

# The Impacts of Temperature, Soil Type and Soil Herbicides on Seed Germination and Early Establishment of Common Milkweed (*Asclepias syriaca* L.)

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## Abstract

The effects of different temperatures (20 °C, 25 °C, 30 °C, 35 °C and photoperiod 26 °C/21 °C), types of soil (sand and loam) and soil herbicides (oxyfluorfen, terbuthylazine and mesotrione) on seed germination percentage, germination rate, as well as seedling length and weight of common milkweed (*Asclepias syriaca* L.) were examined. Over a period of ten days, germinated seeds were counted daily, and seedling length and weight were measured on the final day and germination rate calculated. The results indicated that temperature was the factor that significantly affected the percentage of germinated seeds of common milkweed, seedling length and germination rate, while it had less influence on seedling weight. The results showed that the alternating day/night temperature of 26 °C/21 °C also had a significant impact as the percentage of germinated seeds was the highest at that temperature on both soil types (sand: 71.3%; loam: 61.3%). Data regarding the herbicides tested (oxyfluorfen, terbuthylazine and mesotrione) showed decreasing germination percentage and seedling length with increasing herbicide concentrations on both soil types. Their effect was weakest on seedling weight. Tested herbicides are usable in control of common milkweed at the stages of germination and early establishment.

**Keywords:** loam, mesotrione, oxyfluorfen, sand, terbuthylazine, weed

## Introduction

Common milkweed (*Asclepias syriaca* L.) originates in Central and North America, and is most widespread in southern parts of Canada (Bhowmik and Bandeen, 1976). It was introduced in Europe at the beginning of the 19<sup>th</sup> century and is now present in many South and Central European countries (Tutin *et al.*, 1972) and countries in Serbia's neighbourhood (e.g. Hungary, Romania, Slovakia, Croatia, which have also included the species on their lists of invasive weeds) (Csontos *et al.*, 2009; Hulina, 2010; Szatmari, 2012; Paukova *et al.*, 2013). Distribution and frequency of common milkweed in anthropogenic habitats in Serbia (various types of crops and ruderal habitats in urban and rural areas) have been researched by Malidza *et al.* (2006) and Vrbnicanin *et al.* (2008) and they detected its presence in 15% of the assessed 10×10 km squares on the UTM (Universal Transverse Mercator) grid.

Common milkweed is a perennial species with a large habit (2 m high), opposite and elliptic leaves, and flowers grouped in umbellate inflorescences. The fruit is a follicle covered with hairy wools. Each plant has around 25 follicles, and each follicle some 300 seeds (Gold and Shore, 1995). Common milkweed has adapted to a wide range of climatic and edaphic conditions. It grows well in moderate, humid and dry habitats, as well as in poor and sandy soils. In America it inhabits various types of soils

but mostly the very dry type of loam (Phippen, 2007). Some researchers (Yenish *et al.*, 1997; Hartzler and Buhler, 2000; Csontos *et al.*, 2009) have found that the occurrence of common milkweed depends on neighbouring crops, as well as the type of soil. Common milkweed relative frequency has been found to exceed 70% in soybean, oat and sorghum crops, while it was considerably lower in maize, wheat and alfalfa crops (36, 28 and 6%, respectively). While it made 70% and 51% along railway tracks and roadsides, it only accounted for 14% in meadows (Cramer and Burnside, 1982). In Serbia common milkweed is widespread along field edges, roadsides, embankments and railway tracks throughout Vojvodina province (North Serbia) and, in recent years it has been increasingly observed inside field crops (Malidza *et al.*, 2006; Vrbnicanin *et al.*, 2008).

Species invasions are a principal component of global change, causing large losses in biodiversity as well as economic damage (D'Antonio *et al.*, 2001). Large economic impacts are also associated with many invasive species, which can provoke agricultural losses (Andow *et al.*, 1990). Knowledge about germination would be very helpful for understanding the potential of common milkweed species for spread and invasiveness. Additionally, weeds emergence patterns dictate the extent to which herbicides and nonchemical methods must remain effective to minimize deleterious impacts on crop yield and quality (Norsworthy and Oliveira, 2007). Predicting the

start and the duration of early establishment of seedling can contribute to taking better common milkweed control decisions (Berti *et al.*, 1996) and facilitate optimal timing of control practices (Grundy, 2003). Results about effects of environmental factors on germination and early establishment can be very useful in that context. Several environmental factors, such as temperature, light, pH and soil moisture, are known to affect seed germination (Chauhan *et al.*, 2006; Nandula *et al.*, 2006). Many studies have shown these factors have variable effects on different populations of the same species (Beckstead *et al.*, 1996; Milberg and Andersson, 1998). Although effects of different factors (Zimmerman and Weis, 1983; Norsworthy and Oliveira, 2007), including temperature (Norsworthy and Oliveira, 2007), on common milkweed germination were studied, to our knowledge there is no information about the effect of environmental factors on germination of common milkweed from Balkan Peninsula. As it is well known that one of the most important factors which affect seed germination and early establishment of seedlings is temperature, the objective of this research was to determine the effects of different temperatures on common milkweed seed germination and seedlings length and weight.

According to our knowledge, there are no reports on the effect of use of the soil herbicides (oxyfluorfen, terbuthylazine and mesotrione) on this invasive weed species. This kind of investigation could be helpful for better understanding of weed suppression measures in the field, which is necessary for establishing the best strategies for weed control.

Therefore, the objectives of this research were to: (1) examine the effects of different temperatures and (2) test the effects of soil herbicides on seed germination and early establishment of common milkweed seedlings on different types of soil.

## Materials and Methods

### *Tests of temperature and soil type effects on seed germination and early establishment of common milkweed*

Common milkweed seeds were collected at Tavankut (North Serbia, W 7 382 591, E 5 098 903, n.v. 102 m) in September of 2012. The seeds were cleaned and stored in the laboratory at a temperature of 20 to 22 °C. All tests were conducted on two types of soil. The first soil, sampled from a location at Zemun Polje (Serbia), was a loamy soil (pH 7.17, organic matter 3.96%, sand 49.80%, silt 33.40%, clay 16.80%), medium calcareous, weakly alkaline and highly humic, rich in total nitrogen and well-supplied with available phosphorus and potassium. The other soil type was sampled at the location Tavankut (North Serbia), and its texture was sandy (pH 7.63, organic matter 0.91%, sand 91.44%, silt 1.32%, clay 7.24%), it was medium calcareous, medium alkaline and very weakly humic with moderate total nitrogen content and high supplies of available phosphorus and potassium.

The following temperatures were tested: 20 °C, 25 °C, 30 °C and 35 °C. Twenty seeds were placed into each Petri dish containing 60 g of soil substrate (sand or loam), and 20 ml of distilled water was added to each dish before leaving them in an incubator (Binder CE) in the dark. All the dishes were sealed with parafilm to avoid evaporation. All trial variants were performed in four replications and the trial was repeated twice. The same procedure was used for testing germination of common milkweed seeds under white light illumination in a climate room. The conditions included: 14 h/10 h photoperiod,

26 °C/21 °C temperature, 60 to 70% humidity and 300  $\mu\text{E m}^{-2}\text{s}^{-1}$  light intensity. Distilled water was regularly added to maintain soil moisture. Germinated seeds were counted daily over a period of ten days, and the length and weight of seedlings were measured on the final day. Germination rate was calculated by Maguire's (1962) formula:

$$M = n_1/t_1 + n_2/t_2 + \dots + n_x/t_x$$

where  $n_1, n_2, \dots, n_x$  is the number of germinated seeds over  $t_1, t_2, \dots, t_x$  time elapsed since the start of the experiment expressed in days.

### *Tests of soil herbicides effects on seed germination and early establishment of common milkweed*

The trial included following herbicides: oxyfluorfen (commercial product GALIGAN 240 EC, oxyfluorfen 240  $\text{g L}^{-1}$ , Adama, Israel), terbuthylazine (commercial product RADAZIN TZ-500, terbuthylazine 500  $\text{g L}^{-1}$ , Herbos, Croatia) and mesotrione (commercial product CHIEF, mesotrione 100  $\text{g L}^{-1}$ , Adama, Israel). A series of solutions of specified concentrations calculated on the surface of the plastic tub were prepared, wherein the oxyfluorfen concentration of 960  $\text{g a.i. ha}^{-1}$ , terbuthylazine 750  $\text{g a.i. ha}^{-1}$  and mesotrione 120  $\text{g a.i. ha}^{-1}$  are the recommended field rates.

For each herbicide treatment a sample of sifted soil (250 g) was measured and placed in a thin layer on a plastic tub sized 23×18 cm. From previously prepared solutions of each herbicide concentration, 3 ml were pipetted and transferred into a thin-layer chromatography sprayer which was connected to a compressor. The soil was treated uniformly over the surface, under constant pressure of 3 bars. Control samples were not treated with herbicides. After herbicide application, the soil was hand-mixed and transferred to Petri dishes. Twenty seeds and 20 ml of distilled water were added to each Petri dish, and the dishes were then placed in a climate chamber with the following conditions: photoperiod 14 h/10 h, temperature 26 °C/21 °C, humidity 60 to 70% and light intensity 300  $\mu\text{E m}^{-2}\text{s}^{-1}$ . Distilled water was regularly added to maintain soil moisture. The number of germinated seeds was counted on the 10<sup>th</sup> day and the length and weight of seedlings were measured. All trial variants were performed in four replications and the trial was repeated twice.

### *Data analysis*

Data were analysed by a two-factorial analysis of variance (ANOVA) using STATISTICA 8.0. software package. Normality distribution and homogeneity of variances were checked for all data using Kolmogorov-Smirnov and the Levene test. When F values were statistically significant ( $p < 0.05$ ) treatments were compared using the Fisher's Least Significant Difference (LSD) test.  $\text{ED}_{50}$  were calculated for each herbicide using BIOASSAY 97 software package.

## Results and Discussion

### *Effects of temperature and soil type on seed germination and early establishment of common milkweed*

The germination results obtained with seeds of common milkweed at different temperatures between 20 and 35 °C are presented in Figure 1. The lowest germination percentage (sand: 19.4%; loam: 16.3%) was recorded at the lowest temperature of 20 °C. At 25 °C and 35 °C, the percentage of germination ranged

from 22.5% to 39.4% at both soil types, but it was higher on sandy than on loamy soil at both temperatures. The highest germination of 64.4% was found at temperature of 30 °C in sandy soil, and 51.9% in loamy soil. However, our data showed that photoperiod temperatures of 26 °C/21 °C are the optimal for germination of common milkweed seeds. The percentage of germination under such conditions, on both soil types, was highest, precisely 71.3% on sand and 61.3% on loam. Statistical analysis showed that temperature ( $F = 81.92, p = 0.000000$ ) and type of soil ( $F = 18.50, p = 0.000054$ ) had highly significant influence on the germination of common milkweed seeds.

In a similar way, Farmer *et al.* (1986) tested germination of several populations of common milkweed on different substrates [filter paper, sand, clay, peat, peat-clay (1 : 1), peat-sand (1 : 1)] and temperatures (20 to 40 °C) and concluded that temperature regime was most important to seed germination success, though substrate type did influence germination percentage to some degree. The greatest germination percentage (59% average over all substrates) was obtained with an alternating 20 °C (16 h), 30 °C (8 h) temperature regime. At a constant temperature of 30 °C, germination was lower (32% average over all substrates). Furthermore, these authors, found a greatest percentage of germination on clay and peat-clay, followed by filter paper, sand, peat and peat-sand. Conversely, in our studies, the highest percentage of germination at all temperatures was recorded in sandy soil which could be explained with greater water availability and/or a better seed-soil contact.

Baskin and Baskin (1977) reported that after nine weeks of stratification, regardless of whether seeds were in light or darkness during and/or after stratification, seeds germinated to 94% or more at 35/20 °C and 30/15 °C, to 70% or more at 20/10 °C and to only 0.7 % at 15/6 °C. Evetts and Burnside (1972) concluded that maximum germinating temperatures were 35 °C or 35-40 °C, while Bhowmik (1978) revealed in his experiment that maximum seedling emergence occurred at 27 °C. This relatively high temperature requirement for germination is probably one reason why this species emerges later in the growing season than many other weed species.

The effects of different temperatures and soils on seedling length, seedling weight, and germination rate of common milkweed seeds in our experiment are presented in Table 1. There is no available data to our knowledge about the effect of temperatures and soil type on early establishment of common milkweed, but our research confirmed that those factors have a significant ( $p < 0.05$ ) effect on the seedlings length (temperature:  $F = 17.20, p = 0.000000$ ; soil type:  $F = 16.56, p = 0.000122$ ). Generally, for seedling weight, differences between soil type were

present, but were not significant ( $p > 0.05$ ) while the temperature was significant ( $F = 2.60, p = 0.043304$ ) parameter. The longest seedlings (7.85 cm) were found on sandy soil at 30 °C temperature, while the average seedling length on loam under the same temperature was 4.70 cm. Seedling length at 35 °C and day/night temperatures of 26 °C/21 °C were almost the same while seedlings were shorter at lower temperatures (25 °C and 20 °C). Similarly, seedlings had the greatest weight at 30 °C on sandy soil (0.07 g), and slightly lower on loam (0.06 g), while their weights were almost the same on both soils at the other temperatures.

Germination rates, which represents the sum of germinations per day, depended significantly on temperature ( $F = 50.22, p = 0.000000$ ) and soil type ( $F = 16.76, p = 0.000112$ ). The highest germination rates were observed under day/night temperatures of 26 °C/21 °C (sand: 14.25; loam: 11.30), and 30 °C (sand: 12.86; loam: 10.91), while that parameter had lower values at all other temperatures (20 °C, 25 °C and 35 °C).

*Effects of soil herbicides on seed germination and early establishment of common milkweed*

Effects of different herbicides and soil type on seedling length, seedling weight and germination percentage of common milkweed seeds are presented in Table 2. A higher percentage of germination was overall determined in the sandy soil. In this soil type, oxyfluorfen was proved to be the most effective, thus, at recommended field rate concentration (960 g ha<sup>-1</sup>) inhibition of germination (comparing with control) was the highest (36.1%). The treatment with mesotrione at recommended field rate (120 g ha<sup>-1</sup>) caused the reduction of germination of 22.2%, while the lowest germination inhibition (20.8%) was within the treatment

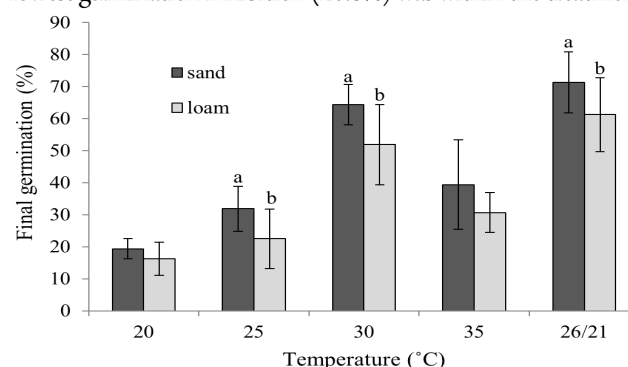


Fig. 1. Final percentage of seed germination (%) of common milkweed at different temperatures and soil type ( $P < 0.05$ , LSD test – a, b – differences between treatments and soil)

Table 1. Effects of different temperatures and soil type on seedling length (cm), seedling weight (g) and germination rate (no. day<sup>-1</sup>) of common milkweed seeds

Treatment	Soil	Seedling length (cm)	Seedling weight (g)	Germination rate (no. day <sup>-1</sup> )
20 °C	sand	2.20±0.83	0.06±0.01	4.15±0.52
	loam	1.96±0.77	0.04±0.01	3.65±0.76
25 °C	sand	5.70±2.69	0.05±0.01	7.56±1.70 a
	loam	4.63±1.23	0.04±0.01	5.05±1.66 b
30 °C	sand	7.85±1.18 a	0.07±0.01	12.86±1.68
	loam	4.70±1.10 b	0.06±0.02	10.91±2.79
35 °C	sand	6.72±1.23 a	0.06±0.01	9.23±3.36
	loam	4.25±0.58 b	0.05±0.02	7.50±2.16
26 °Cday/21 °Cnight	sand	6.15±1.87	0.04±0.01	14.25±2.19 a
	loam	5.23±0.70	0.04±0.01	11.30±2.55 b

P < 0.05 (LSD) test – a, b – differences between treatments and soil

with terbutylazine at its recommended field rate (750 g ha<sup>-1</sup>). Similar was in the loam soil, as the highest inhibition of germination was as well obtained in soil treated with oxyfluorfen (32.2%). However, in this soil type, treatment with terbutylazine was the next most effective one, leading to germination reduction up to 25.4%, while significantly lower inhibition of germination was recorded in the treatment with mesotrione (3.4%).

The length of common milkweed seedlings in both soils decreased as the herbicide concentrations increased (Table 2). In the sandy soil, mesotrione was the most effective concerning the reduction of the seedling length, as in the treatment with recommended field rate of this herbicide, inhibition of this parameter was 61%. Lower inhibitions of the seedling lengths were obtained in the treatment with oxyfluorfen and terbutylazine (52.2 and 49.9% respectively). On the other hand, in loamy soil the lowest inhibition of seedling length was recorded in the treatment with mesotrione (54%), while oxyfluorfen and terbutylazine were proved to be more effective in the reduction of this parameter, with seedling length inhibition of 57.8 and 56.3% respectively.

The calculated ED<sub>50</sub> values showed that the lower application rates of oxyfluorfen caused lower seedling length on loam (ED<sub>50</sub> = 1,000.33) than on sand (ED<sub>50</sub> = 1,061.40) (Fig. 2). Conversely, lower concentrations of terbutylazine caused lower seedling length on sand (ED<sub>50</sub> = 737.63), while on loam the calculated ED<sub>50</sub> value was 797.82. Also, in mesotrione treatments, the lower application rates caused lower seedling length on sand (ED<sub>50</sub> = 108.22) than on loam (ED<sub>50</sub> = 138.46).

Generally, tested herbicides were the least effective in the reduction of the seedling weight, as the weight of seedlings in all trial variants ranged from 0.02 to 0.04 g (Table 2).

Overall, based on the obtained results, it could be concluded that the effectiveness of all three tested herbicides on seed germination and early establishment of common milkweed in the loamy soil were nearly the same. However, in the sandy soil mesotrione was proved to be more effective than other two herbicides. This was probably due to physico-chemical properties of mesotrione (i.e. its high water solubility of 15 g L<sup>-1</sup>) coupled with physico-chemical properties of sandy soil (low organic

matter content 0.91% and high sand content 91.44%), which enable this herbicide to be highly available for the seedlings of common milkweed.

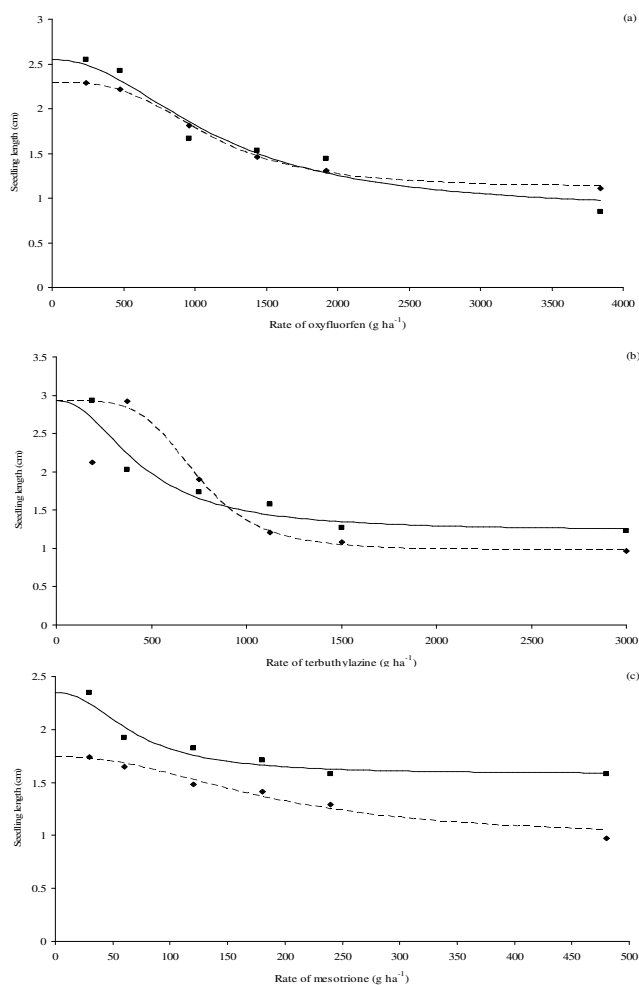


Fig.2. Responses of common milkweed to increasing rates of oxyfluorfen (a), terbutylazine (b) and mesotrione (c) based on seedling length in loam (-) and sand (---)

Table 2. Effects of different herbicides and soil type on seedling length (cm), seedling weight (g) and germination (%) of common milkweed seeds

	Concentration	Seedling length (cm)		Seedling weight (g)		Germination (%)	
	g a.s. ha <sup>-1</sup>	loam	sand	loam	sand	loam	sand
oxyfluorfen	control	3.96±0.55	3.79±0.17	0.04±0.01	0.05±0.01	38.9	45.0
	240	2.55±0.69	2.29±0.68	0.03±0.01	0.03±0.01	34.4	37.5
	480	2.42±0.25	2.22±0.39	0.04±0.01	0.03±0.01	36.9	38.8
	960	1.67±0.90	1.81±0.73	0.03±0.01	0.03±0.01	25.0	28.8
	1440	1.53±0.49	1.46±0.40	0.03±0.01	0.03±0.01	23.8	25.6
	1920	1.44±0.32	1.31±0.32	0.03±0.01	0.02±0.01	18.8	21.9
	3840	0.85±0.52	1.11±0.60	0.02±0.01	0.02±0.01	16.3	18.8
terbutylazine	control	3.96±0.55	3.79±0.17	0.04±0.01	0.05±0.01	38.9	45.0
	187.5	2.93±0.46	2.12±0.43	0.04±0.01	0.03±0.01	33.1	42.5
	375	2.03±0.16	2.92±0.32	0.03±0.01	0.02±0.01	29.4	40.6
	750	1.73±0.34	1.90±0.22	0.03±0.01	0.02±0.01	27.5	35.6
	1125	1.58±0.20	1.21±0.09	0.03±0.01	0.02±0.01	27.5	39.3
	1500	1.27±0.18	1.08±0.26	0.03±0.01	0.02±0.01	25.6	27.5
	3000	1.23±0.22	0.97±0.10	0.02±0.01	0.02±0.01	23.1	20.6
mesotrione	control	3.96±0.55	3.79±0.17	0.04±0.01	0.05±0.01	38.9	45.0
	30	2.35±0.40	1.74±0.54	0.04±0.01	0.03±0.01	39.4	40.6
	60	1.92±0.10	1.65±0.38	0.03±0.01	0.02±0.01	38.1	36.9
	120	1.82±0.39	1.48±0.03	0.03±0.01	0.02±0.01	35.3	35.0
	180	1.71±0.15	1.41±0.59	0.03±0.01	0.03±0.01	34.4	32.5
	240	1.58±0.25	1.29±0.50	0.03±0.01	0.04±0.02	30.6	29.4
	480	1.58±0.49	0.97±0.47	0.04±0.01	0.02±0.01	27.5	26.3

There is a lack of literature data comparable with results obtained in this study, due to different methods used for investigation of herbicide efficiency on seed germination and early establishment of common milkweed. This research was conducted in laboratory conditions, however the results may serve as a guideline to help growers to select the most efficacious herbicide for limiting establishment of this invasive species, especially considering that those three tested soil herbicides are already in intensive usage in plant protection in our country.

### Conclusions

The results of our studies indicate that common milkweed has the ability to germinate under a broad range of temperatures. Obtained data showed that photoperiod temperatures of 26 °C/21 °C are the optimal for germination of common milkweed seeds. Effects of temperature are more expressed on seedlings length and seed germination than on seedlings weight. Also, the results showed that the herbicides oxyfluorfen, terbuthylazin and mesotrione are usable in control of common milkweed at the stages of germination and early establishment.

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