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Germination of *Cleome hassleriana* and *Polanisia dodecandra* Seed Lots in Response to Light, Temperature and Stratification

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Abstract: *Cleome* and *Polanisia* germination is non-uniform, requiring plug growers to continuously manipulate germination conditions. Germination and yield potentials remain significantly lower than bedding plant standards. However, cleomes have been reported to germinate prolifically in gardens, escape cultivation and naturalize. The overarching objective was to compare germination of *Cleome* and *Polanisia* seed lots in response to planting depth, stratification, temperature and photoperiod regimes that simulate field and greenhouse conditions. Three experiments were conducted. First, field germination of *P. dodecandra* fresh and one-year-old seed (0, 1 cm below soil surface) was evaluated (Expt. 1). Second, the effect of stratification (0, 3, 6, 9, 12 weeks at 4°C), photoperiod (0/24, 12/12 and 14/10 h light/dark) and temperature (21/17°C and 19/15°C day/night) regimes on *P. dodecandra* germination were studied (Expt. 2). Third, the effect of stratification (0, 6 weeks), photoperiod (0/24, 12/12, 14/10 h light/dark) and temperature (21/17°C, 19/15°C) on germination of 11 seed lots of *Polanisia* and *Cleome* were compared (Expt. 3). In Expt. 1, buried seeds had significantly greater germination (66%) than non-buried (5%). In Expt. 2, higher germination was observed in stratified seeds (16%) incubated in complete darkness (35%) and at warmer temperature regimes (21%). Expt. 3 confirmed these findings with greater germination in darkness (32%) and warmer temperature (41%), but the effect of stratification varied among seed lots. Significant three-way interactions among seed lot×age×depth (Expt. 1), duration of stratification×temperature×photoperiod (Expt. 2) and seed lot (Expt. 3) confirms lack of uniformity in commercial production and may contribute to spread in non-cultivated environments.

Key words: Environmental cues, invasive species, photoperiod, seed age, seed lots

INTRODUCTION

Since, commercial production of bedding plants is highly mechanized, high and uniform seed germination and yield potential are desirable traits of seed-propagated floriculture crops (Anderson, 2004). Flower breeders continuously select for improved seedling performance to produce plug trays with 100% fill at Stage IV of development (shipping/transplant)

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(Healy, 1999; Styer and Koranski, 1999). While highly domesticated seed-propagated crops (e.g., *Impatiens walleriana* Hook) are selected for ever-increasing germination (98%+) and yield potential (95%+) rates (www.panamseed.com), many ornamental crops still exist with low or uneven seedling performance. Crop examples in this category include *Cyclamen persicum* Mill. *Hypoestes phyllostachia* Bak. and *Cleome hassleriana* Chodat. Despite this, at least one of these species (*Cleome hassleriana*) is an aggressive plant (i.e., self-seeds readily) in the home garden, frequently dominating the seed bank for multiple years (Cutler, 1999) and becoming naturalized in non-cultivated habitats (Steffey, 1984; Gleason and Cronquist, 1991).

Cleomes (*Cleome hassleriana* and *Polanisia dodecandra*, among others) are commonly grown as annual bedding plants (Dole and Willkins, 2005; Steffey, 1984). They are primarily seed-propagated (Ekpong, 2009). *Cleome hassleriana* is native to S. America. Several Open-Pollinated (OP) cultivars are widely distributed across the US with Queen White and Queen Rose among the oldest and most common (Bailey, 1927). Sparkler is the first F_1 hybrid series on the market (White, 2001). Both Queen and Sparkler are selections from *C. hassleriana* populations with the Sparklers being dwarf, compact (White, 2001) mutated selections from Queen (Perkins, pers. comm., 2005). Sparkler has been noted to reseed less than Queen (Anderson *et al*., 2003). *Polanisia dodecandra* Solo (sometimes sold under the mistaken identity of *C. serrulata* Pursh or *C. marshalli*), derives from the species which is native to the western US (Steffey, 1984). Although, Solo retains many non-ornamental qualities (e.g., fetid aroma and small flowers), its marketability as a native N. American spineless species has contributed to its popularity. This species has been observed as a weed in blueberry fields (Becker, MN; Gomez 2004, personal obser.) and has been listed as endangered in Maryland and of special concern in Connecticut (USDA, NRCS, 2009).

Commercial plug production of cleomes is fraught with low, non-uniform seed germination and yield potentials (Clothier, 2001; Lee, 2000; Nau, 1999) and complicates seed lot assessments (Geneve, 1998). Germination rates vary significantly over *C. hassleriana* seed lots and years, ranging from 30-50% (lowest reported levels) to 75-85% (Nau, 1999). Seed germination recommendations also address the possibility of low and variable germination. Nau (1999) stated that *C. hassleriana* seeds should be covered lightly with vermiculite and maintained at 26/21°C (day/night), but also asserts that high germination of cleome can be obtained without covering as long as high temperatures are maintained (21-22°C). The highest percentage of germination is expected to occur in 8-12 days (Nau, 1999). Stratification (4-7°C for <2 weeks) and alternating temperatures are suggested as a mean to overcome low germination (Nau, 1999). Information regarding *P. dodecandra* germination requirements are also conflicting. Clothier (2001) recommends sowing in darkness at 21°C, with radicles emerging in 2-10 days, whereas B and T World Seeds (2009) recommends sowing uncovered (light) at 22°C with germination in 10-14 days. Interestingly, outdoor direct sowing of cleomes results in very high germination.

Comparative studies of seed germination requirements for *Cleome* Sparkler and Queen series and *Polanisia* Solo have not been reported. The overarching objective of this study was to compare germination response of *Cleome* and *Polanisia* to seed age, planting depth, stratification, temperature and photoperiod regimes that simulate field (fall planting) and greenhouse (commercial) conditions. The objective of Expt. No. 1 was to determine germination under two different planting depths (0, 1 cm) for fresh and 1-year-old seeds of *P. dodecandra*; Expt. No. 2 was to determine the effect of duration of stratification on germination of *P. dodecandra.* In Expt. 3 the effects of stratification, temperature and photoperiod regimes on germination of several *Cleome* and *Polanisia* seed lots were determined.

MATERIALS AND METHODS

Germplasm. Seed lots from *C. hassleriana* (Queen White = QW, Sparkler Rose = SR and Sparkler White = SW) and *P. dodecandra* (Solo = S) were used in the experiments. Different seed lots, year of harvest, seed sources, stages of production and storage conditions were represented (Table 1). Seeds for commercial distribution in 2002 were QW, SRP and S; non-commercial seed lots included SRR (non-primed SR) and several SW trial seed lots (SW2, SW3 and SW4) rejected from production due to low germination (Table 1). The non-primed SRR seeds are sparkler seeds that have not been treated with a unique GoldSmart™ priming process designed to accelerate germination and increase plant vigor (www.goldsmithseeds.com). Other non-commercial seed lots used in this study were Open-Pollinated (OP) seeds of sparkler mix and solo plants collected from garden (SMOP) or greenhouse-grown plants (SOP), respectively (Table 1). Seed collected from garden and greenhouse-grown plants were kept in paper bags at 20°C and 14% RH. All the other seed lots were stored in a seed vault under cool, dry conditions (10°C, 20% RH; Extech instruments 45320 Hygrothermometer) when the seeds were received or harvested until use in the experiments (2004-2005). Information regarding seed storage or treatment prior to storage date is proprietary information and unavailable.

Expt. 1. Field Germination

Four seed lots were studied in this experiment (FS, FSOP, S, SOP; Table 1). Fresh seeds (FS, FSOP) were harvested from the prairie (grown without competition) on 17 Oct. 2004 and kept in the lab (20°C, 14% RH) until the start of the experiment on 4 Nov. 2004. Seed lots S and SOP were kept in the seed vault (10°C, 20% RH). Twelve groups from each of FS, FSOP, S and SOP (n = 50 seeds ea.) were planted in 8.9 cm L x 8.9 cm W x 11.4 cm D containers (Jumbo Jr. pots, Belden Plastics, Roseville, MN) half-filled with freshly pasteurized field soil (Waukegin silt loam) collected from the St. Paul, MN Campus agricultural research fields. For each of the four seed lots, six groups (reps) of n = 50 seeds were planted at the soil surface, while the other six groups were planted 1 cm below soil surface. Pots were covered in wire mesh and bridal veil and secured with duct tape (3M Corp., St. Paul, MN) to exclude small mammals and birds.

All 48 pots were randomly distributed in a 1.83×2.44 m plot in the experimental field plots at the University of Minnesota, St. Paul Campus (44°5918N, 93°1035W, St. Paul, MN). Pots were partially buried such that the soil within the pot was level with the exterior soil; the edges of the pot projected above ground level. Seeds remained outside through winter and

Table 1: *Cleome hassleriana* and *Polanisia dodecandra* seed lots used for germination experiments No. 1-3

Seed lot	Harvest (year)	Source[z]	Species	Cultivar	Description[z]	Storage[y]
QW	2002	Seed Co.	*C. hassleriana*	Queen white	Open-pollinated (OP) seeds from prairie plants	Vault
SRP	2002	Seed Co.	*C. hassleriana*	Sparkler rose	Primed	Vault
SRR	2002	Seed Co.	*C. hassleriana*	Sparkler rose	Non-primed; seeds of same origin as SRP	Vault
SMOP	2003	Garden	*C. hassleriana*	Sparkler mix	OP seeds of Sparkler Mix grown in garden	Vault
SW2	2003	Seed Co.	*C. hassleriana*	Sparkler white	Seeds not in production due to low % germ	Vault
SW3	2003	Seed Co.	*C. hassleriana*	Sparkler white	Seeds not in production due to low % germ	Vault
S	2002	Seed Co.	*P. dodecandra*[x]	Solo	Cultivar seed lot	Vault
SOP	2003	Greenhouse	*P. dodecandra*[x]	Solo	OP seed (S plants grown in St. Paul greenhouse)	Vault
FS	2004	Prairie	*P. dodecandra*[x]	Solo	OP seed (S plants grown in prairie)	Lab
FSOP	2004	Prairie	*P. dodecandra*[x]	Solo	Open pollinated seeds from SOP plants	Lab

[z]Sources: Seed Co. = Goldsmith Seed Company (Gilroy, CA); Garden = Horticulture Display Garden (Falcon Heights, MN); Greenhouse = St. Paul Campus Horticulture Greenhouses (Falcon Heights, MN); Prairie =Minneapolis Park and Recreation Board restored prairies in Ridgeway Parkway, Theodore Wirth Parkway and Shingle Creek Park (Minneapolis, MN). [y]Seed vault = 10°C and 20% RH; Lab = 20°C, 14% RH. [x]*Polanisia dodecandra* is sold as *Cleome serrulata*

Table 2: Average precipitation (cm), max./min. temperature (°C), snowfall (cm) and snow depth on the ground (cm) in the field site at St. Paul Campus (45°N Lat., St. Paul, MN) from Oct. 2004 to May 2005 (National Weather Service station 218450 University of Miunesota St. Paul)

Months	Years	Precipitation	Max. Temp	Min. Temp	Snowfall	Snow depth
Oct	2004	9.4	15.3	5.3	0.0	0.0
Nov	2004	z	z	z	0.0	0.0
Dec	2004	0.8	-1.3	-9.1	0.0	0.0
Jan	2005	1.5	-4.8	-12.9	21.8	5.3
Feb	2005	2.1	1.7	-7.0	0.0	5.8
Mar	2005	3.2	5.8	-5.1	0.0	0.0
Apr	2005	z	z	0.0	0.0	z
May	2005	z	z	0.0	0.0	z

z: Missing data

Table 3: Randomization of photoperiod and temperature combinations to the four chambers in each assay (Expt. No. 2) for seed germination of *Cleome, Polanisia*

Growth chamber (ID#)	Assay	Photoperiod (h light/darkness)	Temperature (°C day/night)
1	1	12/12	19/7
	2	14/10	27/15
	3	14/10	19/7
2	1	12/12	27/15
	2	14/10	19/7
	3	12/12	19/7
3	1	14/10	19/7
	2	12/12	27/15
	3	14/10	27/15
4	1	14/10	27/15
	2	12/12	19/7
	3	12/12	27/15

were covered with snow during Jan. and Feb. (Table 2). Germination was recorded in 23 May 2005. Seeds were scored as germinated if the cotyledons were visible above the soil.

Expt. 2. Duration of Stratification on Solo

Fifty seeds from each seed lot (FS, FSOP) were placed in 55 mm filter paper (Whatman, England) folded to form a closed packet. Seed packets were buried in trays containing 3 L of sand (Crystalline Silica Sand Industrial Quartz, Unimin Corp, Le Sueur, MN) thoroughly saturated with ~800 mL of distilled DI water. Tray tops were covered with plastic to prevent water loss and aluminum foil to prevent light exposure during stratification. Trays were kept in a darkened walk-in cooler (4°C). Seeds were stratified for 0, 3, 6, 9 and 12 weeks, removed from the packets and placed in 60×15 mm polystyrene Petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) filled with sand saturated with ~6 mL of distilled water.

Petri dishes were sealed with Parafilm M (Pechiney Plastic Packaging, Menasha, WI, USA) to reduce water loss. Petri plates were randomly assigned to one of six treatment combinations (rep = 4 Petri plates) consisting of two temperature regimes (19/7°C and 27/15°C light/dark cycles) and three photoperiods (0/24, 12/12 and 14/10 h of light/darkness) (Table 3). Petri dishes assigned to the no-light treatment were sown in a green light room at 20°C and covered with aluminum foil. Petri plates assigned to the no-light treatment were split into two groups and each group was placed into one of the two chambers set to the treatment temperature. The experiment was repeated three times (assays). Growth chambers were reassigned a treatment combination at each assay, with 3 weeks between each assay. Seed germination, defined by the emergence of the seed radicle, was recorded after fourteen days.

Expt. 3. Stratification, Photoperiod and Temperature Effects

Seeds were stratified as described above (Expt. 2) for 6 weeks. Control seeds, also in filter paper packets buried in sand, were kept under cool, dry storage (10°C and 20% relative humidity). Seeds were germinated on 60×15 mm polystyrene Petri dishes (as described in Expt. 2). Four Petri plates per seed lot, each containing 50 seeds, were incubated at one of six treatments of a full-factorial combination of two temperature cycles (19/7°C, 27/15°C day/night temperatures) and three photoperiod regimes (0/24, 12/12, 14/10 h of light/darkness). Petri plates assigned to the no-light treatment were split into two groups and each group was placed into one of the two chambers set to the treatment temperature. Germination was recorded fourteen days after incubation. The experiment was repeated three weeks later.

Statistical Analysis

Percent germination was arcsine square root transformed. Analysis were done using the least square means model fit option in JMP IN and Tukeys HSD ($\alpha = 0.05$). For Expt. 2, a full-model (seed lot, age, depth of planting) was used after removing two outliers that were due to seed lot misidentifications. With Expt. 3 data, a statistical model was used to account for potential differences among the 2 assays done 3 weeks apart; the model contained all 3-way interactions. The 3-way interaction of temperature x light x assay was used as the error term.

RESULTS

Expt. 1. Field Germination

A significant effect of the three-way interaction of seed lot×age×depth (Fig. 1) was detected ($p = 0.03$). The magnitude of the difference between surface-planted (0 cm depth) and buried seeds was highly dependent upon seed lot (seed lot×depth, $p<0.001$), even though no significant effects ($p = 0.188$) were detected on % germination due to seed lot alone. The difference between buried and non-buried seed germination is greater in SOP (74%) than S (48%). Percent germination of seeds planted on the surface (5%) was

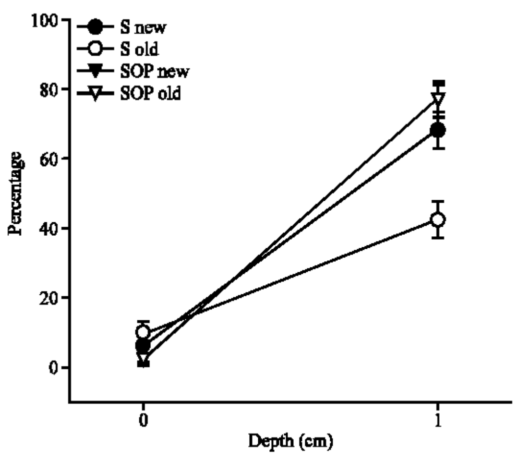

Fig. 1: Least square mean plot (mean % germination±SE) of the three way interaction of seed lot, age and depth in *Polanisia dodecandra* seed lots (S and SOP) germinated outdoors in the Spring of 2005 (Expt. No. 1)

Table 4: Least square means differences of two *Polanisia dodecandra* seed lots (FS and FSOP), varying in age (new and old) and depth of planting (surface, buried-see text) tested in Expt. No. 1 with Tukey HSD test α = 0.05 (*). Means marked with an asterisk denotes they are significantly different from the reference LSM

	FS				FSOP			
	New		Old		New		Old	
Treatments	Surface	Buried	Surface	Buried	Surface	Buried	Surface	Buried
FS, new, surface	0.0	-62.1*	-3.5	-36.2*	3.2	-70.8*	3.9	-71.1*
FS, new, buried		0.0	58.6*	25.9*	65.3*	-8.7	66.0*	-9.0
FS, old, surface			0.0	-32.8*	6.7	-67.4*	7.3	-67.6*
FS, old, buried				0.0	39.4*	-34.6*	40.1*	-34.8*
FSOP, new, surface					0.0	-74.0*	0.7	-74.3*
FSOP, new, buried						0.0	74.7*	-0.2
FSOP, old, surface							0.0	-74.9*
FSOP, old, buried								0.0

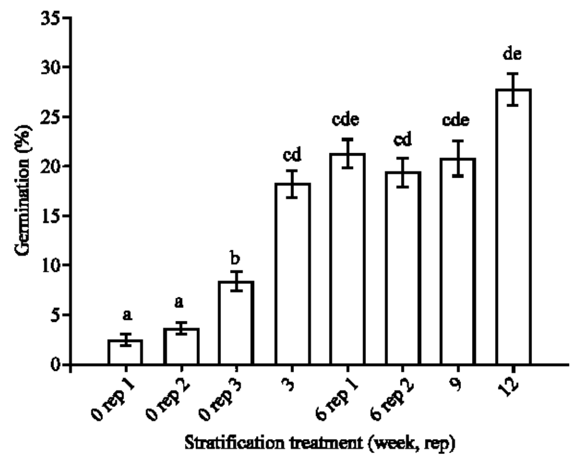

Fig. 2: Least square means plot (mean % germination±SE) in response to duration of stratification (0, 3, 6, 9 and 12 weeks) in *Polanisia dodecandra* (FS and FSOP seed lots pooled) in growth chambers (Expt. No. 2). Letters indicate significant differences tested using a Tukey HSD test α = 0.05. Stratification treatments 0 rep1, 0 rep2 and 0 rep3 were incubated with treatments 6 rep1, 6 rep2 and 12, respectively

The difference between surface and buried seed was 62% in S new, 33% in S old, 74% in SOP new and 75% in SOP old seeds (Table 4). The greatest difference in seed lot germination was observed between SOP old seeds planted on the surface and buried (75%), while the smallest significant difference (26%) was observed between S old buried and surface (Table 4).

Expt. 2. Duration of Stratification on Solo

Germination for all main effects (stratification, photoperiod, temperature) except seed lots were highly significant ($p<0.001$). Significant interactions included seed lot×photoperiod ($p = 0.020$), stratification×photoperiod ($p<0.001$), stratification×temperature ($p<0.001$), photoperiod×temperature ($p = 0.006$) and stratification×photoperiod×temperature ($p = 0.001$). All other interactions were not significant.

Percent germination of FS and FSOP did not differ significantly and therefore, are pooled in the least square means plot for stratification treatments (Fig. 2). Stratification

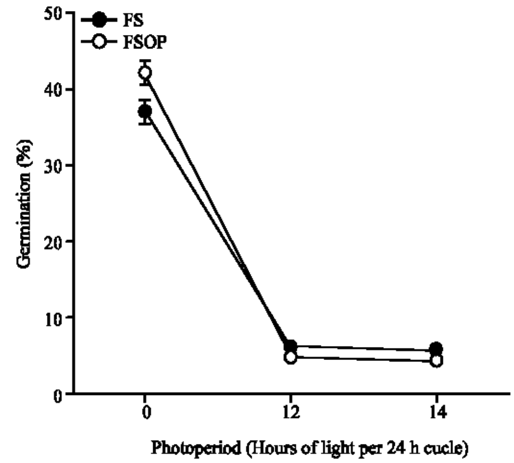

Fig. 3: Percent germination (Least square Mean±SE) of two *Polanisia dodecandra* seed lots (FS and FSOP) incubated at three photoperiod regimes (0, 12 and 14 h of light per 24-h cycle) (Expt. No. 2)

increased mean germination for non-stratified (5%) to stratified (21%; Fig. 2). Stratification for 3, 6 and 9 weeks yielded 20% germination, while 12 weeks increased germination by an additional 8% (Fig. 2). There was a 5% significant increase in % germination of non-stratified seeds incubated in week 12 (0 rep3) over the other two reps (0 rep1, 0 rep2) incubated 6 weeks earlier (Fig. 2). A similar increase (7%) in seeds stratified for 12 weeks and the two reps stratified for 6 weeks (Fig. 2) also occurred.

Complete darkness resulted in the highest germination (40%) in both seed lots, though it was slightly higher in FS than FSOP (Fig. 3). At 12 and 14 h of light per cycle, germination was reduced to 5 and 0.3%, respectively. In addition, as suggested by the significant stratification×photoperiod interaction (Table 5), stratification enhances the effects of darkness on germination. Non-stratified seeds incubated in darkness had 18% germination, whereas stratified seeds had 54% (Fig. 4). Moreover, % germination of non-stratified seeds incubated in darkness was similar to stratified seeds incubated in 12 or 14 h of light (Fig. 4). For example, there was no significant difference in % germination of non-stratified seeds incubated in complete darkness (0 weeks rep1, 0) with stratified at 12 and 14 h photoperiods (Table 5). However, significant differences were found between non-stratified seeds incubated in complete darkness (0 weeks rep2, 0) and 3 weeks stratification at 14 h of light (13%), 6 weeks stratification at 12 (12%) and 14 (11%) h and 9 weeks of stratification at 14 (11%) h of light (Table 5). The greatest difference (60%) was observed between seeds stratified for 12 weeks and incubated without light and non-stratified seeds incubated with light (Table 5). The smallest significant difference (4%) was observed between reps 1, 3 of non-stratified seeds in 14 h light (Table 5).

Percent germination was greater in seeds incubated at warmer (21%) than at cooler (8%) temperatures but the magnitude of the effect varies depending on the stratification treatment (Fig. 5). Average germination percent of stratified seeds at warmer temperature (27/15°C day/night cycles) was 30% and only 10% in non-stratified seeds and 12 weeks of stratification significantly increases germination in both cooler (24%) and warmer (32%) temperatures (Fig. 5). However, the variation in % germination of stratified seeds is greater

Table 5: Least square means differences of two *Polanisia dodecandra* seed lots (FS and FSOP; Expt. No. 2) stratified for 0, 3, 6, 9 or 12 weeks and incubated at one of three photoperiod regimes (0, 12 and 14 h of light per 24 h light/dark cycle)

	0 week rep1			0 week rep2			0 week rep3			3 weeks			6 weeks rep1			6 weeks rep2			9 weeks			12 weeks		
Treatments	0	12	14	0	12	14	0	12	14	0	12	14	0	12	14	0	12	14	0	12	14	0	12	14
0 weeks rep1, 0	0	11*	12*	-7	11*	11*	-11	9*	7	-38*	3	6	-42*	5	1	-42*	5	4	-42*	2	4	-48*	-1	-4
0 weeks rep1, 12		0	1	-17*	1	1	-21*	-2	-3	-48*	-8*	-4	-53*	-6	-10*	-53*	-6	-6	-53*	-8*	-7*	-59*	-12*	-14*
0 weeks rep1, 14			0	-18*	0	0	-23*	-3	-4*	-50*	-9*	-5*	-54*	-7*	-11*	-54*	-7*	-7*	-54*	-9*	-8*	-60*	-13*	-15*
0 weeks rep2, 0				0	18*	18*	-4	15*	14*	-31*	10	13*	-36*	12*	7	-35*	12*	11*	-35*	9	11*	-42*	5	3
0 weeks rep2, 12					0	0	-22*	-2	-4	-49*	-8*	-5*	-54*	-6*	-11*	-53*	-6*	-7*	-53*	-9*	-7*	-60*	-13*	-15*
0 weeks rep2, 14						0	-22*	-3	-4	-49*	-9*	-5*	-54*	-7*	-11*	-54*	-7*	-7*	-54*	-9*	-7*	-60*	-13*	-15*
0 weeks rep3, 0							0	20*	18*	-27*	14*	17*	-31*	16*	12	-31*	16*	15*	-31*	13*	15*	-37*	9	7
0 weeks rep3, 12								0	-1	-47*	-6	-2	-51*	-4	-8*	-51*	-4	-4	-51*	-6	-5	-57*	-10*	-12*
0 weeks rep3, 14									0	-45*	-4	-1	-50*	-3	-7	-50*	-3	-3	-50*	-5	-3	-56*	-9*	-11*
3 weeks, 0										0	41*	44*	-5	43*	39*	-4	43*	42*	-4	40*	42*	-10	36*	34*
3 weeks, 12											0	-35	-45*	2	-2	-45*	2	2	-45*	-1	1	-51*	-4	-6
3 weeks, 14												0	-49*	-2	-6	-49*	-1	-2	-48*	-4	-2	-55*	-8	-10*
6 weeks rep1, 0													0	47*	43*	0	47*	47*	0	45*	46*	-6	41*	39*
6 weeks rep1, 12														0	-4	-47*	0	0	-47*	-2	-1	-53*	-6	-8
6 weeks rep1, 14															0	-43*	4	4	-43*	2	3	-49*	-2	-4
6 weeks rep2, 0																0	47*	47*	0	45*	46*	-6	41*	39*
6 weeks rep2, 12																	0	0	-47*	-2	-1	-53*	-6	-8
6 weeks rep2, 14																		0	-47*	-2	-1	-53*	-6	-8
9 weeks, 0																			0	45*	46*	-6	41*	39*
9 weeks, 12																				0	2	-51*	-4	-6
9 weeks, 14																					0	-52*	-5	-7
12 weeks, 0																						0	47*	45*
12 weeks, 12																							0	-2
12 weeks, 14																								0

*Significant differences tested with Tukey HSD test $\alpha = 0.05$

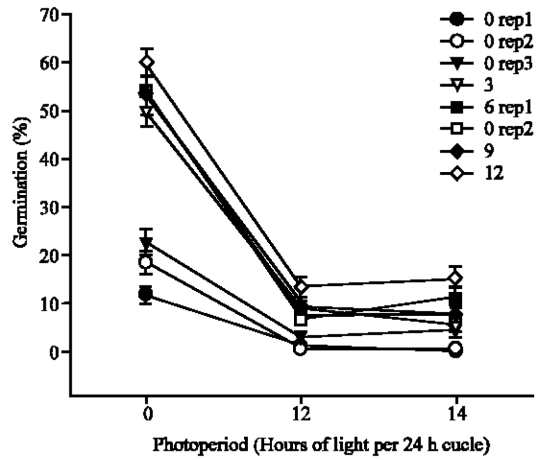

Fig. 4: Percent germination (Least square Means±SE) for *Polanisia dodecandra* seed lots in Expt. No. 2, stratified for 0, 3, 6, 9 and 12 weeks and incubated at three photoperiods (0, 12 and 14 h of light per 24-h cycle)

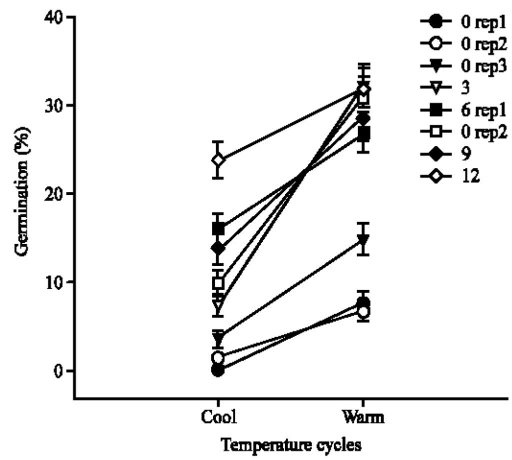

Fig. 5: Percent germination (Least square Mean±SE) for *Polanisia dodecandra* seed lots (FS and FSOP pooled together) stratified for 0, 3, 6, 9 and 12 weeks and incubated at two temperature cycles (cool = 19/7°C and warm = 27/15°C) (Expt. No. 2)

at cooler temperatures than at warmer temperatures. At cooler temperatures, germination ranged from 7 (3 weeks stratification) to 24% (12 weeks), while it was 27 to 32% in warmer temperatures (Fig. 5). In contrast, the variation in germination of non-stratified seeds is greater at warmer (7 to 15%) than cooler (0 to 4%) temperatures (Fig. 5). Moreover, 3 weeks of stratification had greater % germination (32%) than 6 weeks (rep1; 27%) in warmer temperatures, but lower % germination (7%) than the 6 weeks rep1 (16%) in cooler temperatures (Fig. 5). Percent germination of seeds stratified for 12 weeks and incubated at cool temperatures (12 weeks, cool) was not significantly different from non-stratified seeds incubated in warm temperatures (0 weeks rep3, warm) and seeds that were stratified for shorter time periods (3, 6 rep1, 6 rep2 and 9 weeks) and incubated at warmer temperature (Table 6). Moreover, when comparing seeds in cool temperatures, % germination after

Table 6: Least square means differences of two *Polanisia dodecandra* seed lots (FS + FSOP pooled together) stratified for 0, 3, 6, 9 and 12 weeks and incubated at two temperature regimes (cool=19/7°C and warm=27/15°C) (Expt. No. 3)

	0 week rep1		0 week rep2		0 week rep3		3 weeks		6 weeks rep1		6 weeks rep2		9 weeks		12 weeks	
Treatments	Cool	Warm	Cool	Warm	Cool	Warm	Cool	Warm	Cool	Warm	Cool	Warm	Cool	Warm	Cool	Warm
0 weeks rep1, cool	0.00	-7.43*	-1.23	-6.69*	-3.46*	-14.75*	-7.30*	-32.00*	-15.89*	-26.76*	-9.78*	-30.72*	-13.69*	-28.46*	-23.60*	-31.61*
0 weeks rep1, warm		0.00	6.20*	0.73	3.97	-7.32	0.13	-24.57*	-8.46*	-19.33*	-2.35	-23.29*	-6.26	-21.03*	-16.17*	-24.18*
0 weeks rep2, cool			0.00	-5.46*	-2.22	-13.51*	-6.07*	-30.76*	-14.65*	-25.52*	-8.55*	-29.48*	-12.45*	-27.22*	-22.37*	-30.37*
0 weeks rep2, warm				0.00	3.24	-8.05*	-0.61	-25.30*	-9.1 9*	-20.06*	-3.09	-24.02*	-6.99	-21.76*	-16.91*	-24.91*
0 weeks rep3, cool					0.00	-11.29*	-3.85	-28.54*	-12.43*	-23.30*	-6.33*	-27.26*	-10.23*	-25.00*	-20.14*	-28.15*
0 weeks rep3, warm						0.00	7.44	-17.25*	-1.14	-12.01*	4.96	-15.97*	1.06	-13.71*	-8.85	-16.86*
3 weeks, cool							0.00	-24.70*	-8.59*	-19.45*	-2.48	-23.41*	-6.38	-21.16*	-16.30*	-24.30*
3 weeks, warm								0.00	16.11*	5.24	22.22*	1.28	18.31*	3.54	8.40	0.39
6 weeks rep1, cool									0.00	-10.87*	6.11	-14.83*	2.20	-12.57*	-7.71	-15.72*
6 weeks rep1, warm										0.00	16.97*	-3.96	13.07*	-1.70	3.16	-4.85
6 weeks rep2, cool											0.00	-20.93*	-3.90	-18.68*	-13.82*	-21.82*
6 weeks rep2, warm												0.00	17.03*	2.26	7.12	-0.89
9 weeks, cool													0.00	-14.77*	-9.91	-17.92*
9 weeks, warm														0.00	4.86	-3.15
12 weeks, cool															0.00	-8.01
12 weeks, warm																0.00

*Significant differences tested with Tukey HSD test $\alpha = 0.05$

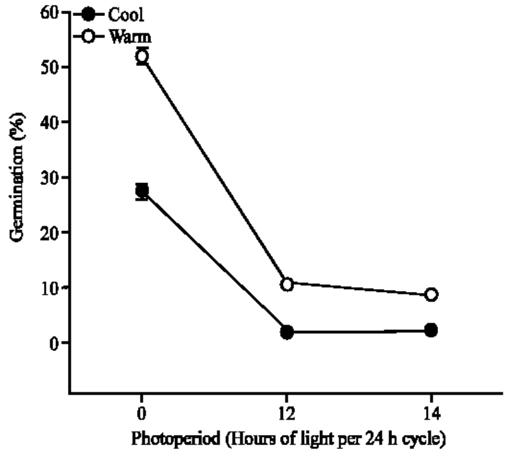

Fig. 6: Percent germination (Least square means±SE) of the significant interaction of temperature and stratification (Expt. No. 2)

12 weeks of stratification was only 8% greater than 6 weeks and 10% greater than 9 weeks of stratification (Table 6). The largest significant difference in % germination (32%) was observed between 0 week rep1, cool and 3 weeks, warm treatments (Table 6). The smallest significant difference (3%) was observed between reps1 and 3 (0 week, cool, Table 6).

Significantly greater germination was obtained at warmer temperatures, but the magnitude of the effect was dependent on light conditions (Fig. 6). Germination was greatest in seeds incubated in complete darkness, with warmer temperatures increasing % germination to 52% relative to cooler temperatures (Fig. 6). At photoperiods of 12 and 14 h of light, warm temperatures increased germination by much less, 9 and 6%, respectively (Fig. 6).

Expt. 3. Stratification, Photoperiod and Temperature Effects

Seed lots varied significantly ($p<0.001$) with mean percent germination ranging from 8% in SW2 to 37% in SRP (Fig. 7). Fresh seed lots (<1 year old), FS and FSOP, had significantly lower % germination (10%) than most of the commercial seed lots, but had % germination comparable to two-years old QW seed (14%). Primed (SRP) and non-primed (SRR) seed lots had similar mean germination (37 and 25%, respectively). Mean germination of two-years old commercially-available seed lot S (24%) were similar to seed harvested in the greenhouse 1 year later from a genetically similar population (SOP = 29%). In addition, 2/3 seed lots rejected for seed production due to low germination (SW2, SW3) had mean germinations comparable to fresh seed (8 and 10%, respectively) and 2-year-old commercial seed (QW), while SW4 (22%) was similar to S (24%, Fig. 7).

Germination at 27/15°C (41%) was significantly greater than at 19/7°C (4%) and in darkness (32%) than at 12 (14%) or 14 (13%) h of light per day. In complete darkness, the difference in germination between the warmer and cooler temperature was 46%, whereas at 12 and 14 h of light the difference in germination between the cooler and warmer temperature was 37 and 24%, respectively. Stratification did not increase germination response ($p = 0.448$); assay ($p = 0.366$) was not significant. Main effects (temperature, photoperiod), temperatures×photoperiod×assay, seed lot×stratification, seed lot×temperature, seed lot×photoperiod, stratification×temperature, seed lot×photoperiod×temperature, seed lot×stratification×photoperiod and seed lot×stratification×temperature were significant.

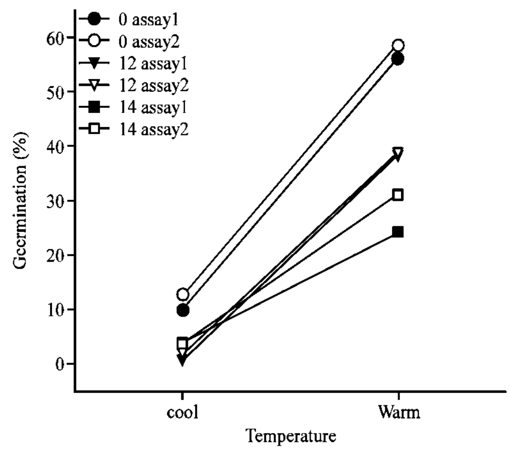

Fig. 7: Interaction plots of light by temperature in two assays of cleome seeds lots germinated in growth chambers (Expt. No. 3)

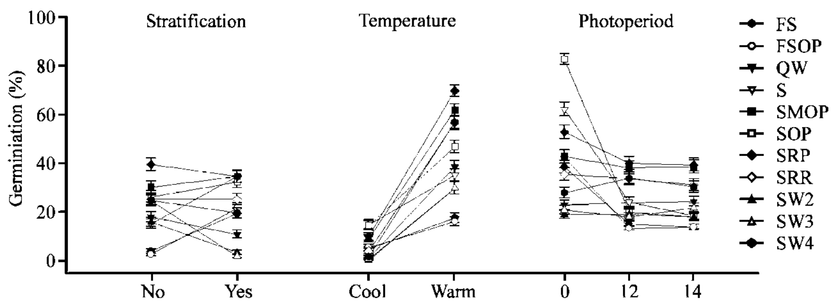

Fig. 8: Analysis of *Cleome, Polanisia* seed lot by stratification, seed lot by temperature and seed lot by light reaction norms (Expt. No. 3)

The effect of stratification, temperature and photoperiod varied depending on seed lot (Fig. 8). For example, SRP had the highest % germination in stratified (34%), non-stratified (39%), warm temperatures (70%) and at photoperiods of 12 and 14 h of light (32% each), but it had the lowest % germination in cool temperatures (10%). In complete darkness, SRP had the third highest % germination (47%) after SOP (80%) and S (57%). The magnitude of the difference between % germination at cool and warm temperatures varied across seed lot, though there was a clear trend towards greater germination at warmer temperatures (Fig. 8). However, some seed lots had greater % germination without stratification (SW2, SW3) or showed no difference between stratification treatment (SRR) and others had greater % germination after being stratified (FS, FSOP, S) (Fig. 8). Differences among seed lots are also observed in response to photoperiods. For example, SW2, SW3, SW4 and SRR had similar germination across photoperiods, while SRP had the highest germination in full darkness (Fig. 8).

DISCUSSION

Cleome seed lots vary in germination (Fig. 7) and in their response to different environmental conditions (Fig. 8). In fact, seed lots in our study, which might have been eliminated from commercial sales due to low germination, germinated comparably to other commercially available seed lots. These observations highlight the difficulty of making generalizations across seed lots and cultivars, corroborating previous reports on the lack of consistency in germination (Dunnett, 1991). Consequently, optimal treatments for germination of some seed lots may be suboptimal for others.

Research on other cleome species also showed variability in germination and investigations to determine optimal germination treatments have provided various recommendations. Germination rates of *C. droserifolia* Del., a species allelopathic to its own seed and select fungi, ranged between 44.3 (control, untreated) to 0.2% (10% *C. droserifolia* extract) in petri dishes incubated at 30/15°C day/night (Hegazy and Fadl-Allah, 1995). In *C. gynandra* L. up to 1 year post-harvest rest period (latency) and after ripening is required (Chweya and Mnzava, 1997), although some germination (1%) is possible from capsules harvested 18 day post-anthesis (Ekpong, 2009). While 1% may be a low percentage, a single cleome can produce 20-100 seeds per capsule, 10-80 capsules per flowering branch and 1-6 flowering branches per growing season (May-September) in Minnesota (Raboteaux and Nadina, 2005). Under ideal conditions (greenhouse) plants can flower continuously. In *C. gynandra*, germination will commence after 6 mos of latency and peak (88%) 3 mos later (Yepes, 1978). Seed of *C. gynandra* stored at 15.8°C and room temperature (30-33°C) both broke dormancy after 3 mos. A 1-5 day pre-heating at 40.8°C or scarification (Ochuodho *et al*., 2004) were effective in breaking dormancy of *C. gynandra* (Ekpong, 2009). Nonetheless, for most cleome seed lots in our study sowing in the dark after cold stratification resulted in the highest percent germination.

All experiments show that seeds germinated best in darkness, which contradicts commercial production recommendations (B and T World Seeds, 2009; Lee, 2000; Nau, 1999). A dark requirement provides an additional challenge to growers since, few crops remain in complete dark for periods in excess of 1-2 weeks (the rare exception is *Cyclamen persicum*; Dole and Wilkins, 2005). Germination of Solo in the dark (covered) was significantly higher than uncovered (light) although very low levels of seed germination still occurred in the field (Fig. 1) and growth chambers (Fig. 3). Studies of *Phalaris arundinacea* L. yielded similar contradictory results on the effect of light due to genotypic differences (Lindig-Cisneros and Zedler, 2001).

Many different mechanisms can be involved in light inhibition (Niedzwieds-Siegen and Lewak, 1992) such as red, blue and red to far-red (R:FR) light ratios (Bell, 1993; Gutterman, *et al*., 1992), seed coat, tegument and radicle physiology (Chacur and Takaki, 1996). Inhibitory effects of light on germination can be variable depending upon the conditions during the early stages of imbibition (Pirovano *et al*., 1996). In some cases, germination can be restored if the seeds are exposed to darkness after being submitted to prolonged periods of light. In cleomes, stratification seems to have a comparable effect. Similar to cleome, germination of some *Cucumis anguria* L. is inhibited by continuous exposure to white light (Chacur and Takaki, 1996). Further experiments would be needed to determine the mechanism(s) of light-inhibited germination in *Polanisia* and some *Cleome*, i.e., light quality, duration and if this is reversible.

Our studies also show that stratification increases germination. Cutler (1999) recommended outdoor sowing of *C. hassleriana* after the last spring frost, implying no need for stratification. In contrast, commercial seed lots have occasionally benefited from stratification (Nau, 1999). Likewise with *P. dodecandra*, a 2 week prechilling has been reported to enhance germination (B and T World Seeds, 2009). Our studies show that 3, 6, 9 and 12 weeks of stratification significantly increase Solo germination over the control

(0 week; Fig. 4). Interestingly, cold also increased germination of Solo in light (12, 14 h), with longer periods of cold (12 weeks) having significantly higher germination (Fig. 4). Therefore, deliberate or natural fall field sowing may result in higher germination than Spring sowing or greenhouse production without stratification. Our research would concur with the use of cold to enhance commercial germination (B and T World Seeds, 2009).

Higher temperatures enhance cleome germination, although at times it may not be sufficient to achieve commercially acceptable germination percents. Stratification followed by a warm temperature incubation cycle (27/15°C, day/night) significantly increased germination of Solo (FS, FSOP) over cool incubation (19/7°C) (Fig. 5). At both temperatures, however, germination rarely exceeded 30%. It is unknown whether continued germination would have happened after the experiments were terminated.

The significant effect of high-order interactions such as the interaction between seed type, age and depth of planting on field % germination in cleome (Fig. 8) underscores the complexity of germination response and highlights the challenges of predicting germination. The presence of genotype×environment interactions, defined as a lack of stability (Gray, 1982) in our experiments demonstrates that cleomes do not perform uniformly across environments. Both reps. of 6 weeks incubation in Expt. 2, for example, changed relative rankings of germination in the cool vs. warm temperatures (Fig. 5). Rank changes indicate instability (Gray, 1982). This is consistent with other studies that show large variation in germination in controlled and natural conditions, e.g., *Cortaderia* Stapf. (Lambrinos, 2002). High order interactions between factors influencing germination have been reported for other species. In *Carex* L., germination was lower at 30°C than 25°C before stratification and increased after stratification at 30°C (Schütz, 2000). Prolonged exposure to light decreased *Hordeum* L. germination when incubated at low temperatures with restricted water (Roth-Bejerano *et al*., 1996). The inhibitory effect of light was greatest at low temperatures and in oxygen depleted conditions for *Amaranthus* L. (Gutterman *et al*., 1992). Liao *et al.* (2000) found that high-order interactions of year, week of harvest and incubation regime were significant for germination of fresh *Chondrilla juncea* L. seeds. OP-derivation of our seed lots could be a contributing factor to lack of stability. A longer process of domestication may result in higher germination and greater stability.

Although studies determining the negative impact of cleomes are lacking, some researchers claim *Cleome hassleriana* is an invasive species. Nonetheless, evidence of naturalization does support prolific germination in certain non-cultivated ecosystems. Low and uneven germination in cleome commercial production appears to contradict prolific reseeding in gardens and naturalization in non-cultivated ecosystems. Seed dormancy and germination characteristics are under strong selective pressure (Meyer *et al*., 1990; Rees, 1996). High germination in sites with light, insensitivity to photoperiods (Lindig-Cisneros and Zedler, 2001) and high level of germination over a wide range of environmental conditions (i.e., greater plasticity or local adaptation) (Greenberg *et al*., 2001; Lambrinos, 2002; Clements *et al*., 2004; Schmitz, 2004) are often cited among characteristics of invasive species. Under these experimental conditions and with the seed lots studied, cleome seeds germinate best in the darkness and are inhibited by prolonged exposure to light. In fact, the increase in germination of the seeds in the dark relative to those in the alternating light treatment suggest that even 12 h of light might be sufficient to induce a light-inhibition germination response in cleome. The relative effects of R:FR light on cleome germination have not been explored and may be relevant in determining germination response in non-cultivated habitat. Relatively undomesticated seed-propagated crops, such as *Cleome* and *Polanisia,* may benefit from directed selection for stability in germination performance under commercial production conditions while limiting germination in non-cultivated environments.

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