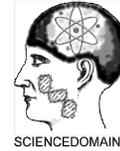


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Seed Germination and Seedling Emergence of Shepherd's needle (*Scandix pecten-veneris*) as Affected by Seed Weight or Burial Depth

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SDS and PL designed the study, performed the experiments and wrote the first draft of the manuscript. Authors TG and EV managed the literature searches and contributed to manuscript revision. All authors read and approved the final manuscript.

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ABSTRACT

Shepherd's needle (*Scandix pecten-veneris* L.) is a very common broadleaf weed of winter cereals and also an edible weed used in many regions in Greece. Knowing the behavior of the weed seeds in the soil may help in designing its management strategy and its future cultivation. Field and laboratory experiments were conducted to evaluate the effect of seed weight or burial depth on seed germination and seedling emergence in 2008 and 2010. For seed weight effect on germination and seedling emergence light and heavy seeds were tested by Petri dish assay and in the field (sowing depth 4 cm). For burial depth study six depths - 2.5, 5, 7.5, 10, 12.5 and 15 cm - were examined in field trials made in two periods of time: 25 November and 15 March for two years. Percentage of seed germination, seedling emergence and mean emergence time were measured. Results showed that light seeds germinated better (74 ± 2.2 - $95\pm 2.2\%$) and earlier (20.5 ± 0.64 - 31.0 ± 0.45 days) than heavy seeds (34 ± 3.2 - $58\pm 5.1\%$ and 25.4 ± 0.57 - 33.8 ± 0.46 days, respectively). The burial depth influenced seedling emergence and mean

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emergence time (MET) in most cases. Low emergence percentage (1.7 ± 1.1 - $33.8\pm 7.2\%$) was found at the depth of 15 cm and high at depth of 2.5, 5, 7.5 cm. Seeds sowed 15.0 cm deep had higher MET (27.0 ± 0.9 - 55.1 ± 1.1 days) than those sowed at 2.5 cm (20.9 ± 0.9 - 41.6 ± 0.5 days).

Keywords: Seedling emergence; burial depth; seed weight; germination; *Scandix pecten-veneris*.

1. INTRODUCTION

Nowadays, concerns for environmental protection and the demand for less use of chemicals in agriculture have increased considerably. Scientists aim at more efficient, sustainable and economical alternative methods in weed control such as integrated weed management systems. However, the development of effective integrated weed management systems depends on a thorough understanding of weed seed biology [1]. Seedling emergence is a key event in determining the success of some weeds in an agroecosystem [2].

Shepherd's needle (*Scandix pecten-veneris*) is a very common annual broadleaf weed of Apiaceae family of winter cereals in Greece [3] and is controlled mainly by chemical methods. To improve its control where this weed is a problem, it is important and useful to know about its germination behavior. However, studies on factors affecting seed germination and emergence of this weed, such as seed weight and burial depth, are scarce. The weight of seed is known to play a major role in plant population dynamics and community structure [4]. Heavy seeds are considered to have better adaptation in competitive conditions [5] while lighter seeds show often high dispersal and much persistence in the seed bank [6]. Another factor that alters the weed seed emergence is burial depth and species-specific emergence responses to this have already been well-documented [7]. Generally, with increasing burial depth plant seed emergence decreases. Carefully chosen cultivations, tailored to the weed species composition in the reservoir of seeds in the soil, can alter seed behavior. This could be achieved by encouraging emergence and hence the premature depletion of the seed bank or by burying weed seeds to depths from which they cannot germinate and successfully emerge [8].

This weed is an edible wild green leafy vegetable widely used in the Mediterranean diet and traditionally is used in various local cuisines [9]. It is consumed either boiled cooked with olive oil or in pies and salads. In several regions of Greece, Shepherd's needle seeds are shown in vegetable gardens (from late October to Spring) and there is an increasing interest in cultivating this species as a vegetable crop.

The objective of this research was to reveal the influence that seed weight or burial depth have on germination, seedling emergence and mean emergence time of *Scandix pecten-veneris* thus, expanding our knowledge into managing the population of this weed species. The results of the study will also help the cultivation techniques of this plant in cases it is grown as a vegetable crop.

2. MATERIALS AND METHODS

2.1 Plant Material

All needed weed fruits were harvested, in a random way, from the natural population that exists at the research farm Velestino, University of Thessaly, in the middle of July in 2007 and 2009 (in central Greece Shepherd's needle seedling emergence occurs from October to April [10] and maturity at the end of spring-May). Each flower produces a fruit (mericarp) consisting of two seeds which remain joined until they ripe and each seed has a long scabrid needle-like appendage (beak) up to 6 cm in length. Only fruits with both well developed seeds were selected and stored in paper bags in laboratory temperature. Seeds collected directly after maturity from mother plant can germinate (at 14°C, plus moisture) 50-60% and over 70-80% when collected approximately 60 days after maturity (unpublished findings). The field experiments were carried out in a field where *Scandix pecten-veneris* had never been seen emerged before. The soil texture (depth 0-30 cm) was Sand 47%, Silt 30%, Clay 23% (Loam). Irrigation was applied after sowing and during the tests at any time it was necessary. For the laboratory tests, the amount of soil used was collected from the above field. For seed weight trials, selected fruits were divided into two classes, small-light and big-heavy, based on the size of the fruit. Seeds derived from them had mean weight 8±2.9 or 15±3.2 mg, made up the small-light class, and 35±3.6 or 53±4.3 mg made up the big-heavy class in 2008 or 2010 trials, respectively. Heavy seeds had a beak about 1/3 to 1/4 bigger in length compared to light seeds and also bigger and heavier the lower reserve part. In seed burial depth tests, all the selected fruits were of medium size-weight (to avoid possible interactions between light or heavy seed weight and burial depth) and derived seeds had mean weight 25±2.1 mg.

2.2 Mean Emergence Time and Seed Weight Trial

Forty seeds of each class were sowed in the field at a depth of 4 cm in a randomized complete block design (RCB), with plot size 0.5 x 0.5 m, block size (including space between plots) 1.2 x 0.5 m and four replications per treatment (weight class) on 24 March 2008 and 12 February 2010. The number of emerged seedlings (cotyledons completely unfolded) was recorded daily until no more seedlings were observed (40 days after sowing). Mean emergence time (MET) was calculated according to the equation of Ellis & Roberts [11] as follow:

$$MET = \frac{\sum Dn}{\sum n}$$

where n is the number of seeds, which were emerged on day D and D is the number of days counted from the beginning of the test. MET is a measure of the rate and spread of seed emergence [12]. Emergence of seeds with high MET is slower and more spread over time than that of seeds with low MET. For these trials, the range of possible MET values with our data was 1 (if the finally measured seedlings emerged all in one day after sowing) to 40 days (if all the finally measured seedlings emerged forty days after sowing).

2.3 Seed Germination and Seed Weight Trial

The effect of seed weight on seed germination was evaluated in laboratory tests carried out in June 2008 and 2010. Twenty seeds of each heavy and light class (described above) were placed in a plastic 9 cm diameter Petri dish (5 dishes-replications per treatment-class in completely randomized design). Then, dishes were filled with 50 mL of dry sieved soil (2 mm

size sieve) and irrigated with 20 mL distilled water. The applied temperature and photoperiod inside the incubator was 15°C/ dark 24 h. Temperature selection was based on previous laboratory experimentation of the authors on the effect of temperature on Shepherd's needle seed germination (unpublished findings). Petri dishes were left at these conditions for 35 days, then the germinated seeds were carefully removed, counted and the final germination percentage was calculated. Germination was recorded only once (end of experiment) and not daily to avoid the risk of breaking the radicles by digging into soil to check germinated seeds.

2.4 Seedling Emergence and Seed Burial Depth Trial

Six burial depths were examined: 2.5, 5, 7.5, 10, 12.5 and 15 cm in field trials carried out during two different periods of time, 25 November and 15 March (sowing dates) for two years 2008-09 and 2010-11. Forty seeds were sowed at each depth in a RCB design with plot size 0.5 x 0.5 m, block size (including space between plots) 2 x 1.5 m and 4 replications per treatment-depth. Emergence of seedlings was recorded daily until no more seedlings were observed (60 days after sowing). Mean emergence time was also calculated as mentioned before and the range of possible MET values for these tests was 1 to 60 days. Soil temperature at a depth of 5 cm was recorded (1 record/30 min) by a data logger (type i-button Dallas Semiconductor) placed near the plots. Mean temperature at 5 cm depth was used under the assumption that in the same period it was probably not too much different of mean temperatures at other depths of the present work. Studies [13] have shown that although temperature varies differently at different depths in the soil during day time, the average soil temperature is similar at 2-20 cm depths. Values on Fig. 1 showed that there was a difference in mean soil temperature between years 2008-09 and 2010-11 during these trials. In March trials the temperature was lower in 2010-11 than 2008-09 (difference of 0 to 5-6°C) from sowing date (15 Mar.) to the end of April. In November 2010-11 test the mean soil temperature for about 10 days (10 to 20 Dec.) was very low and ranged between 1 to 5°C.

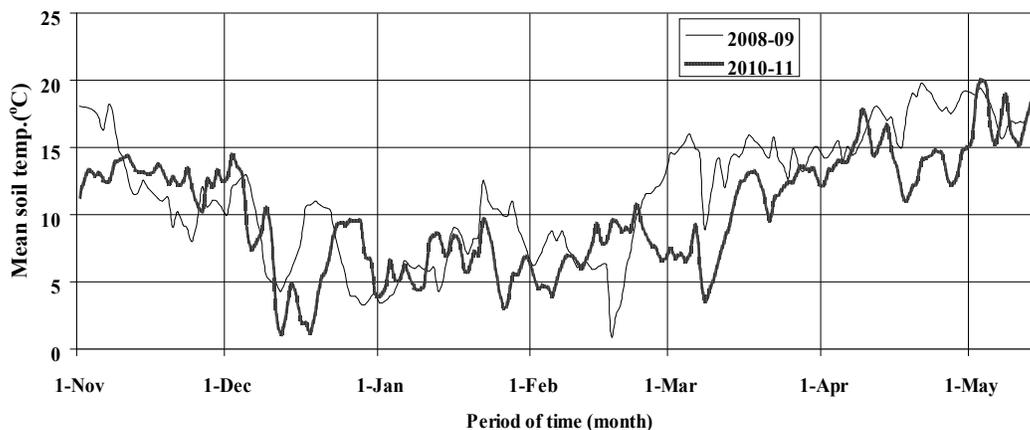


Fig. 1. Mean soil temperature at 5 cm depth in field trials 2008-09 and 2010-11

2.5 Statistical Analysis

Data from trials which examined the effect of seed weight on germination and mean emergence time of Shepherd's needle were analyzed by an ANOVA. Regression analysis was performed for data from seedling emergence and seed burial depth tests. SPSS software (v13.0 for Windows) was used for all statistics. Where *F* test was significant treatment means were given with their \pm SE.

3. RESULTS

3.1 Seed Weight Effect on Seedling Emergence – Seed Germination

Emergence of seedlings was found to be affected by seed weight significantly in both field experiments in 2008 and in 2010 (Table 1 and Fig. 2). The final percentage of light seeds emerged (74 ± 2.2 - $75\pm 2.7\%$) was almost double than that of heavy seeds (34 ± 3.2 - $39\pm 2.3\%$). In 2008 mean emergence time of light seeds was lower (20.5 ± 0.64 days) compared to that of heavy seeds (25.4 ± 0.57 days). Also in 2010 trial, light seeds emerged earlier (31.0 ± 0.45 days) than heavy seeds (33.8 ± 0.46 days) (Fig. 2).

Table 1. One-way ANOVA for the effect of seed weight on seedling emergence and mean emergence time (field trials 2008, 2010)

	Source of var.	Sum of Squares	df	Mean Square	F-value	P-value
Seedling emergence 2008	Between Groups	3200.000	1	3200.000	108.169	.000
	Within Groups	177.500	6	29.583		
	Total	3377.500	7			
Seedling emergence 2010	Between Groups	2556.125	1	2556.125	100.404	.000
	Within Groups	152.750	6	25.458		
	Total	2708.875	7			
Mean emergence time 2008	Between Groups	48.020	1	48.020	32.300	.001
	Within Groups	8.920	6	1.487		
	Total	56.940	7			
Mean emergence time 2010	Between Groups	15.680	1	15.680	18.892	.005
	Within Groups	4.980	6	0.830		
	Total	20.660	7			

Results derived from laboratory tests were similar to those of field experiments. Light seeds had a statistically significant higher level of germination percentage, 78 ± 4.6 - $95\pm 2.2\%$, than heavy seeds, 35 ± 3.5 - $58\pm 5.1\%$ in both years 2008 and 2010 (Table 2 and Fig. 3).

Table 2. One-way ANOVA for the effect of seed weight on seed germination (laboratory trials 2008, 2010)

	Source of var.	Sum of Squares	df	Mean Square	F-value	P-value
Germination 2008	Between Groups	4622.500	1	4622.500	54.382	.000
	Within Groups	680.000	8	85.000		
	Total	5302.500	9			
Germination 2010	Between Groups	3422.500	1	3422.500	43.460	.000
	Within Groups	630.000	8	78.750		
	Total	4052.500	9			

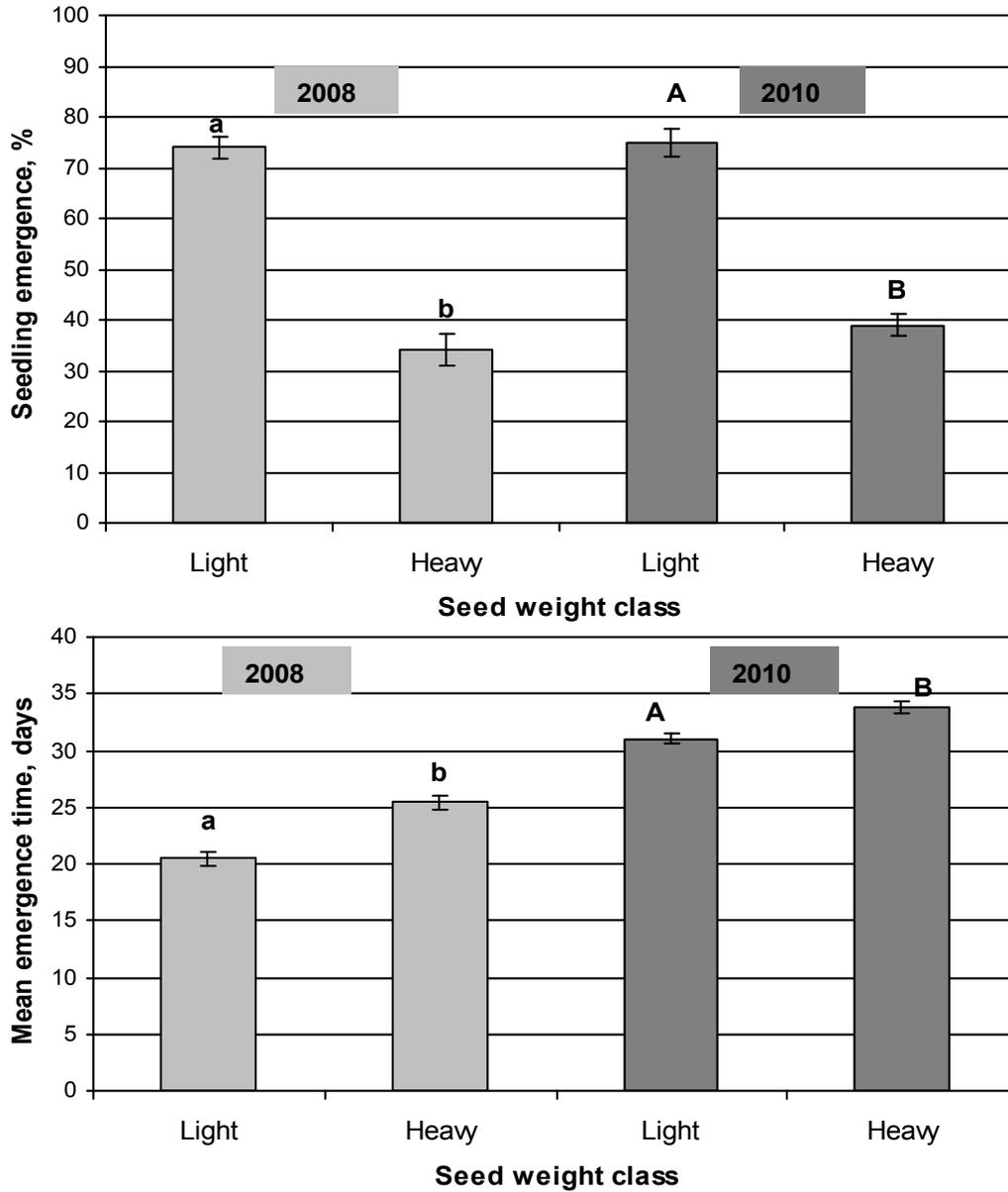


Fig. 2. Seedling emergence as % (above) and mean emergence time in days (below) of Shepherd's needle as affected by seed weight.

For each year, different letters above bars indicate significant difference at P = .05. Error bars are standard error of means of four replications.

3.2 Seed Burial Depth Effect on Seedling Emergence

Significant influence of burial depth on Shepherd's needle emergence was measured in 2008-09 and 2010-11 trials in both periods of time 25 November and 15 March (except Nov. 2010) as shown in Table 3. Emergence percentage generally decreased as burial depth

increased. The lowest percentage 1.7 ± 1.1 to $33.8\pm 7.2\%$ was found at the depth of 15 cm. Emergence above 70% was measured in all trials where seeds were sowed 2.5 to 10 cm deep in soil except in March 2011 test where the highest percentage ($51.7\pm 7.7\%$) was observed only in 2.5 cm depth.

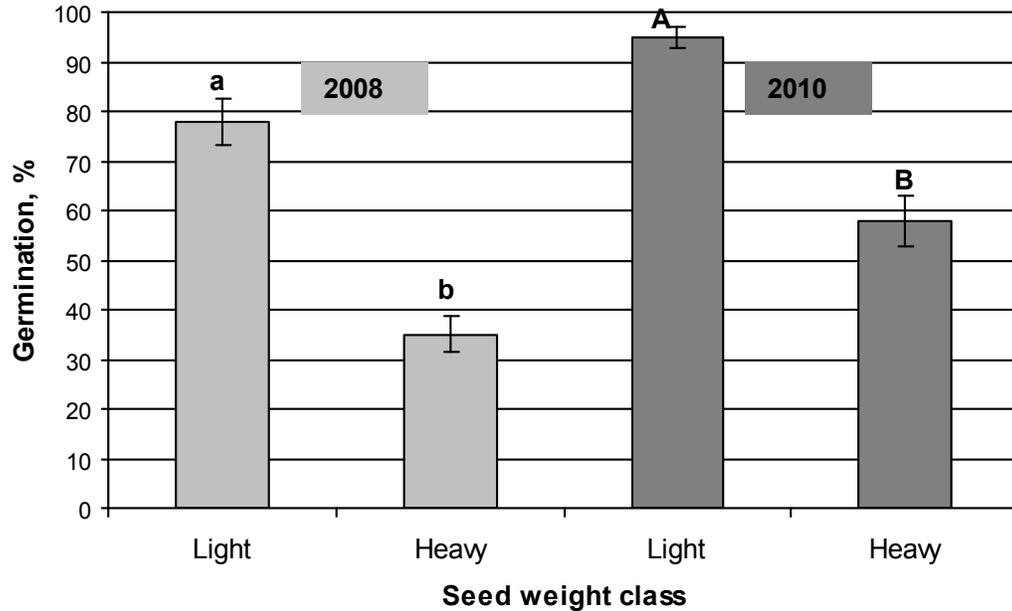


Fig. 3. Seed germination (%) of Shepherd's needle as affected by seed weight. For each year, different letters above bars indicate significant difference at $P = .05$. Error bars are standard error of means of five replications.

Table 3. Seedling emergence (%) of Shepherd's needle as affected by burial depth in two periods of time in 2008-09 and 2010-11 trials

Depth (cm)	2008-09		2010-11	
	25 Nov.	15 Mar.	25 Nov.	15 Mar
2.5	86.3±5.2	75.0±5.4	68.3±9.6	51.7±7.7
5	90.0±4.6	81.3±4.7	65.0±10.8	41.7±6.6
7.5	85.0±2.0	87.5±4.3	80.0±7.4	45.0±9.4
10	72.5±12.7	78.8±5.5	70.0±5.4	26.7±6.6
12.5	51.3±14.3	61.3±2.4	66.7±1.2	10.0±5.0
15	26.3±10.5	33.8±7.2	66.7±3.1	1.7±1.1

Means in each column are followed by \pm standard error.

The data of Table 3 were also examined by regression analysis for each trial separately. The results (Table 4) indicated that there was a significant quadratic trend of seedling emergence response to burial depth for Nov. and Mar. 2008-09 (Fig. 4). For Nov. 2010 regression was not significant ($P=.811$ or $P=.617$). Model comparisons revealed that for Nov. 2008 trial the increase in R^2 from linear to quadratic was significant ($P=.000$) but not for Mar. 2011 ($P=.261$).

Table 4. Regression analysis and model comparisons for seedling emergence and burial depth

Equation	Model Summary					Parameter Estimates			
		R ² , Adj.R ²	F-value	df1	df2	P-value	Constant, P-value	b1, P-value	b2, P-value
Nov. 2008	linear	0.835 0.793	20.197	1	4	.011	111.427 .000	-4.898 .011	-
	quadr.	0.999 0.998	1471.162	2	3	.000	76.710 .000	5.517 .001	-0.595 .000
Mar. 2009	linear	0.561 0.451	5.111	1	4	.087	-	-	-
	quadr.	0.992 0.987	188.517	2	3	.001	53.120 .001	10.051 .002	-0.754 .001
Nov. 2010	linear	0.016 -0.230	0.065	1	4	.811	-	-	-
	quadr.	0.275 -0.208	0.569	2	3	.617	-	-	-
Mar. 2011	linear	0.923 0.904	48.284	1	4	.002	65.807 .000	-4.153 .002	-
	quadr.	0.953 0.922	30.611	2	3	.010	53.890 .013	-0.578 .841	-0.204 .261
Model comparisons									
		R ²	R ² Change	F Change	df1	df2	Sig. F Change		
Nov. 2008	linear	0.835							
	quadr.	0.999	0.164	483.895	1	3	.000		
Mar. 2011	linear	0.923							
	quadr.	0.953	0.030	1.913	1	3	.261		

Mean emergence time (MET) of Shepherd's needle was also affected by burial depth in all trials in both years. When seeds were sowed deeper in the soil, seedling emergence was delayed. MET in 15 cm treatments (Nov.: 54.1±3.4, Mar.: 27.0±0.9 days) during the year 2008-09 was higher than that in 2.5 cm (31.8±2.1, 20.9±0.8 days, respectively) as shown in Table 5. Similar results were taken in 2010-11 study (Table 5).

Data of each trial (Table 5) were also analyzed by regression and results (Table 6) revealed that there was a significant linear trend of mean emergence time response to burial depth for all tests carried out in 2008-09 and 2010-11 (Fig. 5). For Nov. 2008 test the increase in R² from linear to quadratic was not significant ($P=.046$) at $P=.02$.

Table 5. Mean emergence time (days) of Shepherd's needle as affected by burial depth in two periods of time in 2008-09 and 2010-11 trials

Depth (cm)	2008-09		2010-11	
	25 Nov.	15 Mar.	25 Nov.	15 Mar.
2.5	31.8±2.1	20.9±0.9	41.6±0.5	25.4±0.7
5	29.0±1.8	20.1±0.6	47.8±1.9	30.7±2.0
7.5	35.6±0.9	21.7±0.3	48.1±1.8	35.6±1.2
10	39.5±5.7	24.7±0.9	48.5±1.3	35.4±3.0
12.5	45.0±4.5	23.7±0.4	50.5±1.2	32.3±0.8
15	54.1±3.4	27.0±0.9	55.1±1.1	47.0±1.2

Means in each column are followed by ± standard error.

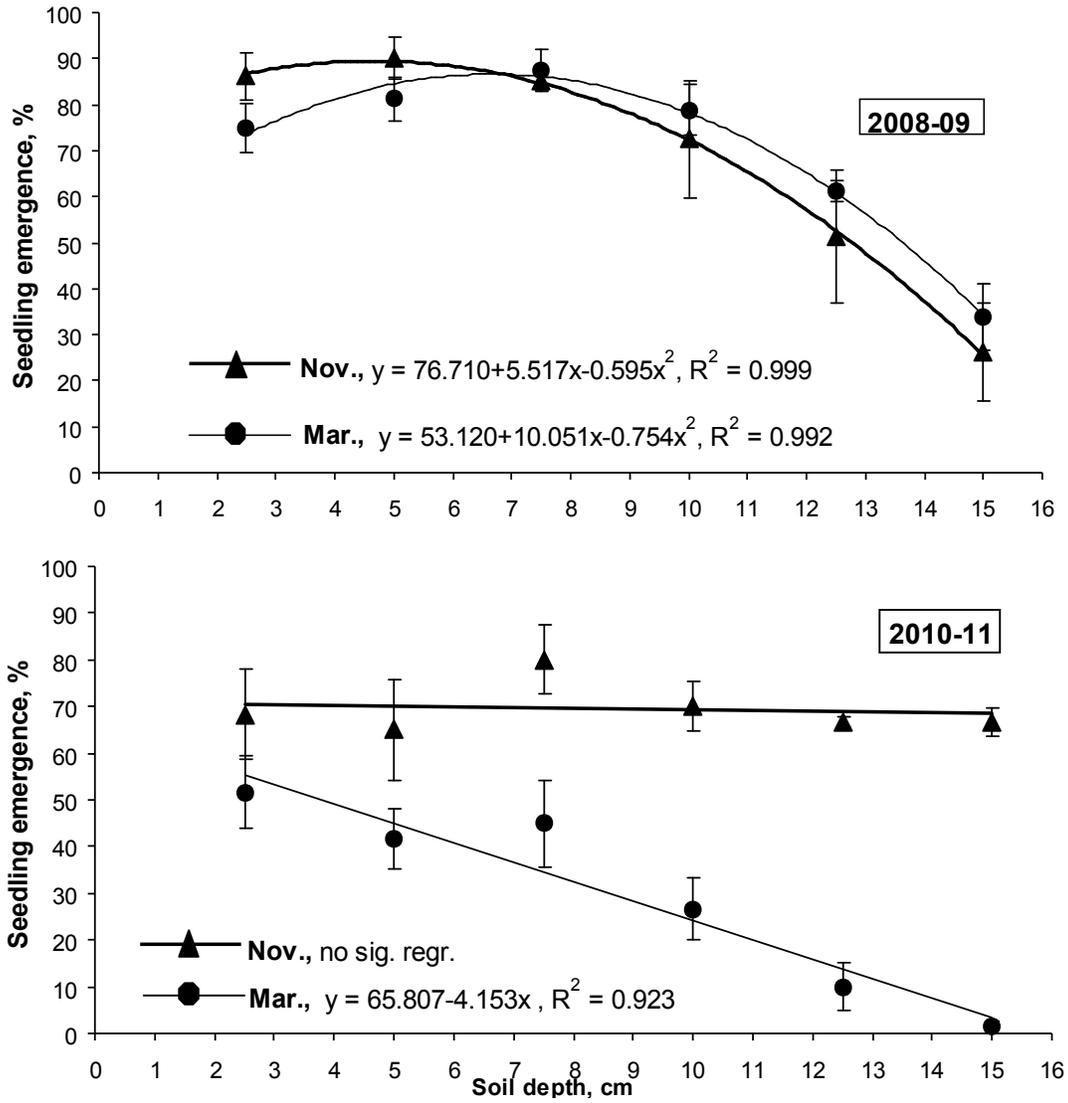


Fig. 4. Response curve of seedling emergence to burial depth according to regression equation.

Error bars are ± standard error of observed means.

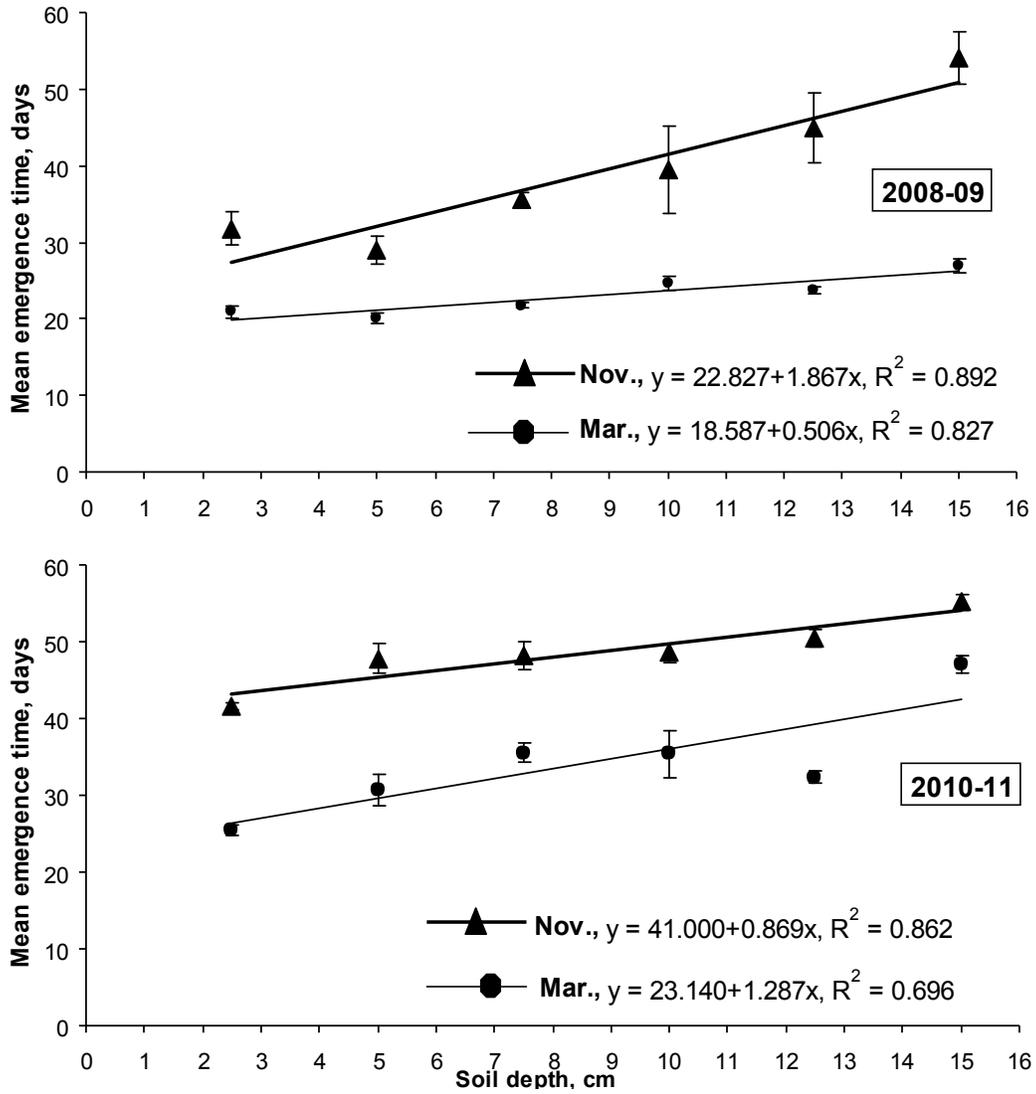


Fig. 5. Response curve of mean emergence time to burial depth according to regression equation.

Error bars are ± standard error of observed means.

Table 6. Regression analysis and model comparisons for mean emergence time and burial depth

Equation		Model Summary				Parameter Estimates			
		R ² , Adj.R ²	F-value	df1	df2	P-value	Constant, P-value	b1, P-value	b2, P-value
Nov. 2008	linear	0.892 0.865	33.116	1	4	.005	22.827 .002	1.867 .005	-
	quadr.	0.977 0.961	63.089	2	3	.004	32.010 .002	-0.888 .374	0.157 .046
Mar. 2009	linear	0.827 0.784	19.161	1	4	.012	18.587 .000	0.506 0.12	-
	quadr.	0.863 0.772	9.459	2	3	.051	-	-	-
Nov. 2010	linear	0.862 0.827	24.918	1	4	.008	41.000 .000	0.869 0.008	-
	quadr.	0.862 0.770	9.358	2	3	.051	-	-	-
Mar. 2011	linear	0.696 0.620	9.151	1	4	.039	23.140 .005	1.287 .037	-
	quadr.	0.706 0.510	3.604	2	3	.159	-	-	-
Model comparisons		R²	R² Change	F Change	df1	df2	Sig. F Change		
Nov. 2008	linear	0.892							
	quadr.	0.977	0.085	10.921	1	3	.046		

4. DISCUSSION

Seed weight had a strong influence on Shepherd's needle seed germination and seedling emergence as derived from the experimental results. Larger-heavier seeds had significantly lower percentage of germination-emergence as compared to small-lighter seeds. Similar results to these findings have been observed for *Erodium brachycarpum* [14]. Contrary to this, it has been reported that heavier seeds of *Abutilon theophrasti* [15], *Lithospermum arvense* [16] and *Panicum racemosum* [17] had greater germination. However, for other species like *Ambrosia artemisiifolia* [18], *Dactylis glomerata* [19], *Andropogon tectorum* [20], *Rumex obtusifolius* [21] weight of seed had no any effect on their germination. Baskin and Baskin [22] suggested that this effect is species dependant and different seed sizes may have low, high or same germination percentage. Shepherd's needle light seeds had lower mean emergence time than heavy seeds and that means earlier germination in soil. Earlier germination of light seeds had also been found in *Pastinaca sativa* [23], *Cakile edentula* [24], *Erodium brachycarpum* [14], *Alliaria petiolata* [25] and *Senna obtusifolia* [26]. However, Stanton [27] found that seed size of *Raphanus raphanistrum* had no effect on emergence time. Stamp [14] attributed the earlier germination of small seeds to their greater access to water as a result of their higher surface to volume ratio. Hence, small seeds imbibed water faster and broke dormancy sooner. Susko and Lovett-Doust [25] stated that more rapid uptake of water as well as thinner seed coats may be responsible for the earlier loss of dormancy in small seeds of *Alliaria petiolata*. In a previous germination study of Shepherd's needle seeds carried out by the authors of this article (unpublished data) it was found that seeds of same size treated with a 98% sulphuric acid solution had higher germination percentage than those of seeds treated with distilled water. It means that sulphuric acid made the seed coats thinner, therefore quick water uptake become easier. So, thinner seed

coat of light Shepherd's needle seeds may be one reason for their earlier germination compared to heavy seeds.

Many studies have reported the negative effect of increasing burial depth on emergence of several weeds species such as *Amaranthus spinosus* and *A. viridis* [1], *Phalaris paradoxa* [28], *Xanthium strumarium*, *Abutilon theophrasti*, *Pharbitis purpurea* [29], *Plantago lanceolata*, *Digitaria sanguinalis*, *Portulaca oleracea*, *Stellaria media* [30] and *Ambrosia artemisiifolia* [18]. Burial depth also influenced Shepherd's needle emergence percentage and MET in our study. In most cases, as seeds were sowed deeper in soil the total emergence percentage decreased and MET increased. It seemed that the 12.5 cm was the critical depth (for the given soil type) for emergence of this weed, below which the seeds poorly could give seedlings and their emergence was too delayed. In the burial tests carried out during March, mean emergence time generally was smaller than that of November trials (independently of burial depth). Differences in mean soil temperature at this period of time could explain this.

Benvenuti and Macchia [31] suggested that affections of burial depth on weed seed germination could be attributed to variation of different soil's layer characteristics, water holding-capacity and gas environment conditions. Although no retrieval of buried seeds was made in this study to check germination afterwards, suicide seed germination or induced secondary seed dormancy could probably also has been another reason for low emergence at deep sowing depths. On 25 November 2010 trial, statistically significant difference in total emergence percentage between treatments was not observed (although there was for MET measurements). During this day of trial installation, soil moisture was very close to saturation due to extended rainfall and the soil used to cover the buried seeds was not compressed properly as in the other trials in order to avoid excessive soil compaction. This may have altered soil's layer characteristics and perhaps, the results of emergence percentage that were observed at this experiment.

5. CONCLUSION

To summarize, seed weight of Shepherd's needle influenced its germination and emergence characteristics, light seeds germinated and emerged quicker and in higher percentage than heavy seeds. It seems that the variable seed size is a biological trait that could allow this weed like other weed species to cope with the variable soil environment conditions found in different fields. Also, the fact that seedling emergence is limited and delayed considerably where seeds sit 12.5 cm or deeper below soil surface could make soil tillage at a depth of 15 cm or more with moldboard plough a useful tool in controlling this weed where it is a problem. In case of cultivation of Shepherd's needle as a vegetable crop, these findings could be very useful when seeds of this plant species are sown in the field.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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