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EFFECT OF SEED MATERIAL PREPARATION ON PLANT EMERGENCE AND YIELD OF MILK THISTLE (*Silybum marianum* (L.) Gaertn.) IN A MODERATE CLIMATE

Jadwiga Andrzejewska[1]✉, Stanisław Ignaczak[1], Tommaso Martinelli[2]

[1] University of Technology and Life Science in Bydgoszcz, Poland

[2] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria – Centro di ricerca per le colture industriali (CREA-CIN), Bologna, Italy

ABSTRACT

In moderate climates where milk thistle is grown as a medicinal plant it emerges slowly and unevenly and thus it is exposed to weed infestation. The aim of this study was to estimate if the storage temperature and thousand seed weight (TSW) of the seed material have an effect on the number of emerging plants. In the 3-year field experiment, seed material that had been stored at 23, 15 and 7°C and separated according to TSW: 26–30, 20–22 and 20–30 g (control) was used. The emergence of plants was evaluated 4 times, from the BBCH growth stage 09–12 to 18–19/37–38. Storage of seed material at 23 and 7°C resulted in an increase in the number of emerging plants in comparison with seed material stored at 15°C. These differences were evident only at the first time of measurements and gradually reduced as growth progressed. A significantly higher number of plants per 1 m^2 was obtained from seed material with TSW 26–30 g as compared with the seed material with TSW 20–22 g. These differences continued at all measurement dates but only in one dry year they did have a positive effect on the achene yield.

Key words: medicinal plants, TSW, seed material storage, soil temperature, BBCH

INTRODUCTION

Milk thistle (*Silybum marianum* (L.) Gaertn.), family Asteraceae is now widespread on most continents, but it is grown as a medicinal plant mostly in Europe and Asia. The fruits (achenes), often referred to as seeds, are both the pharmaceutical raw material for silymarin production and the seed material. The 1000 seed weight can range from 13.9 g [Rahimi and Kamali 2012] to 32.9 g [Andrzejewska and Sadowska 2008], but the most often reported value is in the range 24–26 g [Mel'nikova 1983, Dyduch and Najda 2007, Martinelli et al. 2016]. At maturity the pericarp of the fruit is black to brown coloured and at one edge holds both the elaiosome and the pappus. Radicle emergence takes place at the edge of the fruit that does not hold the elaiosome [Martinelli et al. 2015]. The length of post-harvest maturation depends on the ambient temperature, but at 10–15°C about 40% of seeds have already germinate within a month from the harvest [Young et al. 1978].

In regions of the world where a warm climate makes it possible to overwinter, milk thistle is persistent weed [Dodd 1989, Khan et al. 2009]. Even in moderate climates where it is grown as a medicinal herb this feature of it can also constitute a problem. Its achenes shed easily and can affect adjacent fields as well as successive crops [Habán et al. 2009, Vereš

✉jadwiga.andrzejewska@utp.edu.pl

and Týr 2012, Habán et al. 2015, Týr 2015]. Although milk thistle is also considered a persistent weed, when it is grown for pharmaceutical purposes it is susceptible to weed infestation itself, especially at the early stage of growth. Then other weeds outdistance milk thistle in development and overgrow thistle seedlings, reducing yield and hindering its harvest as well as the drying and cleaning processes of the fruit.

Weed control in crops of medicinal plants is currently a serious problem, because throughout the world there are only a limited number of herbicides intended for these plants. Starting 30 years ago and up until recently in different countries herbicides effective in controlling monocot and dicot weeds had been tested and used in milk thistle crops [Czarnecki and Załęcki 1990, Załęcki et al. 1996, Zheljazkov et al. 2006]. Currently most of those preparations are no longer produced, therefore research on evaluation of the effectiveness of various herbicides in milk thistle cultivation has recently been resumed [Drapalova and Pluhackova 2014]. However, the producers of medicinal plants are obliged to follow the rules of Good Agricultural Practice in Cultivation of Herbal Plants, which require restrictions on the use of pesticides [Anonim 2006]. One of the solutions to weed control may be the use of herbicides prior to emergence and mechanical weed removal after the emergence of the milk thistle. This, however, will be effective only when the plants of milk thistle emerge quickly and the leaves quickly cover the entire field.

In moderate climates the emergence of milk thistle lasts from 7 to 25 days, and the initial plant growth is uneven and mostly depends on the thermal conditions [Czabajska et al. 1989, Andrzejewska and Skinder 2006]. Groves and Kaye [1989] in laboratory experiments observed a germination rate of 20% within the temperature range 15/5°C (day/night), germination of 65% with temperature 20/10°C, and about 90% with the range between 25/15 and 35/25°C. Similar relationships had also been reported by Young et al. [1978].

In agriculture and horticulture there are different methods of seed material preparation that are used that aim to accelerate and even out plant emergence. Similar methods for milk thistle have also been developed, but they are suitable only for laboratory conditions [Purohit et al. 1997, Khan et al. 2013]. For agricultural practice simple methods that allow for the preparation of large amounts of seed material are necessary. Data from the literature [Young et. al. 1978, Mel'nikova 1983, Groves and Kaye 1989, Parmoon et al. 2015] prove that temperatures 20–25°C provide favourable conditions for milk thistle germination. Therefore, the research hypothesis assumed that storing seed material in a heated storehouse may stimulate earlier and more uniform emergence. We also decided to test the effect of cooling milk thistle seed material on the rate of plant emergence. A favourable effect from cooling seed material on the germination of another medicinal plant species – *Heracleum candicans* was found by Joshi and Dhar [2002], but no information is available in this regard about milk thistle. Usually seed material with a higher thousand seed weight (TSW) is also characterized by a higher germination rate and subsequent growth [Lang 1965], but there is no data whether this also pertains to milk thistle.

The aim of this study was to evaluate the effect of seed material storage temperature and TSW on the rate of plant emergence and yield of milk thistle cultivated in a moderate climate.

MATERIALS AND METHODS

Site, field experiment and cultural practices

Field experiment was conducted in 2012, 2013 and 2015 at the Research Station Mochełek (53°13'N, 17°52'E), Poland. Soil at Mochełek is a fine-sandy loam, mixed mesic, Ustic, typic hapludalf with pH 6.0. The soil has a high abundance of potassium and phosphorus and is moderate in magnesium. The experiment using the Polish cultivar 'Silma' was established as a two-factorial in the randomized block design, with 4 replications; the area of a plot was 14.4 m^2, and the yield was collected from 9.0 m^2. The first experimental factor was the temperature of seed material storage for 6 weeks prior to sowing: 23, 15 and 7°C. The second factor was TSW: 26–30, 20–22 and 20–30 g (without separation according to TSW). Separation of seed material according to TSW was performed on a stream separator (K-293 Petkus) using a varied force of air flow. Seed material with the mean TSW amounting to at least 26 g and at the most 22 g was obtained using the air flow force > 90 $m^3 h^{-1}$ and 75–90 $m^3 h^{-1}$, respectively. The TSW of not separated material stayed within the range from 20 to 30 g.

Prior to sowing, the laboratory germinating capacity of each batch of seed material was checked and the seeding rate was planned so that the theoretical number of germinating seeds per m^2 would amounted to 40 plants. Seed material was dressed with Dithane (s.a. Menkozeb), at a rate of 5 g per 1 kg of achenes, to protect against fungal diseases. Sowing was done on 12 April in the first year, on 30 April in the second year, and on 14 April in the third one. Row spacing was 30 cm. Prior to sowing 50 kg ha^{-1} N was applied in the form of ammonium nitrate. During growth, until overgrowing of the inter-rows by milk thistle plants, weeds were removed mechanically.

Plant density was calculated in 2 rows on each plot at the following developmental stages given according to the BBCH scale [Martinelli et al. 2015]:

– stage 09–12: emergence of cotyledons through soil surface–first pair of elliptic true leaves visible (longer than 1 cm each);

– stage 13–15/31: three–five leaves visible (longer than 2 cm) / 10% of ground covered by leaves;

– stage 16–17/33–35: six–seven leaves visible / 30–50% of ground covered by leaves;

– stage 18–19/37–38: eight–nine leaves visible / 70–80% of ground covered by leaves.

The single-stage harvest was performed using a Wintersteiger plot combine harvester on 27 July in 2012, on 12 August in 2013 and on 13 August in 2015. The harvested yield was dried in an airy room and cleaned on a K-293 Petkus stream separator. After separation according to TSW (as in the seed material) the achene yield was measured and the content of silymarin determined.

Silymarin analyses

Silymarin HPLC determination was performed according to Martinelli et al. [2016] on the diluted methanolic extract (final methanol concentration 30%) and was analysed using an Agilent 1100 system (Agilent Technologies, USA) equipped with a C18 column (Kinetex 2.6 μm, 100A, 100 × 3 mm; Phenomenex, USA). The HPLC settings were as follows: flow 340 μl min^{-1}, column temperature 23°C, UV-Vis detector at 288 nm. The mobile phase was composed of methanol (phase A) and 0.1% formic acid (phase B); 0 to 3 minutes isocratic 33% phase A; 3 to 17 minutes gradient 33 to 47% phase A; 17 to 32 minutes isocratic 47% phase A. Flavonolignans quantification was obtained using single purified molecule standards (Sigma-Aldrich, USA). Total silymarin content is the result of the sum of the flavonolignans (silycristin, silydianin, silybin A, silybin B, isosilybin A, isosilybin B) derived from the HPLC analysis.

Statistical analysis

An analysis of variance (ANOVA) was performed using STATISTICA12. Tukeys test was used to assess significant differences between means ($p \leq 0.05$). The analysis of variance for data concerning the plant density was performed separately for each of the times of determination.

RESULTS

Weather conditions

In 2012 and 2013 the moisture conditions were similar and favourable for milk thistle emergence and growth, whereas in 2015 rainfall deficits occurred both prior to milk thistle sowing and during its growth (tab. 1). The thermal conditions during milk thistle growth expressed as the total of air temperatures were almost identical each year (tab. 1). However, the least favourable thermal conditions in the period of milk thistle sowing (April) occurred in 2013. The soil temperature then was 2°C lower in comparison with 2012 and 1.6°C lower as compared with 2015. The mean daily air temperature in 2013 was 1.4°C lower in comparison with 2012 and 0.5°C lower as compared with 2015 (tab. 2).

Since there was no interaction of the effects of the experimental factors either on the number of plants or on the yield and content of silymarin, the results were compared and described independently for each of the factors.

Table 1. Basic data on conditions of milk thistle growth at the Experimental Station Mochełek in the years of the study

Specification	2012	2013	2015
Number of days of milk thistle growth	106	104	121
Total precipitation from 1 January to the day of sowing (mm)	119	104	82
Total precipitation during milk thistle growth (mm)	272	291	136
Total daily air temperatures during milk thistle growth (°C)	1611	1669	1667

Table 2. Weather conditions in the initial growth period of milk thistle at the Experimental Station Mochełek

	Year	April				May			
		1–10	11–20	21–30	mean/sum	1–10	11–20	21–31	mean/sum
Mean daily soil temperature (°C) at a depth of 5 cm	2012	4.3	7.7	12.2	8.1	14.5	13.9	17.5	15.4
	2013	0.2	7.7	10.4	6.1	13.0	15.5	15.4	14.6
	2015	4.8	7.7	10.4	7.7	12.1	12.6	15.2	13.4
	1996–2012		–		8.1		–		14.6
Mean daily air temperature (°C)	2012	3.2	7.6	14.3	8.4	14.1	12.6	16.5	14.5
	2013	0.6	10.1	10.3	7.0	14.0	15.4	13.3	14.2
	2015	4.3	7.8	10.6	7.5	12.0	11.9	13.2	12.4
	1996–2014		–		8.1		–		13.2
Precipitation (mm)	2012	11.3	2.1	13.1	26.5	9.9	15.5	0.0	25.4
	2013	0.5	11.4	1.7	13.6	18.0	9.1	64.6	91.7
	2015	5.5	1.1	9.0	15.6	8.7	12.4	0.5	21.6
	1996–2014		–		28.7		–		61.1

Effect of storage temperature

The method for seed material preparation of milk thistle significantly affected the plant density in the first (2012) and the third (2015) years of the study (tabs 3 and 4). In 2012 the highest plant density was obtained from seed material stored at the highest and lowest temperature, i.e., 23 and 7°C. However, at the stage 18–19/37–38 a significant difference was recorded in favour of seed material stored at 23°C as compared with storage at lower temperatures. Plant density from seed material stored at 15°C was always the lowest (tab. 3). In 2013, when sowing occurred at the latest time, none of the experimental factors had an effect on the rate of emergence. Between 09–12 BBCH and 13–15/31 BBCH in 2013 the number of plants per 1m^2 more than doubled (tab. 3). In 2015 an influence of temperature of seed material storage on plant emergence was noted only at the first time of measurement (09–12 BBCH). It was found then that plants from seeds stored at 23°C emerged in the greatest numbers, and those from seeds stored at 7°C in the lowest numbers. However, at further developmental stages of milk thistle these differences were eliminated (tab. 3).

The average from the years' results of measurements show that increasing the temperatures of seed material storage from 15 to 23°C had a favourable effect on the number of plants after emergence at the stage 09–12 BBCH (tab. 3). Measurements made at successive developmental stages showed a decreasing effect of the increasing storage temperature on the number of plants (differences in successive developmental stages were: 6.2, 5.4, 5.1 and 4.9 per m^2). A decrease in temperature of seed material storage from 15 to 7°C also caused an increase in the number of plants determined at the stage 09–12 BBCH. Again in the course of growth, differences in the number of plants were gradually reduced (3.1, 2.4, 2.5, 2.2 per m^2).

Effect of TSW

In 2012, more numerous plant emergences were obtained from the unseparated seed material and from that with TSW 26–30 g than from the seed material with TSW 20–22 g. Only at the first measurement (09–12 BBCH) there was no significant difference in the density of plants obtained from the unseparated seed material and that with TSW 20–22 g (tab. 4). No differences were recorded in 2013. In 2015, a significant effect of achene weight on the plant density was again found. At each measurement the highest density was obtained from the seed material with TSW 26–30 g, and the lowest from the seed material with TSW 20–22 g (tab. 4).

Means from the years of the study indicate that when the seed material with TSW 26–30 g or not separated material was applied, the emergence was equal. However, the use of seed material with TSW 20–22 caused significantly weaker plant emergence in comparison with the emergence from the seed material with TSW 26–30 g. It is important that the differences remained until the last measurement performed at stage 18–19/37–38 BBCH (tab. 4).

Table 3. The number of milk thistle plants per 1 m^2 at successive developmental stages depending on the storage temperature of seed material

Year/temperature (°C)		Developmental stage acc. to BBCH scale			
		09–12	13–15/31	16–17/33–35	18–19/37–38
2012	23	32.5 a*	37.4 a	36.1 a	37.7 a
	15	17.8 b	24.5 b	24.3 b	24.6 c
	7	28.0 a	33.2 a	31.0 a	31.1 b
2013	23	12.9 a	27.4 a	27.4 a	26.4 a
	15	11.2 a	26.6 a	26.9 a	25.7 a
	7	12.0 a	26.2 a	26.6 a	25.7 a
2015	23	20.0 a	21.3 a	22.6 a	21.9 a
	15	17.9 ab	18.9 a	19.6 a	21.2 a
	7	16.0 b	17.8 a	20.6 a	21.4 a
Mean from the years	23	21.8 a	28.7 a	28.7 a	28.7 a
	15	15.6 b	23.3 a	23.6 a	23.8 a
	7	18.7 a	25.7 a	26.1 a	26.1 a

* Values marked with different letters differ significantly in respect of data from one developmental stage in each year and the mean from the years

Yield of achenes and silymarin content

Yields of achenes of milk thistle were similar in 2012 and 2013, and considerably lower in 2015 (tab. 5). However, only in 2015 did seed material with TSW 26–30 g give significantly higher yields than were obtained from the seed material with TSW 20–22 g.

The yield of achenes was separated just as the seed material had been before, and it was found that if the achenes had a weight equal to or less than 22 g, the content of silymarin did not achieve the level 3% and was on average 1% lower than in the achenes with higher TSW (tab. 6).

Table 4. The number of milk thistle plants per 1m^2 at successive developmental stages depending on seed material TSW

Year/TSW (g)		Developmental stage acc. to BBCH scale			
		09–12	13–15/31	16–17/33–35	18–19/37–38
2012	26–30	29.6 a**	35.9 a	33.8 a	35.0 a
	20–22	22.5 b	26.5 b	25.0 b	25.5 b
	20–30	26.3 ab	32.8 a	32.7 a	32.9 a
2013	26–30	11.8 a	28.1 a	28.4 a	26.4 a
	20–22	12.1 a	25.9 a	25.7 a	25.4 a
	20–30	12.2 a	26.1 a	26.8 a	25.9 a
2015	26–30	22.2 a	25.0 a	25.2 a	26.0 a
	20–22	13.2 c	14.0 c	16.0 c	16.9 c
	20–30	18.7 b	19.0 b	21.6 b	21.6 b
Mean from the years	26–30	21.2 a	29.7 a	29.1 a	29.1 a
	20–22	15.9 b	22.1 b	22.2 b	22.6 b
	20–30	19.1 ab	26.0 ab	27.0 a	26.8 ab

* Values marked with different letters differ significantly in respect of data from one developmental stage in each year and the mean from the years

Table 5. Yield of achenes of milk thistle (kg ha^{-1}) depending on the method for seed material preparation

	Preparation of seed material	2012	2013	2015	Mean
Storage temperature (°C)	23	1226 a	1197 a	661 a	1028 a
	15	1167 a	1174 a	748 a	1030 a
	7	1178 a	1209 a	733 a	1040 a
TSW (g)	26–30	1193 a	1208 a	799 a	1067 a
	20–22	1174 a	1196 a	636 b	1002 a
	20–30	1204 a	1178 a	706 ab	1029 a
	mean	1190	1194	714	1033

* Values marked with different letters vertically differ significantly

Table 6. Content of silymarin (% DM) in achene yield of milk thistle depending on their TSW

TSW (g)	2012	2013	2015	Mean
26–30	3.90 a	4.16 a	3.32 a	3.79 a
20–22	2.87 b	2.57 b	2.97 b	2.80 b
20–30	3.82 a	4.45 a	3.49 a	3.92 a
Mean	3.53	3.73	3.24	3.51

* Values marked with different letters vertically differ significantly

DISCUSSION

In 2012 and 2015, when the spring weather conditions were similar to the long term average in the study area, it was found that the method for seed material preparation had an effect on the number of emerged plants. An increase in the temperature of seed storage up to 23°C and the use of seed material with a TSW higher than 26 g had a favourable effect on the number of emerged plants. It is notable that the seed material was stored at 23°C, but it was later sown into soil with a temperature of 7–8°C and, in spite of this, an improvement in germination was noted.

Cooling the seed material during the storage period showed contrasting results between years. During the first year a higher plant emergence was observed, in the second year no response of milk thistle to this factor was noted and in the third one a lower number of plantlets was observed. Based on the data the authors have at their disposal it is difficult to explain such a plant response, since the soil conditions, including the soil temperature and the rainfall distribution and total, in 2012 and 2015 were similar.

In 2013 sowing was performed at the latest date, due to long-lying snow. Full emergence was only recorded in the middle of May, at which time the moisture conditions were exceptionally favourable and this probably eliminated the effect of the experimental factors on the plant density. In an earlier study [Andrzejewska and Skinder 2006] conducted in the same location it was shown that a delay of sowing by 3 weeks resulted in an increase in the number of plants by 6–10 per m^2, but at the same time it caused a decrease in achene yield by 10–15%.

Only in 2015 did the achene yields of milk thistle depend on plant density, influenced both by the TSW and weather conditions. Milk thistle growing at high density forms fewer branches and as a result, the seed yield remains on a similar level as that found at a lower density. For the conditions in question, the desirable plant density of milk thistle is within the range of 20 to 25 [Andrzejewska and Skinder 2006] and it is considerably higher than that applicable in regions situated much farther south than Poland [Foldesi and Barsi 1983, Carrubba and la Torre 2003, Omidbaigi et al. 2003]. In our experiment, according to its aim, the plant density was assessed to the moment milk thistle plants covered the soil of interrows. Plant density was not counted prior to harvest, but in the mentioned earlier study it was shown that the final density is usually lower by 10–15% than that determined after full emergence [Andrzejewska and Skinder 2006].

The content of silymarin in fruits ranged from 2.57 to 4.16%, which means that it stayed within the typical range of the cultivar 'Silma' [Andrzejewska and Sadowska 2008]. It is interesting to notice that to a high degree the content of silymarin was related to TSW. The considerably lower content of silymarin in achenes with the weight 20–22 g probably resulted from the fact that the fruits were collected before they reached maturity, and this has a substantial effect on the accumulation of flavonolignans [Martinelli et al. 2015].

From the point of view of the yield level and quality, the proposed methods for seed material preparation seem to have no economic justification, as usually an increase in yield was not obtained. Nevertheless, if farmers apply mechanical weed control, each activity aimed at fast and full soil coverage by milk thistle leaves is desirable. One such method might seem to be for instance doubling of the seeding rate, but this is not recommended due to the cost of seed material.

This study indicates the need for undertaking work addressed at improving milk thistle seed material quality, especially as the human benefit values of this species show that its prospective use can be much wider than at present [Andrzejewska et al. 2015]. The problem of seed material quality applies particularly to the regions where saline soils occur [Sedghi et al. 2010] and the regions with a cooler climate than in the Mediterranean basin. One possible solution could be the production of seed material in Italy, for instance, where the conditions of seed maturation are considerably more favourable than in countries situated further to the north. Better and more uniform emergence was obtained in Poland from Italian seed material than from native seeds (personal observation). However, although *S. marianum* is a self-pollinating plant by about 98% [Hetz et al. 1995], even a small per cent of allogamy may become a problem wherever forms of this species growing in the wild commonly occur.

CONCLUSION

In a moderate climate improvements in spring emergence of milk thistle plants can be obtained by the use of seed material stored at 23°C for 6 weeks and by using achenes with a TSW higher than 22 g. However, these treatments mostly have no effect on the yield of achenes. The quality of pharmaceutic material in turn may be considerably improved by removing achenes with a TSW lower than 22 g from the harvested yield.

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