conn 1990; Conn and Deck 1995). Best (1977) reported that seeds of *D. sophia* germinated most readily in late autumn and early spring, while germination was rare during summer. Li *et al.* (2005) found that the level of dormancy in *D. sophia* decreased during afterripening and stratification, but 5 days of darkness at 4°C induced a secondary dormancy.

To our knowledge, there is no comprehensive study on seed dormancy and germination of *M. africana*. Greater knowledge about the environmental factors, which influence seed germination and seedling emergence of *D. sophia*, *M. africana*, and *T. arvense* would contribute to our understanding of the ecological life cycles of these species. Forecasting the potential seedling density to optimise weed management strategies would then be possible. Therefore, the objective of this study was to determine the influence of: cold stratification, dry storage, depth, and duration of seed burial in the soil, gibberellic acid, KNO_3, physical and chemical scarification on seed dormancy as well as to determine the germination of these species.

Materials and Methods

Seed collection and germination tests

Experiments were conducted in August 2011 at the seed laboratory of the Isfahan University of Technology, Isfahan, Iran. Seeds were collected in June 2011 from *M. africana*, *T. arvense*, and *D. sophia* plants growing as weeds in the wheat fields around Isfahan (latitude 32°32′ N and longitude 52°22′ E, and 1,630 m above sea level). Immediately after seed collection, seeds were cleaned and stored in paper bags at –20±1°C until they were used in further experiments (about 2 months).

Seeds were sterilised in a 1% sodium chloride solution (household bleach solution) for 15 min followed by rinsing three times in sterile distilled water. Germination treatments were undertaken with 4 replicates. Each replicate consisted of 50 seeds placed in 70-mm Petri dish containing a layer of Whatman No. 1 filter paper and 5 ml of distilled water or the solution appropriate for the experiment. Distilled water or the test solution was added when necessary, to prevent seeds from drying out. All dishes were wrapped with plastic film to minimise water loss due to evaporation. The dishes (4 replicates per treatment) were placed in germination incubators (Arvin Tajhiz Espadana, Iran) at a fluctuating day/night temperature of 30/20°C under 16 : 8 h (light : dark) photoperiod, and an irradiance of 4,000 lux provided by cool white fluorescent tubes. The criterion for germination was visible radicle protrusion from the seed coat. The number of germinated seeds were counted after 14 days since after this period, seed germination becomes constant. Ungerminated seeds were pinched with forceps to determine if they contained a firm embryo (indicating viability) or a soft embryo (indicating non-viability) (Baskin *et al*. 2004).

Experiment 1. Seed burial outdoors

To investigate the effect of depth and duration of seed burial on seed dormancy an outdoor experiment was conducted from August 1, 2011 to February 1, 2012 at the university research station. Matured seeds of the three species were placed in polyethylene mesh bags (each species in one bag) and then buried at a depth of 1 and 10 cm in sandy soil under fallow conditions. Each bag, containing more than 300 seeds was dug up in 30-day intervals and subjected to a germination test. Local climate data taken during the experiment are presented in table 1. The species under study are mainly problematic species of winter cereals. In addition, the weeds that emerge before or simultaneous to the crops have higher competitive effects on crops. Thus, the necessary data can help in the control of the species early in the season. The focus of the present study was to provide data on the first flushes of the seedlings that emerge in autumn.

Experiment 2. Dry-storage

To evaluate the effect of dry-storage on seed dormancy, seeds were placed in transparent sealed Petri dishes and dry stored at a constant temperature of 20°C for 6 months. Every 30 days, four dishes were randomly selected and subjected to a germination test.

Table 1. Average minimum air temperature, maximum air temperature, and precipitation (mm) during the experiments

Month	Average minimum air temperature [°C]	Average maximum air temperature [°C]	Precipitation [mm]
August 01-September 01, 2011	20.00	37.60	0
September 01-October 02, 2011	15.53	31.94	0.90
October 02-November 01, 2011	9.71	24.58	0.12
November 01-December 01, 2011	4.52	16.48	30.60
December 01-December 31, 2011	0.03	10.32	43.00
December 31, 2011-January 30, 2012	0.58	14.19	14.30

Experiment 3. Cold stratification

To evaluate the effect of cold stratification on seed dormancy, seeds were placed on a layer of filter paper in Petri dishes moistened with 3 ml of distilled water. The Petri dishes were sealed with aluminum foil and stratified at 5°C for 6 months in a dark refrigerator. Every 30 days, four Petri dishes per species were randomly selected and subjected to a germination test.

Experiment 4. Gibberellic acid

To evaluate the effect of GA_3 on seed dormancy, different concentration of GA_3 (0, 10, 20, 50, 100, 150, and 200 ppm) were added to Petri dishes containing seeds stratified at 5°C (as in Experiment 3) and dry stored at 20°C (as in Experiment 2) both for 6 months, and seeds buried 1 and 10 cm for 4 months (as in Experiment 1). The seeds were then sealed with parafilm and subjected to the germination test. The solution was added to the Petri dishes, when necessary, during the germination test.

Experiment 5. Potassium nitrate

To evaluate the effect of KNO_3 on seed dormancy, seeds were placed on a layer of filter paper, in Petri dishes moistened with 3 ml of different concentrations of KNO_3 solutions (0, 0.2, 0.02, 0.002 and 0.0002 M). The Petri dishes then were sealed with plastic film and subjected to the germination test. Potassium nitrate solution was added to the Petri dishes when necessary during germination test.

Experiment 6. Scarification

The effect of physical and chemical scarification on seed dormancy was investigated in two sets of tests. To scarify seeds physically, the samples of mature seeds were rubbed with sandpaper. To scarify the seeds chemically with acid, sample of dry seeds were rinsed with H_2SO_4 (97%) for periods of 0, 5, 10, 15, 30, and 60 sec followed by washing under tap water for 5 min. The scarified seeds were then subjected to the germination test.

Statistical analysis

A completely randomised design was used for all the experiments. Differences among species, treatments, and possible interactions in all the experiments, were tested using the Generalized Linear Model (GLM) procedure (SAS Institute Inc., 2008). The proportion of germinating, viable seeds was treated as a categorical variable, assuming binomial distribution, logit link function and a type 1 sum of square option. The Pearson chi-square correction (PSCALE) was applied to correct over dispersion. Standard errors of means (SEM) were calculated using the least-squares (LS) means statement with Stderr option in the GLM procedure.

Results

Experiment 1. Seed burial outdoors

Statistical analysis showed significant differences among species, depth and time of burial as well as all interactions among these factors (Table 2). The percentage of the ger-

Table 2. Analysis of deviance for the effect of soil burial, moist stratification, and dry storage on seed germination of *D. sophia*, *M. africana*, and *T. arvense*

Source	Scaled deviance	df	F value	Pr > F
Buried seeds in the soil (Experiment 1)				
Species	562.6371	2	40.86	< 0.0001
Treatment	557.8409	1	4.80	0.0304
Species × treatment	547.5522	2	5.14	0.0071
Time	417.8862	6	21.61	< 0.0001
Species × time	263.0455	12	12.90	< 0.0001
Treatment × time	195.0226	6	11.34	< 0.0001
Species × treatment × time	137.3936	12	4.80	< 0.0001
Dry stored seeds at 20°C (Experiment 2)				
Species	718.2010	2	65.42	< 0.0001
Time	173.4505	6	81.05	< 0.0001
Species × time	76.54715	12	7.21	< 0.0001
Stratified seeds at 5°C (Experminent 3)				
Species	135.3822	2	11.05	< 0.0001
Time	100.7336	6	5.77	< 0.0001
Species × time	72.18407	12	2.38	0.0134

mination of all surface-sown seeds (1 cm) was quite low in all species as well as seeds of *T. arvensis* and *M. africana* buried at a depth of 10 cm (the range was from 0 to 18%). In contrast, the germination percentage of *D. sophia* seeds buried at a 10 cm depth of soil reached 55%, 60 days after burial (DAB), and then decreased until the end of the experiment. Due to the loss of seed samples of *M. africana* in February, there was no observation for 180 DAB (Fig. 1).

Experiment 2. Dry-storage

The differences among species and their response to dry storage treatment over time, were statistically significant (Table 2). The germination percentage of *D. sophia* constantly increased from 90 days after dry storage (DADS) at 20°C until the end of the experiment. For seeds of *M. africana*, the germination percentage increased from 120 to 150 DADS, and then tended to decrease. The seeds of *T. arvense* showed a negligible increase in germination; only 150 and 180 DADS (Fig. 2).

Experiment 3. Cold stratification

Although statistical analysis showed significant differences among species and their response to stratification at 5°C (Table 2), the increase in the germination of all three species was not notable (Fig. 3).

Experiment 4. Gibberellic acid

Significant differences were found among species and their responses to the seed treatments (Table 3). In *D. sophia*, only seeds buried 1 cm deep showed a significant increase in germination with increasing GA_3 concentra-

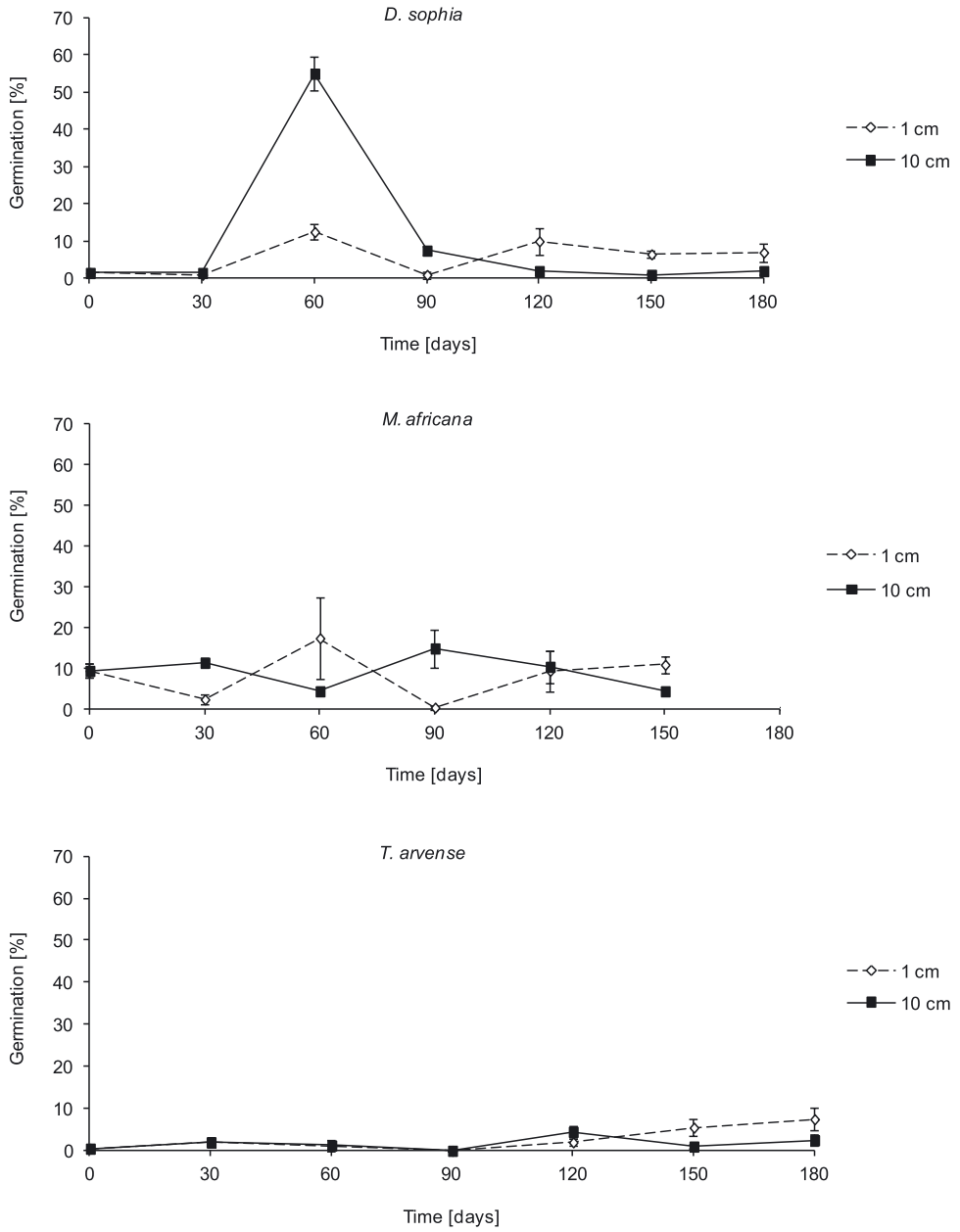

Fig. 1. Effect of duration and depth of burial at 1 and 10 cm, on seed dormancy of *D. sophia*, *M. africana*, and *T. arvense*. Vertical bars represent standard errors of means (SEM)

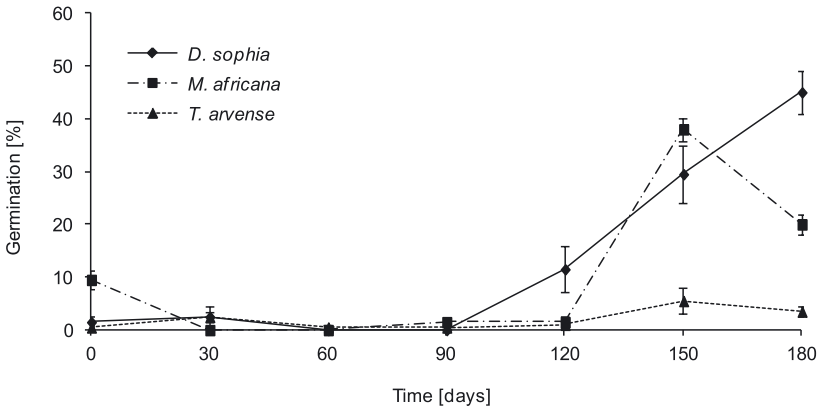

Fig. 2. Effect of dry storage at 20°C on seed dormancy of *D. sophia*, *M. africana*, and *T. arvense*. Vertical bars represent SEM

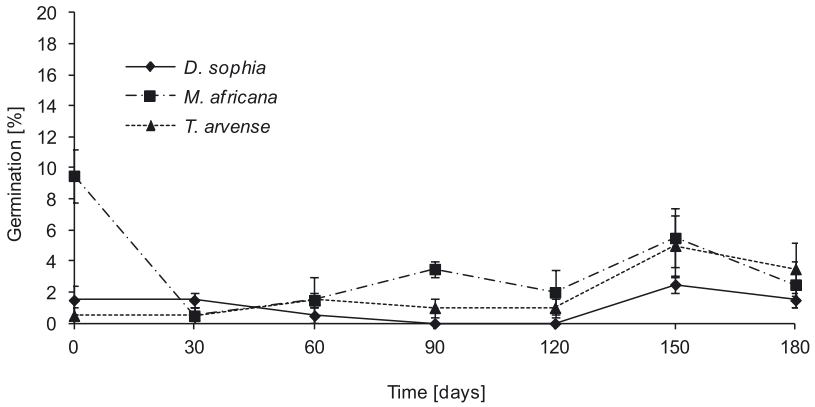

Fig. 3. Effect of moist stratification on seed dormancy of *D. sophia*, *M. africana*, and *T. arvense*. Vertical bars represent SEM

Table 3. Analysis of variance for the effect of GA_3 on seed germination of *D. sophia*, *M. africana*, and *T. arvense* buried at 1 and 10 cm soil depths for 4 months, and dry stored at 20°C, and moist stratified at 5°C for 6 months

Source	Scaled deviance	df	F value	Pr > F
Species	2,060.7	2	72.19	< 0.0001
Treatment	1,722.1	4	84.65	< 0.0001
Species × treatment	1,136.5	8	73.21	< 0.0001
GA_3	781.5	6	59.17	< 0.0001
Species × GA_3	675.3	12	8.84	< 0.0001
Treatment × GA_3	554.6	24	5.03	< 0.0001
Species × treatment × GA_3	338.6	48	4.5	< 0.0001

tions. However, total germination percentages were not much higher than 40% of the seeds sown (Fig. 4A). In *M. africana*, germination tended to increase with GA_3 concentrations in all treated seeds. The highest germination percentage (90.5%) occurred in seeds buried 1 cm deep at the GA_3 concentration of 150 ppm (Fig. 4B). Application of 10 ppm of GA_3 increased seed germination in all treated and intact seeds of *T. arvense*. The highest germination percentages (67%) were recorded in intact seeds having GA_3 concentrations of 150 ppm (Fig. 4C).

Experiment 5. Potassium nitrate

As far as seed germination was concerned, there were significant differences among species in response to KNO_3 (Table 4). Although seed germination was not affected by KNO_3 in *D. sophia* and *T. arvense*, it induced germination in seeds of *M. africana* at all concentrations, except at 0.2 M which yielded no germination (Table 5).

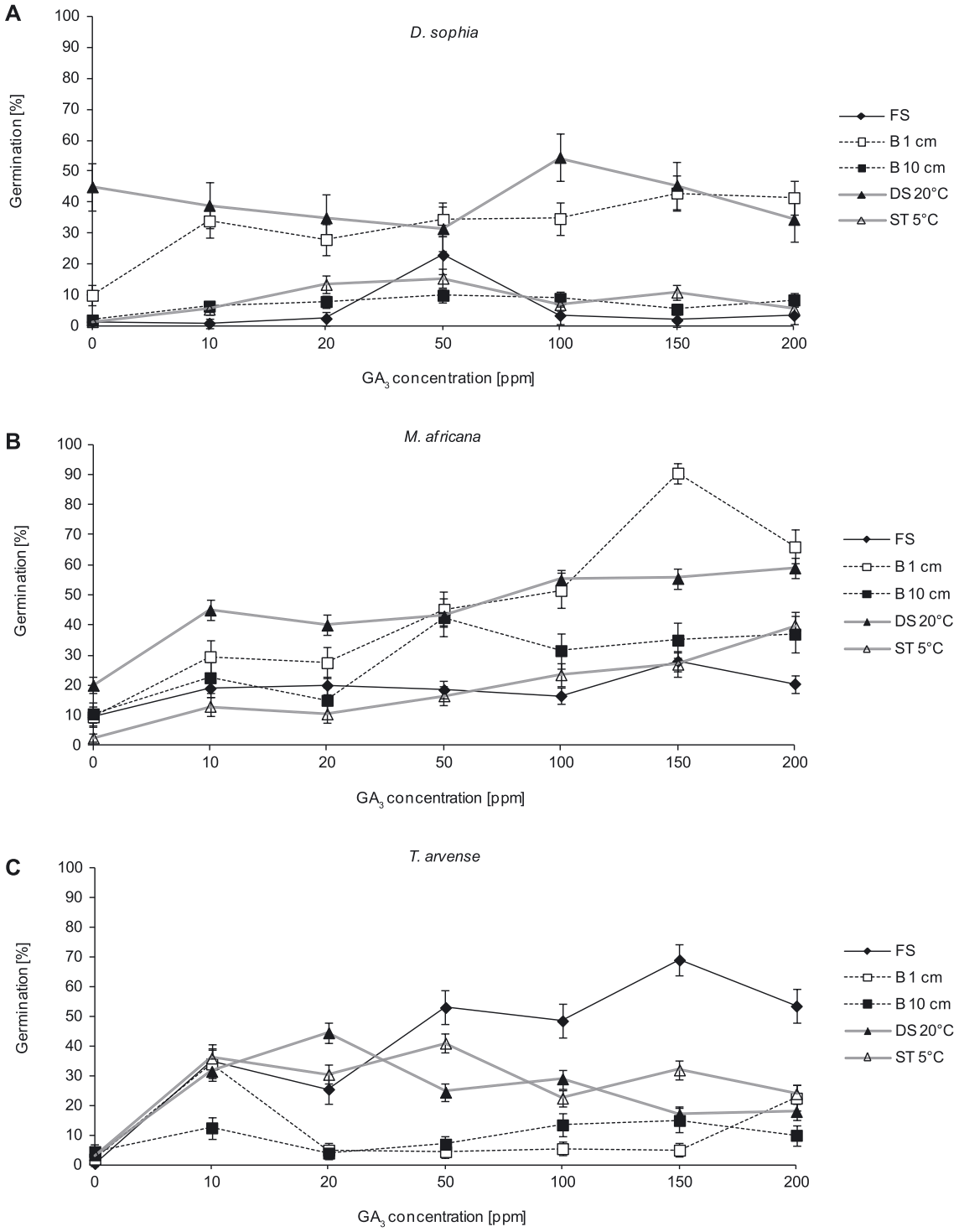

Fig. 4. Effect of GA_3 concentrations on germinability of seeds of *D. sophia*, *M. africana*, and *T. arvense* buried at 1 cm (B_1) and 10 cm (B_{10}) soil depths for 4 months, and dry seeds stored at 20°C (DS), and moist seeds stratified at 5°C (ST) for 6 months, and fresh seeds (FS). Vertical bars represent SEM

Table 4. Analysis of deviance for the effect of KNO_3 on seed germination of *D. sophia*, *M. africana*, and *T. arvense*

Source	Scaled deviance	df	F value	Pr > F
Species	247.7	2	242.12	0.0001
KNO_3	68.5	4	44.8	0.0001
Species × KNO_3	46.5	8	2.75	0.0146

Table 5. The effect of KNO_3 on seed germination of *D. sophia*, *M. africana*, and *T. arvense*

KNO_3 concentration [M]	Species		
	D. sophia	*M. africana*	*T. arvense*
	germination [%]		
0	1.5 (0.9)	9.5 (1.7)	0.5 (0.5)
0.0002	0.0 (0.0)	37.5 (3.0)	0.5 (0.5)
0.002	0.0 (0.0)	38.5 (2.2)	2.0 (1.4)
0.02	0.0 (0.0)	39.5 (10.4)	0.0 (0.0)
0.2	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Values in parenthesis represent SEM

Experiment 6. Scarification

Physical scarification caused a 22% increase in the germination percentage in *D. sophia* and 8% in *T. arvense* but had no effect on the germination of *M. africana* (data not shown). Chemical scarification with H_2SO_4 did not affect the germination in any of the species (data not shown).

Discussion

All three species showed different pattern of germinability depending on the duration of burial in the soil (Fig. 1). The differing patterns suggest there are differences in the level of seed dormancy among the species. Buried seeds of *D. sophia* at the depth of 10 cm showed increased germination after 30 days of burial in the soil, when air temperature in the preceding weeks was above 15°C (Table 1). Germination reached 55% in October with a subsequent reduction until the end of the experiment. Such reduction pattern indicate that there was a release of primary dormancy followed by induction. There was no consistent pattern to the changes in the germinability of *M. africana*. However, the germinability in the buried seeds of *T. arvense* started to increase after 90 days of burial. This increase suggests a deeper level of dormancy that needed a longer period of burial in the soil to release. The lower germination in seeds buried at 1 cm deep, might be due to high temperature fluctuation close the soil surface (Stoller and Wax 1973).

Dormancy level differences among species in Experiment 1 are consistence with results obtained from Experiment 2. An increase in germinability was observed after 90 days in *D. sophia* and after 120 days in *M. africana* and *T. arvense* seeds, dry stored at 20°C (Fig. 2). This shows that after-ripening of seeds during storage at ambient temperatures is critical for the germination of these species. Li *et al.* (2005) also observed dormancy release in *D. sophia* seeds after dry storage at room temperature. Best (1977) reported the release of dormancy in seeds of *D. sophia* in late autumn and early spring. Release of dormancy and increased germination in seeds of *T. arvense*, dry stored at 15°C and buried over winter, has also been reported (Andersson *et al.* 1997).

Stratification at 5°C did not cause a substantial increase in germinability in any of the species (Fig. 3). This implies, that seed dormancy combined with low temperatures in the habitat during the first autumn following dispersal, prevented seed germination of *D. sophia*, *T. arvense* and *M. africana*. Therefore, the low germination percentage of annual weeds in autumn at high latitudes, is attributed to decreasing temperatures which hamper the breaking of seed dormancy. Similar results were obtained by Taab and Andersson (2009) who studied dormancy in seeds of *Solanum nigrum* and recorded a negligible release of dormancy at 5°C. In contrast to our results, Milberg (1997) and Li *et al.* (2005) reported dormancy release in seeds of *D. sophia* and *T. arvense* stratified at 3°C. This contradiction could be due to the fluctuating effect or to the higher (25°C) temperature used for the germination test as compared with the constant temperature of 22°C used in our study.

The results showed that at certain concentrations, GA_3 may act as a stimulant (Fig. 4). For seeds of *D. sophia* buried 10 cm deep, for 4 months, GA_3 did not substantially increase germination (Fig. 4A). This might be due to a dormancy induction in the buried seeds after 4 months, as shown in Experiment 1 (Fig. 1). However, germination was higher for seeds buried 1 cm deep. This means that the dormancy had been reduced enough for seeds to respond to germination stimuli factors like GA_3. It is obvious, that seeds buried deep in the soil may stay dormant due to lack of enough oxygen for germination. The higher concentration of carbon dioxide prevents the germination process in deeply buried seeds. However, this might not be the case for seeds buried at shallower depths. It does not appear that GA_3 increases the germination of seeds dry-stored at 20°C for 6 months. As shown in Experiment 2 (Fig. 2), these seeds might have reached a maximum release of dormancy after this period, as well as maximum capacity to germinate (45%) at the present germination condition test. Thereafter, germination stimuli factors, like GA_3, have no further effect on stimulating germination. Germination response to GA_3 in *D. sophia* might be dormancy dependant.

For *M. africana*, seeds buried in the soil depth of 1 and 10 cm for 4 months, and seeds after dry storage at 20°C, there was a pronounced increase in germinability in response to GA_3 concentrations (Fig. 4B). Germination in

seeds dry stored at 20°C increased when the GA_3 concentration was 10 ppm, and there was little increase at higher concentrations. This treatment was found to best release dormancy in this species in Experiment 2 (Fig. 2). Germination reached its maximum at a GA_3 concentration of 50 (43%) and 150 ppm (90.5%) in seeds buried 10 and 1 cm, respectively. Maximum germination was 40% in seed stratified at 5°C for 6 months, at a GA_3 concentration of 200 ppm. The response to GA_3 was not notable in intact seeds. The germination response to GA_3 in *M. africana* might be associated with changes in the dormancy level with a variable response at higher concentrations.

All treated seeds of *T. arvense*, except seeds buried at a 10 cm soil depth for 4 months, showed increased germination (> 30%) at a GA_3 concentration of 10 ppm (Fig. 4C). This was followed by a considerable decrease in germination in seeds buried at a 1 cm soil depth at a GA_3 concentration of 20 ppm. There was also the tendency for a decreased germination at GA_3 concentrations above 20 ppm in dry stored seeds at 20°C, and at concentrations above 50 ppm in seeds stratified at 5°C. Surprisingly, intact seeds of *T. arvense*, representing a higher level of dormancy, showed an increase in germination with increased GA_3 concentrations. The maximum germination (69%) was achieved at a GA_3 concentration of 150 ppm. It is concluded, that a GA_3 concentration of more than 10 ppm might not be necessary to stimulate germination in treated seeds of *T. arvense*. The intricate interaction between stimulating, inhibiting, and limiting factors demonstrate that a series of requisites must be met for the seed to germinate. For instance, Derkx and Karssen (1993) reported that GA_3 sensitivity could be dependent on seed pretreatment conditions in *Arabidopsis thaliana*. They also showed an increased germination for GA_{4+7} concentrations with an increased effect in seeds with lower dormancy.

Potassium nitrate did not affect germination in seeds of *D. sophia* and *T. arvense*, whereas it increased germination in seeds of *M. africana*. Although nitrate may not influence the level of dormancy (Bouwmeester and Karssen 1993), it may remove constraints for seed germination (Benech-Arnold *et al.* 2000). For some seed populations, once the degree of dormancy is low, dormancy must be released by the effect of promoters such as nitrate for the germination process to proceed. Evidence for changes in sensitivity to the effect of nitrate, with changes in the degree of dormancy, was given by Hilhorst (1990), and Derkx and Karssen (1993). Germination of *S. nigrum* and *D. sophia* were also found to increase when potassium nitrate was applied (Roberts and Lockett 1978; Li *et al.* 2005). Germination in seeds of *M. africana* was higher (9.5%) in comparison with *D. sophia* and *T. arvense* (Table 5). The tested seeds of *M. africana* might have weaker levels of dormancy. These seeds responded to potassium nitrate for germination.

Physical scarification had little effect on promoting germination in *D. sophia* and *T. arvense*, and no effect on the germination of *M. africana* (data not shown). Chemical scarification with H_2SO_4 had no effect on the promotion of germination in any of the species (data not shown). Similar to our results, Moyo *et al.* (2009) reported that for *Sclerocarya birrea*, scarification with: H_2SO_4, boiling water, dry heat, and prolonged soaking of seeds did not improve germination. Von Teichman *et al.* (1986) showed that acid scarification was ineffective in enhancing seed germination of *S. birrea*. In experiments conducted by Ghadiri and Torshiz (2000), no significant increase occurred in germination when seeds of *Glycyrrhiza glabra* were chemically scarified with sulfuric acid for 15 min. We concluded, that type of dormancy in seeds of the studied species might be under the control of physiological phenomena rather than being a coat-imposed dormancy.

To sum up, the type of seed dormancy of the species is likely to be physiological and regulated by temperature. Buried seeds e.g. *D. sophia*, may go through a cycle of dormancy reduction and induction until seeds with a lower level of dormancy face the proper environmental conditions for germination, and finally germinate. This response can be used to predict the seedling emergence timing of the species in the field. Seed dormancy may be affected by GA_3 which may cause an increase in germinability depending on the pretreatment conditions and level of dormancy. Potassium nitrate increased germinability in seeds with reduced dormancy, as shown in *M. africana*. Therefore, applying KNO_3 may cause increased germinability of seeds in the soil seed bank. This could be used to stimulate seed germination and consequently, deplete the soil seed bank.

Acknowledgements

The authors thank Bahar Javidizadeh and Zeinab Teimori for their invaluable assistance in many aspects of this study.

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