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Population, Temperature, and Substrate Influences on Common Milkweed (*Asclepias syriaca*) Seed Germination[1]

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Abstract. **Seed from 21 common milkweed (*Asclepias syriaca* L. #[3] ASCSY) populations from six states were germinated on six substrates (filter paper, sand, peat, clay, and mixtures) under three temperature regimes. The variation in germination response between populations and experimental conditions was analyzed. The greatest percent germination (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) and much more variable among seeds from different populations. At 25 C, average percent germination dropped to 1.2% over all substrates. Germination appeared to be strongly correlated with seed source (population), temperature, and substrate type, and poorly correlated with seed size. No association was found between geographical origin and germination response.**

Additional index words. **Seed size, stratification, ASCSY.**

INTRODUCTION

Common milkweed has been of interest to agriculturalists for many years because of its potential economic value as a new crop (1, 4, 7)[4] and its negative value as a weed (9, 12). Both as a weed and a potential crop, the germination characteristics of common milkweed are of fundamental concern.

Seed germination is influenced by a number of factors which include temperature, moisture, light, dormancy requirements, substrates, seed size, and parental effects. For the germination of common milkweed seed, temperature, moisture, light, and dormancy requirements have been well documented (3, 5, 6, 9, 12, 14, 17)[5,6]. From these works and our own preliminary tests, the most effective treatment of common milkweed seed appears to involve moist storage at 5 C for a minimum of 1 to 2 weeks and germination in the dark in an alternating 20 to 30 C temperature regime.

Variation in germination caused by substrate type, differential seed size, and population of origin has been examined in other species (8, 10, 11, 13, 18, 19)[7], but has not been reported for common milkweed.

The purpose of this study was to compare the germination response of common milkweed seed from geographically diverse populations under different environmental conditions. In particular, we were interested in the interaction between seed source, temperature, and substrate.

MATERIALS AND METHODS

To test the variability of seed germination among 21 common milkweed populations (Table 1) in relation to various substrates and temperature conditions, seed, deflossed by hand, from 22 to 95 pods (a total of 8 to 30 randomly selected stalks) per population were counted and weighed. Ten samples (representing 10 pods) with mature seed were pooled and from this pooled sample 750 seed were used to represent each population. We removed potential within population error due to poor seed fill by using only large well-filled seed. Various combinations of substrates (peat, sand, filter paper, and clay) were selected to give a range in texture and water retention. Since our preliminary germination tests, as those of other authors, had shown essentially no germination in dry storage, all seed were stratified in distilled water at 5 C for 1 week.

Each 750-seed sample was divided into five subsamples of 150 seed each, which provided six sets of 25 seed for testing on six sterilized experimental substrates: filter paper, loosely packed sand, sand-peat (1:1, v/v), peat, peat-clay (1:1, v/v), and clay. Sufficient substrate was used to cover the bottoms of the petri dishes. Seed were placed on top of the substrates.

One set of six 10-cm petri dishes (a total of 150 seed) per population was placed in a growth chamber at a constant 25 C, two replicates (300 seed) were placed in a growth chamber at a constant 30 C, and two replicates (300 seed) were placed in an alternating temperature regime of 20 C (16 h) and 30 C (8 h). The moisture content of the substrate in each petri dish was maintained at saturation level for the duration of the experiment and all dishes were kept in the dark. Germination (emergence of the radicle), was recorded every 3 days for 15 days. The germinated seed were removed.

Data were analyzed with a SPSS statistical package (16) A factorial analysis of variance and a Scheffe's multiple comparison procedure (21) were performed on arcsin transformed germination percentages.

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[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

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RESULTS AND DISCUSSION

Statistical tests were performed on the effect of temperature, substrate type, and origin of population on germination. Because the simultaneous analysis of all populations exceeded the capacity of our analysis of variance program (16), the data were divided into two groups. One group included populations from Ohio, Pennsylvania, West Virginia, and Kentucky. The second group included populations from New York and Virginia. Since both groups exhibited a similar pattern and the first group included populations which were geographically more diverse, an analysis of variance for the first group is shown in Table 2.

Population, temperature, and substrate main effects were shown to be significant (Table 2). The interaction variance for the population by temperature terms was significant, indicating that all populations did not respond to the temperature regimes in the same manner. The interaction variances for population by substrate, substrate by temperature, or population by temperature by substrate were not significant. These analyses suggest that differential germination response is influenced primarily by germination temperature and origin of the population.

In a general study of seven annual weedy species in northern and central California, Jain (13) also demonstrated a large amount of variation in germinability between populations for each species examined. For example, with 16 populations of brome grass (*Bromus mollis* L. # BROMO), mean germination percentages ranged from 0 to 100%. Curly dock (*Rumex crispus* L. # RUMCR) and broadleaf dock (*R. obtusifolius* L. # RUMOB) collected from different habitats exhibited significant differences in germination response (8). Dorne (11) found that the germination response of perennial goosefoot (*Chenopodium bonus-henricus* L. # CHEBH) was negatively correlated with populations from increasing altitude, in which seed collected at 600 m showed 53% germination while seed collected at 2100 m showed germination of only 18% under experimental conditions.

The summarized results for the temperature and substrate effects for all populations for two temperature regimes are shown in Table 3. Data for germination at 25 C were not included in the table due to very low percent germination (1.2% over all substrates). The alternating 20 to 30 C regime yielded the greatest germination percentages (59.3% averaged over all substrates). The constant 30 C regime promoted greater differences among populations, as evidenced by the consistently greater coefficient of variation.

Work by other authors also suggests differential germination response to varied substrate type. Rorison (18) tested seven species on different soil types and found that although all species would germinate on a given soil type, significant differences were observed in germination response. In another study, Hermann and Chilcote[7] found a similar response in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco]. In our study, the 20 to 30 C and the constant 30 C temperature regimes, clay and peat-clay produced the greatest percent germination, followed by filter paper, sand, peat, and peat-sand.

Scheffe's test of similar response (21) was performed on all populations averaged over all substrates for the two temperature regimes (Table 4). The 20 to 30 C regime divided the 21 populations into five groups while the 30 C regime divided the 21 populations into six groups. Pairs of populations geographically close to each other occasionally occurred in the same group. At 20 to 30 C, the pair 66 and 67 were grouped together, but at 30 C, they fell into separate groups. Populations 10, 16, 17, 52, and 55 were all located in Giles County, Virginia, but had both

Table 1. Mean seed weights of 21 common milkweed populations.

Population code	Location (county and state)	Mean seed weight
		(mg)
ASH	Delaware, OH	5.5
BR	Jefferson, PA	5.8
CC	Clay, WV	5.4
CIN	Warren, OH	5.4
CL	Harrison, WV	4.9
FR	Franklin, KY	4.6
GR	Westmoreland, PA	5.4
JO	Elk, PA	4.3
LO	Medina, OH	6.2
MO	Rowan, KY	4.0
NF	Niagra, NY	5.4
10	Giles, VA	5.4
16	Giles, VA	6.3
17	Giles, VA	7.4
18	Pulaski, VA	6.0
52	Giles, VA	5.3
55	Giles, VA	5.8
56	Wythe, VA	6.2
66	Carroll, VA	5.8
67	Carroll, VA	4.1
460	Giles, VA	6.3
Overall mean		5.5
Standard deviation		0.8

Table 2. Partitioned variances from a factorial analysis of variance performed on all substrates and temperatures for 10 common milkweed populations after 15 days germination.

Source of variation[a]	Df	Variance[b]	F-ratio
P	9	0.011**	21.2
T	2	0.107**	639.8
S	5	0.001*	3.8
P × T	18	0.005**	4.2
P × S	45	0.001 NS	1.3
T × S	10	0.001 NS	1.7
P × T × S	90	0.000 NS	0.7

[a]P, T, and S refer to population, temperature, and substrate main effects, respectively.

[b]* and ** indicate the 0.05 and 0.01 level of significance, respectively; NS indicates a nonsignificant value.

Table 3. Common milkweed percent seed germination by substrate and temperature treatment averaged for all populations after a 15-day germination period[a].

Temperature		Filter paper	Sand	Clay	Peat	Peat-clay	Peat-sand	All substrates
		Substrates						
(C)		(%)						
20–30	$\bar{x}$	60.4	59.9	66.3	51.9	67.9	49.1	59.3
	sd	20.2	23.3	22.7	19.3	18.9	19.6	7.5
	cv	33.0	38.0	34.0	37.0	28.0	40.0	
30	$\bar{x}$	33.3	29.5	34.1	29.3	39.4	27.2	32.1
	sd	20.5	17.0	22.0	15.8	21.2	17.9	4.4
	cv	62.0	59.0	65.0	54.0	54.0	66.0	

[a]Mean ($\bar{x}$), standard deviation (sd), and coefficient of variation (cv) calculated on untransformed data.

Table 4. Mean germination response for seed of 21 common milkweed populations averaged regardless of substrate at two specific temperatures[a].

20–30 C		30 C	
Population	Germination	Population	Germination
	(%)		(%)
52	23.9 ab	CC	7.3 a
JO	35.5 ab	MO	8.0 ab
MO	36.2 ab	52	9.9 ab
FR	36.6 ab	FR	10.3 ab
CC	42.3 abc	JO	13.1 a-d
56	44.2 abc	LO	19.2 a-d
460	45.9 abc	460	21.1 a-d
CL	51.8 abc	16	23.7 a-e
LO	51.9 abc	CL	25.0 a-e
ASH	60.0 a-d	BR	25.7 a-e
BR	62.0 bcd	ASH	30.8 a-e
18	64.3 b-e	GR	31.6 a-e
16	65.0 b-e	66	33.0 b-e
GR	69.4 b-e	18	39.0 cde
NF	72.4 b-e	56	40.1 cde
10	75.0 b-e	NF	43.3 def
66	79.0 cde	CIN	43.5 def
67	80.2 cde	55	49.0 def
55	88.0 de	10	51.4 ef
CIN	93.3 e	67	53.2 f
17	93.8 e	17	74.9 f

[a]Populations grouped according to similarity of response by method of Scheffe's at 0.5 level of significance.

the highest and the lowest germination percent and were not grouped together at 20 to 30 C. Populations from Ohio paired with populations from Virginia (LO, ASH, 460; CIN, 17). When considering all populations, no consistent geographical associations or north-south clines were found.

Crouch and Vander Kloet (10) found significant correlation between seed weight and latitude and consequently with germination response within a section of the blueberries (*Vaccinium* sp.). In perennial goosefoot, Dorne (11) noted no correlation between mean seed weight and altitude. Seed weight in common milkweed is known to vary between populations, with average seed weights ranging from 4.2 mg to 7.3 mg per seed (6, 23). In our collections (Table 1), average seed weight per population varied from 4.0 mg to 7.4 mg. The correlation coefficient between seed weight and germination temperature was not significant. At 30 C, the populations MO and FR represent both light seeds and poor germination, while population 17 had the highest germination and the greatest seed weight. However, population 67 had the lowest seed weight and relatively high germination while population 460 showed one of the highest average seed weights and relatively low germination. At 20 to 30 C, seed weighing an average of 5.4 mg and those weighing 7.4 mg both showed 93% germination. Though seed weights below 5 mg generally gave lower germination, at 20 to 30 C, the lowest germination rate encountered (23.9%), was from a population (52) with an average seed weight of 5.3 mg.

Alternative hypotheses to explain seed germination response include the effects of temperature on the developing seed (2), effects of photoperiod, temperature, and level of solar radiation on the parent plants (15), inbreeding depression (22)[8], or internal genetic control (20). Our experiment was not designed to test for these influences.

Results of our experiments show that temperature regime and origin of the population are most important to seed germination success in common milkweed, though substrate type does influence germination rates to some degree.

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