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

Original Research Article

ABSTRACT

Shepherd's needle (*Scandix pecten-veneris* L.) is a very common broadleaf weed of winter cereals in Greece. Knowing the behavior of the weed seeds in the soil may help in designing its management strategy. 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 ($74\pm2.2-95\pm2.2\%$) and earlier (20.5 ± 0.64 - 31.0 ± 0.45 days) than heavy seeds ($34\pm3.2-58\pm5.1\%$ and $25.4\pm0.57-33.8\pm0.46$ days respectively). The burial depth influenced seedling emergence and mean emergence time (MET) in most cases. Low emergence percentage ($1.7\pm1.1-33.8\pm7.2\%$) was found at the depth of 15cm and high at depth of 2.5, 5, 7.5 cm. Seeds sowed 15 cm deep had higher MET ($27.0\pm0.9-55.1\pm1.1$ days) than those sowed at 2.5 cm ($20.9\pm0.9-41.6\pm0.5$ days).

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> Keywords: <mark>seedling emergence; burial depth; seed weight; germination; Scandix pecten-</mark> <mark>veneris.</mark>

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15 **1. INTRODUCTION**

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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]

24 Shepherd's needle (Scandix pecten-veneris) is a very common annual broadleaf weed of 25 Apiaceae family of winter cereals in Greece [3] and is controlled mainly by chemical 26 methods. To improve its control where this weed is a problem, it is important and useful to 27 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 28 29 of seed is known to play a major role in plant population dynamics and community structure 30 [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 31 32 factor that alters the weed seed emergence is burial depth and species-specific emergence 33 responses to this have already been well-documented [7]. Generally, with increasing burial 34 depth plant seed emergence decreases. Carefully chosen cultivations, tailored to the weed 35 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 36 37 bank or by burying weed seeds to depths from which they cannot germinate and 38 successfully emerge [8].

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40 The objective of this research was to reveal the influence that seed weight or burial depth 41 have on germination, seedling emergence, and mean emergence time of Scandix pecten-42 veneris thus, expanding our knowledge into managing the population of this weed species. 43

44 2. MATERIALS AND METHODS

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48 All needed weed fruits were harvested, in a random way, from the natural population that 49 exists at the research farm Velestino, University of Thessaly, in the middle of July in 2007 50 and 2009 (in central Greece Shepherd's needle seedling emergence occurs from October to 51 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. Each seed has a long scabrid 52 53 needle-like appendage (beak) up to 6cm in length, which acts as a spring dispersal mechanism as the seed ripens. Only fruits with both well developed seeds were selected 54 55 and stored in paper bags in laboratory temperature. The field experiments were carried out 56 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 57 58 after sowing and during the tests at any time it was necessary. For the laboratory tests, the 59 amount of soil used was collected from the above field. For seed weight trials, selected fruits 60 were divided into two classes, small-light and big-heavy, based on the size of the fruit. 61 Seeds derived from them had mean weight 8±2.9 or 15±3.2 mg, made up the small-light 62 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 63 seeds and also bigger and heavier the lower reserve part. In seed burial depth tests, all the 64 selected fruits were of medium size-weight (to avoid possible interactions between light or 65 66 heavy seed weight and burial depth) and derived seeds had mean weight 25±2.1 mg.

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2.2 Mean emergence time and seed weight trial

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70 Forty seeds of each class were sowed in the field at a depth of 4 cm in a randomized 71 complete block design (RCB), with plot size 0.5 x 0.5 m, block size (including space between 72 plots) 1.2 x 0.5 m and four replications per treatment (weight class) on 24 March 2008 and 73 12 February 2010. The number of emerged seedlings (cotyledons completely unfolded) was 74 recorded daily until no more seedlings were observed. Mean emergence time (MET) was 75 calculated according to the equation of Ellis & Roberts [9] as follow: 76

$MET=\sum Dn / \sum n$

77 where n is the number of seeds, which were emerged on day D, and D is the number of 78 days counted from the beginning of the test.

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80 2.3 Seed germination and seed weight trial

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82 The effect of seed weight on seed germination was evaluated in laboratory tests carried out 83 in June 2008 and 2010. Twenty seeds of each heavy and light class (described above) were 84 placed in a plastic 9 cm diameter Petri dish (5 dishes-replications per treatment-class, in 85 completely randomized design). Then, dishes were filled with 50 mL of dry sieved soil (2 mm 86 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 87 88 laboratory experimentation of the authors on the effect of temperature on Shepherd's needle 89 seed germination (unpublished findings). Petri dishes were left at these conditions for 35 90 days, then the germinated seeds were carefully removed, counted and the final germination 91 percentage was calculated. Germination was recorded only once (end of experiment) and 92 not daily to avoid the risk of breaking the radicles by digging into soil to check germinated 93 seeds.

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2.4 Seedling emergence and seed burial depth trial

97 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 98 99 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 100 per treatment-depth. Emergence of seedlings was recorded every day until no more 101 102 seedlings were observed. Mean emergence time (MET) was also calculated as mentioned 103 before. Soil temperature at a depth of 5 cm was recorded (1 record/30 min) by a data logger 104 (type i-button Dallas Semiconductor) placed near the plots. Values on Fig. 1 showed that 105 there was a difference in mean soil temperature between years 2008-09 and 2010-11 during 106 these trials. In March trials the temperature was lower in 2010-11 than 2008-09 (difference of 107 0 to 5-6°C) from sowing date (15 Mar.) to the end of April. In November 2010-11 test the 108 mean soil temperature for about 10 days (10 to 20 Dec.) was very low and ranged between 109 1 to 5°C.



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

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113 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 ±SE.

120 121 **3. RESULTS**

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123 **3.1 Seed weight effect on seedling emergence – seed germination**

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Emergence of seedlings was found to be affected by seed weight significantly in both field experiments, in 2008 and in 2010 (Fig. 2 and Table 1). The final percentage of light seeds emerged $(74\pm2.2-75\pm2.7\%)$ was almost double than that of heavy seeds $(34\pm3.2-39\pm2.3\%)$. 128 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±0.45 129 130 days) than heavy seeds (33.8±0.46 days) (Fig. 2).



Seed weight class

132 133 Fig. 2. Seedling emergence as % (above) and mean emergence time in days (below) of 134 Shepherd's needle as affected by seed weight. 135 For each year, different letters above bars indicate significant difference at P = .05. Error 136 bars are standard error of means of four replications 137 138

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140 Table 1. Two-way ANOVA for the effect of seed weight on seedling emergence and 141 mean emergence time (field trials 2008, 2010)

	Source of var.	<mark>Sum of</mark> Squares	df	<mark>Mean</mark> Square	F-value	P-value
	Treatments	<mark>3200</mark>	<mark>1</mark>	<mark>3200</mark>	<mark>738.462</mark>	<mark>.000</mark>
Seedling	Replications	<mark>164.5</mark>	<mark>3</mark>	<mark>54.833</mark>	<mark>12.654</mark>	<mark>.033</mark>
2008	Error	<mark>13</mark>	<mark>3</mark>	<mark>4.333</mark>		
	Total	<mark>3377.5</mark>	<mark>7</mark>			
Seedling emergence 2010	Treatments	<mark>2556.125</mark>	<mark>1</mark>	<mark>2556.125</mark>	<mark>78.751</mark>	<mark>.003</mark>
	Replications	<mark>55.375</mark>	<mark>3</mark>	<mark>18.458</mark>	<mark>0.569</mark>	<mark>.673</mark>
	<mark>Error</mark>	<mark>97.375</mark>	<mark>3</mark>	<mark>32.458</mark>		
	Total	<mark>2708.875</mark>	<mark>7</mark>			
	Treatments	<mark>48.020</mark>	<mark>1</mark>	<mark>48.020</mark>	<mark>21.631</mark>	<mark>.019</mark>
Mean	Replications	<mark>2.260</mark>	<mark>3</mark>	<mark>0.753</mark>	<mark>0.339</mark>	<mark>.801</mark>
time 2008	Error	<mark>6.660</mark>	<mark>3</mark>	<mark>2.220</mark>		
time 2000	Total	<mark>56.940</mark>	<mark>7</mark>			
Mean emergence time 2010	Treatments	<mark>15.680</mark>	<mark>1</mark>	<mark>15.680</mark>	<mark>13.876</mark>	<mark>.034</mark>
	Replications	<mark>1.590</mark>	<mark>3</mark>	<mark>0.530</mark>	<mark>0.469</mark>	<mark>.725</mark>
	Error	<mark>3.390</mark>	<mark>3</mark>	<mark>1.130</mark>		
	Total	<mark>20.660</mark>	<mark>7</mark>			

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143 Results derived from laboratory tests were similar to those of field experiments. Light seeds 144 had a statistically significant higher level of germination percentage, 78±4.6-95±2.2%, than 145 heavy seeds, 35±3.5-58±5.1% in both years 2008 and 2010 (Fig. 3 and Table 2).



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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 149 are standard error of means of five replications

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151 Table 2. One-way ANOVA for the effect of seed weight. on seed germination 152 (laboratory trials 2008-2010)

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	Source of var.	<mark>Sum of</mark> Squares	df	<mark>Mean</mark> Square	F-value	P-value
Germination	<mark>Between</mark> Groups	<mark>4622.5</mark>	<mark>1</mark>	<mark>4622.5</mark>	<mark>54.38</mark>	<mark>.00</mark>
<mark>2008</mark>	Within Groups	<mark>680</mark>	<mark>8</mark>	<mark>85</mark>		
	Total	<mark>5302.5</mark>	<mark>9</mark>			
Germination	<mark>Between</mark> Groups	<mark>3422.5</mark>	<mark>1</mark>	<mark>3422.5</mark>	<mark>43.46</mark>	<mark>.00</mark>
2008	Within Groups	<mark>630</mark>	<mark>8</mark>	<mark>78.75</mark>		
	Total	<mark>4052.5</mark>	<mark>9</mark>			

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155 **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 $33.8\pm7.2\%$ 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 ($51.7\pm7.7\%$) was observed only in 2.5 cm depth.

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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

	2008-09		2010		
Depth (cm)	25 Nov.	15 Mar.	25 Nov.	15 Mar.	Grand Mean
2.5	86.3 <mark>±5.2</mark>	75.0 <mark>±5.4</mark>	68.3 <mark>±9.6</mark>	51.7 <mark>±7.7</mark>	70.3±7.2
5	90.0 <mark>±4.6</mark>	81.3 <mark>±4.7</mark>	65.0 <mark>±10.8</mark>	41.7 <mark>±6.6</mark>	<mark>69.5</mark> ±10.6
7.5	85.0 <mark>±2.0</mark>	87.5 <mark>±4.3</mark>	80.0 <mark>±7.4</mark>	45.0 <mark>±9.4</mark>	<mark>74.4</mark> ±9.9
10	72.5 <mark>±12.7</mark>	78.8 <mark>±5.5</mark>	70.0 <mark>±5.4</mark>	26.7 <mark>±6.6</mark>	<mark>62.0</mark> ±11.9
12.5	51.3 <mark>±14.3</mark>	61.3 <mark>±2.4</mark>	66.7 <mark>±1.2</mark>	10.0 <mark>±5.0</mark>	<mark>47.3</mark> ±12.8
15	26.3 <mark>±10.5</mark>	33.8 <mark>±7.2</mark>	66.7 <mark>±3.1</mark>	1.7 <mark>±1.1</mark>	<mark>32.1</mark> ±13.4
Grand mean	68.5 ±10.2	69.6 ±8.0	69.4 ±2.2	29.4 ±8.2	59.2

176 Means in each column are followed by ± standard error

177Part of data of Table 3 (six grand means) were also examined by regression analysis. The178observed means plotted in Fig. 4, show that seeds in depth between 2.5 and 9 cm had an179emergence of about 70% but deeper than 9 cm emergence decreased significantly.180Regression analysis (Table 4) indicated that there was a significant (P=.004) quadratic trend

181 with $R^2 = 0.975$ of seedling emergence response to burial depth whereas, these parameters 182 were minimized for the linear trend (*P*=.021 and $R^2=0.775$)



Table 4. Regression analysis for seedling emergence and burial depth

Equation		Model	<mark>Sumr</mark>	nary		Parame	ter Estima	ates
	R ²	F-value	df1	df2	P-value	Constant	<mark>b1</mark>	<mark>b2</mark>
Linear	<mark>0.775</mark>	<mark>13.76</mark>	<mark>1</mark>	<mark>4</mark>	<mark>.021</mark>	<mark>86.267</mark>	<mark>-3.086</mark>	
Quadratic	<mark>0.975</mark>	<mark>58.764</mark>	<mark>2</mark>	<mark>3</mark>	<mark>.004</mark>	<mark>61.2</mark>	<mark>4.434</mark>	<mark>-0.43</mark>

Fig. 4. Response curve of seedling emergence to burial depth according to regression equation. Error bars are standard error of observed grand means.

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 15cm 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).

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

213	2008-09		3-09	2010			
215	Depth (cm)	epth (cm) 25 Nov.		25 Nov.	15 Mar.	Grand Mean	
216 217	2.5	31.8 <mark>±2.1</mark>	20.9 <mark>±0.9</mark>	41.6 <mark>±0.5</mark>	25.4 <mark>±0.7</mark>	29.9 ±4.5	
218	5	29.0 <mark>±1.8</mark>	20.1 <mark>±0.6</mark>	47.8 <mark>±1.9</mark>	30.7 <mark>±2.0</mark>	<mark>31.9±</mark> 4.5	
219	7.5	35.6 <mark>±0.9</mark>	21.7 <mark>±0.3</mark>	48.1 <mark>±1.8</mark>	35.6 <mark>±1.2</mark>	<mark>35.2±</mark> 5.4	
220	10	39.5 <mark>±5.7</mark>	24.7 <mark>±0.9</mark>	48.5 <mark>±1.3</mark>	35.4 <mark>±3.0</mark>	37.0 ±4.9	
221	12.5	45.0 <mark>±4.5</mark>	23.7 <mark>±0.4</mark>	50.5 <mark>±1.2</mark>	32.3 <mark>±0.8</mark>	37.9 ±6.0	
222	15	54.1 <mark>±3.4</mark>	27.0 <mark>±0.9</mark>	55.1 <mark>±1.1</mark>	47.0 <mark>±1.2</mark>	<mark>45.8±</mark> 6.5	
223	Grand mean	39.2 ±3.8	23.0 ±1.06	48.6 ±1.8	34.4 ±2.9	<mark>36.3</mark>	

Means in each column are followed by ± standard error

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 226 The six grand means of MET (Table 5) were also analyzed by regression and results (Table



6) revealed that there was a significant linear trend (P= .003 and R² = 0.91) of mean

emergence time response to burial depth. Curve estimation showed also a significant

quadratic trend (P= .015) with higher value of R² = 0.94 and this equation was used in Fig. 5



Table 6. Regression analysis for mean emergence t	ime and burial depth
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Equation	Model Summary					Parameter Estimates		
	R ²	F-value	df1	df2	P-value	Constant	<mark>b1</mark>	<mark>b2</mark>
Linear	<mark>0.91</mark>	<mark>40.35</mark>	1	<mark>4</mark>	<mark>.003</mark>	<mark>26.353</mark>	<mark>1.135</mark>	
Quadratic	<mark>0.94</mark>	<mark>23.608</mark>	<mark>2</mark>	<mark>3</mark>	<mark>.015</mark>	<mark>29.670</mark>	<mark>0.14</mark>	<mark>0.057</mark>

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247 **4. DISCUSSION**

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249 Seed weight had a strong influence on Shepherd's needle seed germination and seedling 250 emergence as derived from the experimental results. Larger-heavier seeds had significantly 251 lower percentage of germination-emergence as compared to small-lighter seeds. Similar 252 results to these findings have been observed for Erodium brachycarpum [11]. Contrary to 253 this, it has been reported that heavier seeds of Abutilon theophrasti [12], Lithospermum 254 arvense [13] and Panicum racemosum [14] had greater germination. However, for other species like Ambrosia artemisiifolia [15], Dactylis glomerata [16], Andropogon tectorum [17], 255 256 Rumex obtusifolius [18] weight of seed had no any effect on their germination. Baskin and 257 Baskin [19] suggested that this effect is species dependant and different seed sizes may 258 have low, high or same germination percentage. Shepherd's needle light seeds had lower 259 mean emergence time than heavy seeds and that means earlier germination in soil. Earlier 260 germination of light seeds had also been found in Pastinaca sativa [20], Cakile edentula [21], 261 Erodium brachycarpum [11], Alliaria petiolata [22] and Senna obtusifolia [23]. However, 262 Stanton [24] found that seed size of Raphanus raphanistrum had no effect on emergence 263 time. Stamp [11] attributed the earlier germination of small seeds to their greater access to 264 water as a result of their higher surface to volume ratio. Hence, small seeds imbibed water 265 faster and broke dormancy sooner. Susko and Lovett-Doust [22] stated that more rapid 266 uptake of water, as well as thinner seed coats, may be responsible for the earlier loss of 267 dormancy in small seeds of Alliaria petiolata. In a previous germination study of Shepherd's 268 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 269 270 percentage than those of seeds treated with distilled water. It means that sulphuric acid 271 made the seed coats thinner, therefore quick water uptake become easier. So, thinner seed 272 coat of light Shepherd's needle seeds may be one reason for their earlier germination 273 compared to heavy seeds.

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275 Many studies have reported the negative effect of increasing burial depth on emergence of 276 several weeds species such as Amaranthus spinosus and A. viridis [1], Phalaris paradoxa 277 [25], Xanthium strumarium, Abutilon theophrasti, Pharbitis purpurea [26], Plantago 278 lanceolata, Digitaria sanguinalis, Portulaca oleracea, Stellaria media [27], Ambrosia 279 artemisiifolia [15]. 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 280 281 emergence percentage decreased and MET increased. It seemed that the 12.5 cm was the 282 critical depth (for the given soil type) for emergence of this weed, below which the seeds 283 poorly could give seedlings and their emergence was too delayed. In the burial tests carried 284 out during March, mean emergence time generally was smaller than that of November trials 285 (independently of burial depth). Differences in mean soil temperature at this period of time 286 could explain this.

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Benvenuti and Macchia [28] 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

292 secondary seed dormancy could probably also has been another reason for low emergence 293 at deep sowing depths. In November 2010 trial, statistically significant difference in total 294 emergence percentage between treatments was not observed (although there was for MET 295 measurements). During field experiment installation soil moisture was very close to 296 saturation due to extended rainfall and the soil used to cover the buried seeds was not 297 compressed properly as in the other trials in order to avoid excessive soil compaction. This 298 may have altered soil's layer characteristics and, perhaps, the results that were observed.

- **5. CONCLUSION** 300
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302 To summarize, seed weight of Shepherd's needle influenced its germination and emergence 303 characteristics, light seeds germinated and emerged guicker and in higher percentage than 304 heavy seeds. It seems that the variable seed size is a biological trait that could allow this 305 weed like other weed species to cope with the variable soil environment conditions found in 306 different fields. Also, the fact that seedling emergence is limited and delayed considerably 307 where seeds sit 12.5 cm or deeper below soil surface could make soil tillage at a depth of 15 308 cm or more with moldboard plough a useful tool in controlling this weed where it is a 309 problem.

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COMPETING INTERESTS 311

313 Authors have declared that no competing interests exist.

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