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Short communication. Secondary dormancy in *Diplotaxis erucoides:* a possible adaptative strategy as an annual weed

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Abstract

The germination of stored *Diplotaxis erucoides* seed was studied under controlled conditions of temperature and light by conducting germination tests over three years after collection. The *D. erucoides* seed was not dormant at harvest, but secondary dormancy appeared during storage as indicated by reduced germination 12 months after collection (from 92% down to 39%). This could be overcome by prolonged storage or by the use of gibberellic acid. Such shifts between dormancy and non-dormancy can be interpreted in terms of the soil seed bank dynamics in relation to intraspecific competition and/or with its adaptation as an annual weed in periodically disturbed soil in cultivated areas.

Additional key words: germination, gibberellic acid, seeds, soil seed bank, storage time.

Resumen

Comunicación corta. Dormición secundaria en *Diplotaxis erucoides:* una posible estrategia adaptativa como mala hierba anual

Se estudió la evolución de la germinación/dormición de semillas de *Diplotaxis erucoides* mediante ensayos periódicos en condiciones controladas (temperatura y luz), durante tres años desde el momento de su recolección. Las semillas recién recolectadas no presentaron dormición, pero a los 12 meses apareció una dormición secundaria (la germinación disminuyó de un 92% a un 32%), desapareciendo posteriormente durante el almacenamiento o mediante aplicaciones exógenas de ácido giberélico. Los cambios entre estado no durmiente y durmiente de las semillas se interpretan como una dinámica de las semillas en el banco del suelo, como posible estrategia relacionada con la competición intraespecífica y su adaptación como mala hierba anual.

Palabras clave adicionales: ácido giberélico, almacenamiento, banco de semillas, germinación, semillas.

Diplotaxis erucoides (L.) DC. is a widespread weedy crucifer around the Mediterranean extending from the Iberian Peninsula, across central and southern Europe and North Africa to western Asia (Martínez-Laborde, 1993). It is naturalized in North and South America (Martínez-Laborde and Méndez, 2001). It is a frequent weed of numerous crops, notably in vineyards (Mendiola and Olmedo, 1987; Wilmanns, 1991) and it can reach high levels of infestation on both unirrigated and irrigated land. Like many annual weeds (Fenner, 1992), its short life cycle, its ability to prosper in highly disturbed environments, and the high number of small seeds produced per plant provide it with remarkable colonizing and invasive capacity (Gadgil and Solbrig, 1972).

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As with other allied species, *D. erucoides* might be considered a valuable phytogenetic resource because of its potential as a salad vegetable (Branca, 1995), a source of seed oil (Kumar and Tsunoda, 1980) or nectar for honey production (Rigual, 1972). Glucosinolates, which are known to have a potential for cancer prevention (Rosa, 1999) have also been found in this species (Al-Shehbaz and Al-Shammary, 1987).

The phenology, pollination and reproductive success of *D. erucoides* were studied by Farré and Sebastiá (1992), Kunin (1992) and Sans and Bonet (1993). The effect of soil characteristics, controlled light, temperature and moisture on seed germination was studied by Peltier (1969), Branca (1995) and Pérez-García *et al.* (1995). In an ecological approach, Sans and Masalles (1994) discussed the adaptive value of its discontinuous germination. The main objective of this work

was to investigate if the germination behaviour of *D. erucoides* seeds changes over time, by studying the effect of storage duration and environmentally controlled factors of temperature and light on germination.

Ripe seed of *D. erucoides* was collected, at random in October 1996 from a wild growing population, in the Botanical Garden of the Pannonian University of Agricultural Sciences in Mosonmagyaróvár (Hungary). Seed were cleaned and stored at room temperature (about 25°C) for 15 days and then stored in a cold chamber at 5°C in a hermetically sealed container until used for testing. Seed moisture content of seed at room temperature, determined by oven drying two replicates (of 150 seeds each) for 17 h at 103°C before storage at 5°C, was 7.38 ± 0.22% on the fresh weight basis.

Four replicates of 25 seeds each were placed in Petri dishes (7 cm in diameter) on two sheets of filter paper disks moistened with 3.5 ml of distilled water. More water was added, as necessary, during the germination tests. The Petri dishes were incubated at 10, 15, 20, and 25°C constant temperatures and 25/15°C alternating temperatures, under a 16/8 h light/dark regime and an irradiance of 35 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes OSRAM L 58W/20. Germination tests were conducted every six months over three years, i.e. at 6, 12, 18, 24, 30 and 36 months after seed collection.

Seed stored for 12 months was soaked in different gibberellic acid (GA_3) solutions of increased concentrations (from 0 to 250 mg L^{-1}), at room temperature (approx. 25°C) for 24 h and then incubated at 25°C with a 16/8 h photoperiod. Seed stored for 12, 24 and 36 months were also soaked in a GA_3 solution at 250 mg L^{-1}), or distilled water, under the same conditions and were also incubated at 25°C with 16/8 h light/dark photoperiod.

In all trials, seeds showing radicle emergence were counted every two days and removed from the Petri dishes. The final germination percentage was scored after a 42-day incubation period. For each trial, germination percentages were arcsine transformed and were subjected to analysis of variance (Sokal and Rohlf, 1995). Comparison of means was carried using the least significant difference test (LSD) at the 1% level of probability.

Figure 1 shows the final germination of *D. erucoides* seed incubated at 25°C measured at six monthly intervals starting immediately after collection over 36 months of storage. The highest germination of 92% was from the first assay with fresh seed. Later assays had lower values between 72% and 85%, except for the assay at 12 months after collection, at which seed germination was significantly lower at only 39% ($P \leq 0.001$).

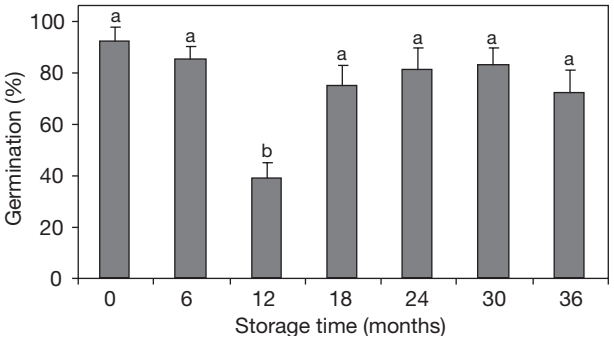

Figure 1. The germination of *Diplotaxis erucoides* seed with increased storage duration. Results are mean ± standard deviation after 42 d incubation at 25°C under a 16/8 h light/dark regime. The germination percentages with the same letter are not significantly different at 1% level using LSD test.

Incubation temperatures of 10, 15, 20 and 15/25°C were also tested after storage for 0, 12, 24 and 36 months (Fig. 2). At all assays mean germination was always lower than at 25°C, except for the assay after 12 months storage where germination obtained at 15/25°C was 40%, similar to that obtained at 25°C (39%, Fig. 1). Incubation at 10°C gave extremely low germinations (always lower than 5%).

To try to overcome the fall in germination after 12 months storage, seed was soaked in increasing concentrations of GA_3 (0-250 mg L^{-1}) prior to incubation at 25°C (Fig. 3). The germination promoting effect of GA_3 was evident as the germination was always higher than that of the control (41%). Significantly higher

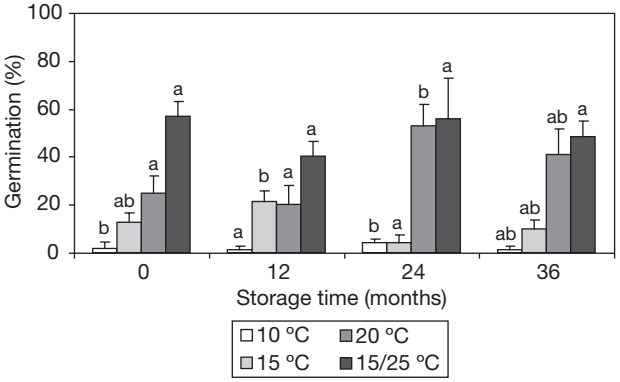

Figure 2. The germination of *Diplotaxis erucoides* seed with increased storage duration at different incubation temperatures. Results are mean ± standard deviation after 42 d incubation under a 16/8 h light/dark regime. For each incubation temperature, the germination percentages with the same letter are not significantly different at 1% level using LSD test.

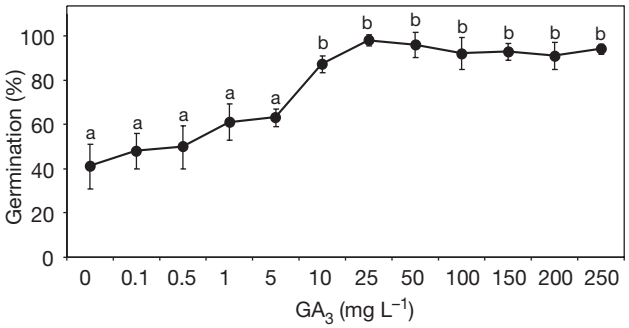

Figure 3. The germination of *Diplotaxis erucoides* seed incubated in GA_3 solutions of increased concentration after 12 months storage. Results are mean ± standard deviation after 42 d incubation at 25°C under a 16/8 h light/dark regime. The germination percentages with the same letter are not significantly different at 1% level using LSD test.

germinations (87-98%) were obtained with GA_3 concentrations ≥ 10 mg L^{-1}.

Complementary assays were performed on seeds stored for 12, 24 and 36 months, soaking them in water or in a standard GA_3 solution of 250 mg L^{-1} before incubation at different temperatures (Table 1). As expected from the reduction in germination shown in Figure 1, germination at a temperature of 25°C of seed soaked in water was significantly higher at 24 and 36 months (79% and 65% respectively) than germination after storage for 12 months (41%). Treatment with GA_3 increased germination to 94%.

The fresh seed of *D. erucoides* was not dormant at harvest as the initial germination at 25°C was 92% (Fig. 1). Germination at lower temperatures tended to be lower (Fig. 2), in accordance with the results of Pérez-García *et al.* (1995).

The reduction in germination observed 12 months after harvest (39%) suggests that secondary dormancy occurred during storage (Fig. 1). The promoting effect of GA_3 which increased germination from 41% (no GA_3) to 87-98% (Fig. 3) confirmed that the fall in germination was due to seed dormancy. Pérez-García *et al.* (1995) also found the lowest germination of *D. erucoides* populations of different geographical origin after 13-16 months storage.

However, this dormancy was also reversed in storage, as indicated by the increased germination after 18 months or longer storage. The recovery of germination after 18 months storage, was not complete, since in no instance did germination reach the values obtained before the onset of dormancy (Fig. 1). This was probably due to a persistent, slight level of dormancy and/or to moderate loss of viability. Pérez-García *et al.* (1995)

Table 1. The germination of *Diplotaxis erucoides* seed incubated after 24 h immersion in H_2O or GA3 solution (250 mg L^{-1}) after 12, 24 and 36 months storage. Results are mean ± standard deviation after 42 d incubation under a 16/8 h light/dark regime

Temperature	Months of storage					
	12 months		24 months		36 months	
	H_2O	GA_3	H_2O	GA_3	H_2O	GA_3
10°C	23 ± 1.03	92 ± 3.27	10 ± 2.00	96 ± 2.82	4 ± 2.82	77 ± 1.34
15°C	21 ± 2.83	91 ± 3.83	4 ± 0.00	95 ± 3.56	11 ± 4.68	78 ± 2.00
20°C	18 ± 2.52	93 ± 3.83	51 ± 1.74	96 ± 4.90	24 ± 3.48	82 ± 2.20
25°C	41 ± 0.00	94 ± 2.31	79 ± 1.20	90 ± 2.64	65 ± 1.32	74 ± 3.00
15/25°C	32 ± 3.64	93 ± 3.83	62 ± 3.24	96 ± 1.90	39 ± 2.54	78 ± 2.00

Analysis of variance

Clinical treatment (CT)		Storage time (ST)		Temperature (T)		Significant interactions (P)	
H_20	32.27	12	59.80	10°C	50.33	ST:CT	0.0006
GA_3	88.33	24	67.90	15°C	50.00	ST:T	0.0008
		36	53.20	20°C	60.67	CT:T	< 0.0001
				25°C	73.83	ST:CT:T	< 0.0001
				15/25°C	66.67	CV	0.11
Standard error	0.87		1.06		1.37		

P: probability. CV: coefficient of variation.

also reported an increase in germination 10 months after an initial decrease. A decrease in seed dormancy level was also detected by Ellis *et al.* (1993) in other cruciferous seeds after prolonged storage at low temperature.

Laboratory data suggest that, in nature, most *D. erucoides* seeds might readily germinate shortly after being released. However, those which were not exposed to adequate environmental conditions would temporarily enter the soil seed bank. The build-up of a soil seed bank from which seeds can germinate gradually appears as a proposed adaptive strategy controlling the population demography of *D. erucoides* and other annual weeds growing in disturbed areas (Sans and Masalles, 1994).

At 12 months after dispersal, however, a fraction of the seed might show dormancy at approximately the same time that a new generation of seed is being released in the population. This strategy may be interpreted as a mechanism that diminishes the number of seeds available for germination and consequently the number of seedlings emerging at that time, which in turn would reduce intrapopulation competition.

In the field, onset of dormancy might be induced by low temperatures during autumn and winter, as suggested by the appearance of experimental dormancy after storage at 5°C in a cold chamber. Burial experiments —not carried out so far— should be conducted to confirm this behaviour. In the laboratory, however, dormancy disappeared during the second year and did not recur during the third year. This suggests that the germination behaviour of *D. erucoides* seed does not seem to fit the cycling between dormancy and non-dormancy model observed in seed of several species in correlation with seasonal temperature cycles (Courtney, 1968; Roberts and Neilson, 1982; Van Hezewijk *et al.*, 1994; Baskin and Baskin, 1998). Thus, some kind of endogenous control could be implied in this mechanism, as proposed by Froud-Williams *et al.* (1986) in *Poa trivialis*. According to the model suggested by this data, and the work of Pérez-García *et al.* (1995), each year recruitment of *D. erucoides* seedlings would be assured by a stabilized pool of seeds consisting of a fraction of newly dispersed seeds, together with non-dormant, one-year-old seed and two-year-old, or older seed, avoiding excessive loss of viability in the soil seed bank. In any case, additional experiments, including burial tests and based on different populations from a wider range of geographical origins, should be conducted to confirm this germination behaviour.

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