Germination capability of immature seeds of *Lotus ornithopodioides* L. and *Scorpiurus subvillosus* L.

A. Cristaudo*, F. Gresta*, G. Avola and V. Miano***
*Department of Agronomy, Agrochemistry and Animal Production Sciences, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy
**Institute for Mediterranean Agriculture and Forest Systems (ISAFOM) of Italian Counsil of Research, Stradale V. Lancia, Blocco Palma I, Zona Industriale, 95121 Catania, Italy

SUMMARY – *Lotus ornithopodioides* L. and *Scorpiurus subvillosus* L. are two hard seed pasture legumes that are very palatable to livestock. In order to overcome the obstacle of hard seed, immature seeds of these species were collected from mother plants and stored at 5°C until the beginning of the germination trial. Seed germination was evaluated at 7 different temperatures of germination with a step of 5°C, for four different storage periods. At the same time, a batch of seeds stored at 5°C for 300 days was subjected to 35°C for 40 days and then tested, both intact and scarified, at the same temperature as above. Immature seeds of both species showed germination values up to 100%. Optimal temperatures for both species ranged between 10 and 25°C. With the increase of storage period, germination seems quite stable in *Lotus* and decreases in *Scorpiurus*. Intact seed placed at 35°C completely lost germination capability due to the establishment of hardseededness.

Keywords: *Lotus ornithopodioides*, *Scorpiurus subvillosus*, germination, immature seeds, hardseededness, storage.

RESUME – *"Capacité de germination de semences non mûres de* Lotus ornithopodioides *L. et* Scorpiurus subvillosus *L.".* Lotus ornithopodioides *L. et* Scorpiurus subvillosus *L. sont deux légumineuses du pâturage, très agréables pour le bétail et avec des graines dures. Dans le but de surmonter l'obstacle à la germination de ces graines dures ont été rassemblées des graines non mûres de ces espèces sur des plantes mères et elles ont été conservées à 5°C jusqu'au commencement des essais de germination. La germination des graines a été évaluée à 7 températures différentes avec un écart de 5°C, pour quatre périodes de stockage différentes. En même temps, un lot de graines conservées à 5°C pendant 300 jours ont été soumises à 35°C pendant 40 jours et ensuite ont été testées, intactes ou scarifiées, aux mêmes températures que précédemment. Les graines non mûres des deux espèces ont montré un pourcentage de germination jusqu'à 100%. Les températures optimales pour ces deux espèces sont comprises entre 10 et 25°C. Avec l'augmentation de la période de stockage, la germination paraît assez stable chez* Lotus *et est réduite chez* Scorpiurus*. Les graines intactes placées à 35°C sont incapables de germer à cause de l'établissement de la dormance imposée par la dureté des enveloppes séminales.*

Mots-clés : Lotus ornithopodioides*,* Scorpiurus subvillosus*, germination, graines non mûres, graines dures, stockage.*

Introduction

Many forage legume seeds show dormancy, due to their hard impermeable seed coat which prevents water entering, gaseous exchanges and radical emission (Ballard, 1973; Baskin and Baskin, 1998; Egley, 1995). Seed coat, in fact, is one of the most important factors regulating field germination, as well as vigour and longevity potentials of seed (Dübbern De Souza and Marcos-Filho, 2001), permitting seed germination only when conditions are suitable for supporting seedling growth. Many authors suggest that the hard-coated condition in legume is inherited (Ramsay, 1997), even if it can be regulated by environmental factors (Baskin and Baskin, 1998). Hard coat imposes dormancy in the late period of seed development through the dehydration of the seed (Bewley and Black, 1994; Samarah, 2005) so that, in many legumes, embryo achieves germination capability long before seed coat dormancy is imposed (Gresta *et al*., 2007b). Hardseededness can be removed by any method that can soften or scarify the covering, such as mechanical or acid scarification (Patanè and Gresta, 2006).

Among forage legumes, birdsfoot lotus (*Lotus ornithopodioides* L.) and prickly scorpion's tail

(*Scorpiurus subvillosus* L.), two self-reseeding annual legumes palatable for grazing animals, show high hardseededness level, up to 90% (Meloni *et al*., 2000; Gresta *et al*., 2007a). *L. ornithopodioides* and *Scorpiurus subvillosus* are widespread in Mediterranean basin and little is known in comparison to more traditional annual legumes such as *Trifolium* and *Medicago* species. For the above mentioned reasons, both species are suitable for improvement of pastoral systems in the Mediterranean environments.

The present research was conducted to explore the germination capability of immature seeds of *L. ornithopodioides* and *S. subvillosus* in relation to different storage temperatures and periods.

Materials and methods

Immature and mature seeds of *L. ornithopodioides* and *S. subvillosus* were collected from natural populations during May-June 2005 in Eastern Sicily (Italy, latitude 38° 54' N, longitude 15° 03' E, at an average altitude of 550 m a.s.l.). Seeds were harvested respectively at the greenish-yellow (immature seeds - IS) and brown (fully mature seeds - MS) pod stages of development. Seeds, after extraction from pods, were stored at 5°C for 10, 150, 300 and 500 days after harvest (DAH), times in which germination tests were performed. At the same time, a batch of seeds stored at 5°C for 300 days was subjected to 35°C for 40 days (IS_{35}) and then tested, both intact and scarified. For all the above mentioned treatments, tests were conducted in 9 cm diameter Petri dishes, using 25 seeds for each, four times replicated, at constant temperature of 5, 10, 15, 20, 25, 30 and 35°C, in continuous darkness. Seed germination was evaluated for 15 days. Germinated seeds were counted and removed daily throughout the experimental period, under a green safe light. Germination was defined as radicle protrusion through the seed coat by about 2 mm. Finally, the total percentage of germinated seeds was calculated. Cumulative germination data was adapted by means of four parameters Weibull function (SigmaPlot 9.0, Systat software Inc.) and the derived data of maximum germination percentage and time required to achieve 50% of germination (T_{50}) are discussed.

Results and discussion

Immature seeds of both species showed germination values up to 100%, while no germination was observed on fully mature seeds (Table 1).

This finding shows that at immature stage tested embryo is already able to germinate and seed coat has not yet imposed dormancy. *Lotus* immature seed showed high germination values (90-100%) over a wide range of temperatures (from 10 to 25°C) without any influence of storage periods, but 10°C at 500 DAH (54%).

Generally lower and higher temperatures determined minor germination percentages, which were also negatively affected by storage time. Optimal germination temperature, as emerges from T_{50} values, was in the range of 20-25 °C in which 1.4-2 days is needed for achieving 50% (Table 2). On the contrary, at the lowest temperature tested (5°C) a much slower germination process was observed (T_{50} between 8 and 11 days).

Scorpiurus immature seed showed the highest germination values (85-96%) in a narrower thermal range (10-20°C), compared to *Lotus*, and no germination at all was detected at temperature over 25°C. A marked and generalized decrease of germination with the increase of storage period was also observed at all temperatures. Optimal germination temperature was between 15-20°C and 50% of germination was achieved after 4 days, while at 5°C the highest T_{50} was recorded (10-12 days).

In *Lotus*, with the increase of storage period, germination seemed quite stable at temperatures from 15 to 30°C and decreased at extreme temperatures (5 and 35°C). This decrease was more conspicuous at the 5°C, especially at 500 DAH where germination declined to 8%.

Table 1. Maximum germination percentage as estimated by parameter *a* of the Weibull function of *Lotus* and *Scorpiurus* immature (IS) and mature (MS) seeds ± standard error

	DAH	Temperature													
		5°C		10°C		15°C		20°C		25°C		30°C		35°C	
Lotus ornithopodioides (IS)															
	10	87.4	±0.6	97.4	±0.4	99.8	±1.5	96.7	±3.1	90.8	±0.8	79.2	±0.7	64.8	±0.3
	150	62.3	±1.7	97.5	±0.9	100.0	±0.1	99.0	±0.1	99.0	±0.1	89.6	±2.5	66.4	±1.6
	300	40.1	±0.6	91.8	±0.4	97.7	±0.4	96.3	±0.4	94.3	±0.4	84.2	±0.5	50.6	±0.6
	500	8.7	±0.8	54.3	±0.3	96.9	±1.2	98.5	±0.4	99.7	±0.2	95.4	±0.9	46.3	±0.9
Scorpiurus subvillosus (IS)															
	10	41.3	±0.3	84.7	±0.5	95.5	±1.3	93.4	±2.4	19.3	±0.5	--		--	
	150	43.4	±0.3	91.5	±0.9	91.7	±0.2	84.4	±0.6	30.0	±0.9	--		--	
	300	50.3	±0.2	73.8	±2.0	86.0	±0.6	80.4	±0.4	34.2	±0.4	--		--	
	500	34.7	±0.7	58.2	±0.2	72.5	±0.4	47.8	±0.2	24.2	±0.4	--		--	
Lotus ornithopodioides (IS_{35})															
I*	300 + 40	--		--		--		--		--		--		--	
S*	300 + 40	--		73.8	±2.0	88.2	±0.8	92.4	±0.8	91.9	±0.0	79.8	±0.2	64.0	±0.1
Scorpiurus subvillosus (IS_{35})															
I	300 + 40	--		--		--		--		--		--		--	
S	300 + 40	--		81.9	±0.5	90.1	±1.0	70.0	±0.0	30.1	±0.2	--		--	
Lotus ornithopodioides (MS)															
I	10	--		--		--		--		--		--		--	
S	10	--		85.0	±0.9	87.2	±0.4	92.6	±2.4	90.3	±0.4	84.2	±0.5	70.0	±0.7
Scorpiurus subvillosus (MS)															
I	10	--		--		--		--		--		--		--	
S	10	--		91.9	±0.4	94.0	±1.2	84.0	±0.0	32.6	±0.8	--		--	

I* = intact seed; S* = scarified seed

Table 2. Days required to achieve 50% germination (T_{50}) as estimated by parameter *b* of the Weibull function of *Lotus* and *Scorpiurus* immature (IS) and mature (MS) seeds ± standard error

	DAH	Temperature													
		5°C		10°C		15°C		20°C		25°C		30°C		35°C	
Lotus ornithopodioides (IS)															
	10	8.2	±0.2	3.8	±0.0	3.7	±0.1	5.3	±0.1	4.4	±0.1	2.7	±0.1	4.3	±0.0
	150	11.4	±0.1	5.2	±0.0	2.8	±0.0	2.3	±0.1	1.7	±0.0	2.5	±0.1	4.5	±0.1
	300	11.1	±0.1	4.8	±0.0	3.6	±0.0	2.2	±0.0	2.3	±0.0	3.8	±0.0	3.6	±0.1
	500	10.9	±0.2	4.9	±0.0	4.2	±0.1	2.0	±0.0	1.4	±0.0	2.6	±0.1	3.7	±0.1
Scorpiurus subvillosus (IS)															
	10	9.8	±0.0	4.9	±0.0	4.0	±0.1	4.6	±0.1	5.0	±0.1	--		--	
	150	11.5	±0.0	4.2	±0.0	3.9	±0.0	3.8	±0.1	6.9	±0.1	--		--	
	300	11.2	±0.0	4.2	±0.0	4.0	±0.1	4.4	±0.0	7.1	±0.1	--		--	
	500	12.2	±0.1	6.5	±0.0	4.0	±0.0	3.7	±0.0	7.3	±0.1	--		--	
Lotus ornithopodioides (IS35)															
I*	300 + 40	--		--		--		--		--		--		--	
S*	300 + 40	--		4.2	±0.0	3.0	±0.1	2.5	±0.1	2.9	±0.0	3.2	±0.0	5.0	±0.0
Scorpiurus subvillosus (IS35)															
I	300 + 40	--		--		--		--		--		--		--	
S	300 + 40	--		4.0	±0.0	2.9	±0.3	1.9	±0.0	4.5	±0.0	--		--	
Lotus ornithopodioides (MS)															
I	10	--		--		--		--		--		--		--	
S	10	--		5.3	±0.1	3.2	±0.0	2.2	±0.0	2.5	±0.0	3.2	±0.0	6.8	±0.5
Scorpiurus subvillosus (MS)															
I	10	--		--		--		--		--		--		--	
S	10	--		3.8	±0.0	2.6	±0.1	2.0	±0.0	4.3	±0.2	--		--	

I* = intact seed; S* = scarified seed

Immature seed of both species, when exposed at constant temperature of 35°C for 40 days completely lost germination capability due to the establishment of hardseededness. Scarification in these seeds, in fact, resulted in a germination percentage comparable with those of mature scarified seed and immature seeds as well. However, compared to these last, scarified IS_{35} and scarified MS did not show any germination at 5°C, in the 15 days of germination test, probably because of a slackening of the metabolic activity of dried seeds exposed to low temperatures. This decrease of germination percentage and increment of T_{50} also occurs in immature seeds stored at 5°C, with the increasing of DAH. Immature seeds of both *Lotus* and *Scorpiurus* seeds, showed same germination pattern in response to temperature, except 5°C. Moreover scarified seeds of both species revealed a shorter T_{50} compared to immature seeds, since the seedling has no obstacle during the protrusion of radicle.

Conclusions

L. ornithopodioides and *S. subvillosus* showed seed coat dormancy established after physiological maturity of embryo. Consequently, immature seeds are readily germinable and keep their germination characteristics almost unchanged (except 5°C) for more than one year when stored at low temperature.

The exposure to high temperature (35°C) of immature seeds induces hardseededness conditions comparable with those achieved when the seed matures on mother plant. This phenomenon is probably due to the seed dehydration that makes the seed coat impermeable.

References

Ballard, L.A.T. (1973). Physical barriers to germination. *Seed Science and Technology*, 1: 285-303.

Baskin, C.C. and Baskin, J.M. (1998). *Seeds: ecology, biogeography and evolution of dormancy and germination*. Academic Press, San Diego.

Bewley, J.D. and Black, M. (1994). *Seeds: Physiology of Development and Germination*. Plenum Press, New York.

Dübbern de Souza, F.H. and Marcos-Filho, J. (2001). The seed coat as a modulator of seed-environment relationships in Fabaceae. *Revista Brasileira de botânica*, 24: 365-375.

Egley, G.H. (1995). Seed germination in soil: Dormancy cycle. In: *Seed development and germination*, Kigel, J. and Galili, G. (eds). Marcel Dekker, Inc., New York, pp. 529-543.

Gresta, F., Avola, G. and Abbate, V. (2007a). Germination ecology of *Scorpiurus subvillosus* L. seeds: The role of temperature and storage time. *Plant Ecology*, 190: 123-130.

Gresta, F., Avola, G., Anastasi, U. and Miano, V. (2007b). Effect of maturation stage, storage time and temperature on seed germination of *Medicago* species. *Seed Science and Technology*, 35: 698-708.

Meloni, M.C., Piluzza, G. and Bullitta, S. (2000). The potential role of alternative legumes from Asinara island for multiple uses in difficult environments. *Cahiers Options Méditerranéennes*, Vol. 45: 427-430.

Patanè, C. and Gresta, F. (2006). Germination of *Astragalus hamosus* and *Medicago orbicularis* as affected by seed- coat dormancy breaking techniques. *Journal of Arid Environments*, 67: 165-173.

Ramsay, G. (1997). Inheritance and linkage of a gene for testa-imposed seed dormancy in faba bean (*Vicia faba* L.). *Plant Breeding*, 116: 287-289.

Samarah, N.H. (2005). Effect of drying methods on germination and dormancy of common vetch (*Vicia sativa* L.) seed harvested at different maturity stages. *Seed Science and Technology*, 33: 733-740.