Seedbanks and Plant Development of *Stylisma pickeringii*, an Endangered Plant in Illinois

(Seedbanks—Objectives 1 and 2)

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ABSTRACT

Stylisma pickeringii var. *pattersonii* is an endangered Illinois sand prairie plant that occurs in Cass, Henderson, and Mason Counties of Illinois. One colony at Mason County is prone to frequent disturbance by discing and chemical treatment, and another colony at Mason County is an undisturbed area exposed to less recent ground and chemical disturbance. *Stylisma pickeringii* var. *pattersonii* produces seed of 3 different colors (yellow, tan, maroon), and seeds exhibit a dormancy. Germination did not decrease for *S. pickeringii* var. *pattersonii* seeds after 1 year of cold storage. No attempts have been made to measure the amount of seed produced or to predict the longevity of viable seed in the soil seed bank. The objectives of this study were to estimate the number of *S. pickeringii* var. *pattersonii* seeds in the soil seed bank at Henderson and Mason Counties, and to predict the longevity and viability of seeds in cold storage and in the field. For seed production, soil cores were taken with a 3 cm diameter soil corer at Mason and Henderson Counties. Cores in Mason County were taken at both the disturbed and undisturbed colonies at depths of 0.0 –10.0 cm and at a depth of 30.0 cm (divided into increments of 0.0–2.5 cm, 2.5–5.0 cm, 5.0–10.0 cm, 10.0–15.0 cm, 15.0–20.0 cm, 20.0–25.0 cm, and 25.0–30.0 cm). Soil cores in Henderson County were taken at a depth of 10.0 cm (divided into increments of 0.0–5.0 cm and 5.0–10.0 cm). Soil scrapes (10 cm x 10 cm x 7.5 cm) were taken at both the disturbed and undisturbed colonies at Mason

County and at Henderson County. Soil cores and scrapes were sieved with ASTM #5 and #10 screen mesh soil sieves. Number of *S. pickeringii* var. *pattersonii* seeds per sample were counted and sorted by color. Estimated number of *S. pickeringii* var. *pattersonii* seeds/m^2 was calculated. For seed longevity in cold storage, seeds of *S. pickeringii* var. *pattersonii* collected from the undisturbed colony in Mason County on September 9, 1999, August 25, 2000, August 21, 2001, and August 21, 2002 were kept at 4°C with less than 50% relative humidity. Scarified and unscarified (control) seeds from each harvest year were germinated on petri dishes at 25° C in a 12-hour photoperiod, and counted every other day for two weeks. For seed longevity in the field, seeds of *S. pickeringii* var. *pattersonii* were collected on September, 20, 2001. Half of the seeds were kept in cold storage (control), and half were rolled into bundles of fiberglass screening (9 holes/cm^2). Fiberglass bundles were buried at a depth of 10 cm in the native soil of the undisturbed colony in Mason County in July 2002. The bundles were dug up and control seeds were removed from cold storage at four-month intervals. Seeds were germinated in petri dishes at 25°C in a 24-hour photoperiod of 5 $\mu mol\ s^{-1}\ m^{-2}$ measured with a Licor quantum sensor on the electric lamp setting. Petri dishes were kept in a growth room and counted every 2-3 days. Seeds that did not germinate were cut lengthwise with a razor blade and soaked in 2,3,5-triphenyltetrazolium chloride (TTC) (1%) solution to test for viability. Redness of seeds was evaluated at 1, 2, 5, and 21 hours after application. No significant colony differences were found in the number of *S. pickeringii* var. *pattersonii* seeds in each increment at depths of 30.0 cm and 0.0–10.0 cm for cores at disturbed and undisturbed colonies at Mason County and Henderson Counties. All *S. pickeringii* var. *pattersonii* seeds in cores were found at depths of 0.0–15.0 cm in disturbed and undisturbed colonies.

No significant difference was found in the number of *S. pickeringii* var. *pattersonii* seeds for soil scrapes, and seed production estimates were 2,340 seeds/m^2, 2,040 seeds/m^2, and 1,310 seeds/m^2 for the disturbed Mason County colony, the undisturbed Mason County colony, and the Henderson County colony, respectively. Seed color was not significantly different between colonies. Scarified seeds with 2 years of cold storage had highest germination (95%). Germination was 0% for unscarified seeds stored for 4 months in field conditions and for uscarified seeds stored for 4 months in cold storage. After 8 months, germination was 6 % and 0% for seeds in field conditions and cold storage, respectively. After 4 and 8 months, seeds in cold storage were significantly redder after 5 hours of TTC application than seeds in field conditions.

INTRODUCTION

Survival strategies of a plant can include adaptations in seed biology. The success of a plant population can depend on the availability of a viable seed source. In nature this seed source comes from current year's seed production or from stored seed in the soil seed bank. The seed bank of an area includes the viable, un-germinated seeds that are deposited by seed plants into the soil for natural storage (Moore *et al.*, 1998). These stored seeds may remain dormant but viable in the soil until favorable conditions promote germination. The most important conditions to lengthen the life of stored seeds are low moisture content of seed, low storage temperature, and modification of the storage atmosphere. Proper storage will prevent the seed from imbibing moisture, and thus prohibit fungal activity, heating, and germination (Hartmann *et al.*, 1997).

Stylisma pickeringii var. *pattersonii* is an endangered Illinois sand prairie plant that occurs in Cass, Henderson, and Mason Counties of Illinois. In Cass County, the

colony is limited to one clump (approximately 1.5 m^2) (Todd, 2002). The size of the colony at Henderson County has not been measured but appears to be larger than the Mason County colonies (approximately 55,000 m^2) (Todd, 2002). Plants at Mason County are divided into two colonies by a county road. One colony had a recent disturbance in 1999 by discing and chemical treatment (pendimethalin, trade name: Prowl). The area of the disturbed colony is approximately 10,700 m^2. The other colony is an undisturbed area exposed to less recent ground and chemical disturbance. The area of the undisturbed colony is approximately 44,500 m^2 (Todd, 2002).

Stylisma pickeringii var. *pattersonii* produces seed of three different colors (yellow, tan, maroon) with yellow seeds having the highest germination rate (55-100%) (Heisler *et al.*, 1999; Todd, 2002). Seeds of *S. pickeringii* var. *pattersonii* exhibit a dormancy where the seed coat inhibits germination (Heisler *et al.*, 1999). Frequently, seed coat inhibition can be alleviated by scarification, a process that breaks the seed coat and allows exchange of materials. In the lab, scarification of *S. pickeringii* var. *pattersonii* seed is best accomplished with an acid soak (Todd, 2002; Todd *et al.*, 2002). In the field, scarification could be accomplished with repeated abrasion by the sandy soil of sand prairies and by freezing and thawing. One year of storage did not affect germination of *S. pickeringii* var. *pattersonii,* and seeds harvested and scarified in 1999 (80% germination) were not significantly different for germination from seeds harvested in 1999 and scarified after 1 year of cold storage (90% germination) (Todd, 2000; Todd *et al.*, 2002). Germination differs between growing seasons. Seeds of *S. pickeringii* var. *pattersonii* harvested and scarified in 2000 had significantly higher germination (100%) than seeds harvested and scarified in 1999 (85%).

Seed production of *S. pickeringii* var. *pattersonii* in the field is abundant and seed has been collected from 2 colonies in Mason County (Todd, 2002). It was estimated that 7,205 and 3,560 seedlings emerged in the disturbed and undisturbed areas, respectively, in 2000 (Todd *et al.*, 2001; Todd, 2002). Flower densities at peak flowering in Mason County on July 5, 2001 were 103 flowers/m^2. Seed development in the field has determined *S. pickeringii* var. *pattersonii* flowers produce fruit that are usually 1- or 2-seeded (Myint, 1966; Todd, 2002). No attempts have been made to measure directly the amount of seed produced or to predict the longevity of viable seed in the soil seed bank.

The objectives of this study were to estimate the number of *S. pickeringii* var. *pattersonii* seeds contained in the soil seed bank at Henderson and Mason Counties, Illinois, and to predict the longevity of viable seed in cold storage and in the field. Results from this project will provide a better understanding of seed biology for this endangered plant, and allow for more insightful management decisions.

MATERIALS AND METHODS

Seed production. On December 18, 2001 at both the disturbed and undisturbed colonies in Mason County, 10 preliminary soil cores were taken at a depth of 30.0 cm and divided into increments of 0.0–2.5 cm, 2.5–5.0 cm, 5.0–10.0 cm, 10.0–15.0 cm, 15.0–20.0 cm, 20.0–25.0 cm, and 25.0–30.0 cm, to determine the depth where most seeds occur. On May 7, 2002 in Mason County, 20 soil cores were taken at a depth of 0.0–10.0 cm. On July 9, 2002 in Henderson County, 30 soil cores were taken at depths of 10.0 cm and divided into increments of 0.0–5.0 cm and 5.0–10.0 cm. In Henderson County only the first 20 cores were used, and numbers of seed in both increments were combined to calculated number of seeds at a depth of 0.0–10.0 cm. Soil cores were taken with a 3 cm

diameter soil corer and sieved with ASTM #5 and #10 screen mesh soil sieves. Number of *S. pickeringii* var. *pattersonii* seeds per sample was counted. Estimated number of *S. pickeringii* var. *pattersonii* seeds/m^2 was calculated.

On May 7, 2002, July 10, 2002, August 21, 2002, September 9, 2002, and April 8, 2003, 10 soil scrapes were taken randomly throughout both the disturbed and undisturbed colonies in Mason County. On July 9, 2002 10 soil scrapes were taken randomly throughout the colony in Henderson County. For soil scrapes, the first 7.5 cm of soil and organic matter from an area of 100 cm^2 was scrapped onto cardboard and deposited in paper and plastic bags. The samples were sieved with ASTM #5 and #10 screen mesh soil sieves. Number of *S. pickeringii* var. *pattersonii* seeds per sample was counted, and seeds that were found were sorted by color. Estimated number of *S. pickeringii* var. *pattersonii* seeds/m^2 was calculated.

Seed longevity in cold storage. Yellow seeds of *S. pickeringii* var. *pattersonii* were collected from the undisturbed colony in Mason County on September 9, 1999, August 25, 2000, August 21, 2001, and August 21, 2002. After harvest, seeds were kept in a Dry Keeper seed storage unit at 4°C with less than 50% humidity until scarification. Seeds from each harvest year were scarified in concentrated sulfuric acid (18.0 M H_2SO_4) for 2 hours and rinsed for 2 minutes in distilled water. An equal number of seeds from each harvest year were left un-scarified to use as controls. Twenty seeds from each harvest year were counted and divided into 4 replications of 5 seeds each. Seeds were dusted with Thiram (50% active ingredient, tetramethylthiuram disulfide) for fungal control and placed on 2 sheets of Whatman #1 filter paper moistened with 5 ml of deionized water in 100 x 15 mm Fisher Brand petri dishes. Petri dishes were sealed with

parafilm and placed in clear plastic Rubbermaid® containers (25 x 15 cm). Containers were stored at 25°C in a 12-hour photoperiod of 99 μmol s^{-1} m^{-2} (measured with an Apogee quantum sensor) from fluorescent lamps. For 4 weeks from October 23 to November 12, 2002, seed germination was counted every other day for 2 weeks and once a week for the last 2 weeks. Seeds were considered germinated when the radicle emerged at least 2 mm from the seed.

Seed longevity in the field. On September 20, 2001 yellow seeds were collected from the undisturbed colony in Mason County and kept in a Dry Keeper seed storage unit at 4°C with less than 50% humidity. On July 9, 2002, 180 seeds were removed from cold storage and placed on 7 x 9 cm pieces of fiberglass screening (9 holes/cm^2) and rolled lengthwise into cylindrical bundles. Ends of the bundles were tied with 20 gauge copper wire. On July 10, 2002, 3 fiberglass bundles were strung on the stake of a metal flag and buried at a depth of 10 cm in the native soil of the undisturbed colony in Mason County. Six groups with 3 bundles of 10 seeds each (for a total of 180 seeds) were buried in a line 1 meter apart with the ends of the line staked with rebar. The bundles were unburied at approximately four-month intervals. On November 26, 2002, the first 3 bundles of 10 seeds each were unburied. On April 8, 2003, the second 3 bundles of 10 seeds each were unburied. On each date, *S. pickeringii* var. *pattersonii* seeds were removed from bundles and 30 control seeds were removed from cold storage. Seeds were dusted with Thiram (50% active ingredient, tetramethylthiuram disulfide) for fungal control and placed on 2 sheets of Whatman #1 filter paper moistened with 5 ml of deionized water in 100 x 15 mm Fisher Brand petri dishes that were sealed with parafilm and placed in clear plastic Rubbermaid® containers (25 x 15 cm). Containers were stored at 25°C in a 24-hour

photoperiod of 5 μmol s^{-1} m^{-2} (measured with a Licor quantum sensor) from fluorescent lamps. Every 2 to 3 days the number of germinated seeds was counted. Seeds were considered germinated when the radicle emerged at least 2 mm from the seed. Those seeds that did not germinate were cut lengthwise with a razor blade and soaked in 2,3,5-triphenyltetrazolium chloride (TTC) (1%) solution to test for viability (Lakon, 1949). Redness of seeds, indicating positive presence of dehydrogenase enzymes, was ranked from 0 (no color change) to 5 (entire seed red) at 1, 2, 5, and 21 hours after TTC application. Seeds were considered viable if they ranked a 1 or higher after 5 hours.

In all studies data were analyzed with ANOVA using Microsoft Excel (Microsoft Corporation, 2000) and SAS 8.2 (SAS Institute, Inc., 2001). Mean separations were determined with Duncan's multiple range test at 5% level. Means and percentages were calculated plus or minus standard error.

RESULTS

Seed production. No significant colony differences were found in the number of *S. pickeringii* var. *pattersonii* seeds in each increment at depths of 30.0 cm for preliminary cores at disturbed and undisturbed colonies at Mason County (Table 1). Total seeds/colony in each increment at depths of 0.0–30.0 cm are estimated based on preliminary cores (Table 1). No significant difference was found in the number of *S. pickeringii* var. *pattersonii* seeds for soil cores at depths of 0.0–10.0 cm between the more disturbed Mason County colony, the undisturbed Mason County colony, and the

Henderson County colony (Table 2). Total seeds/colony at depths of 0.0–10.0 cm are estimated based on soil cores (Table 2).

No significant difference was found in the number of *S. pickeringii* var. *pattersonii* seeds for soil scrapes between the different collection dates for the more disturbed Mason County colony, the undisturbed Mason County colony, and the Henderson County colony, hence averages were calculated across dates (Table 3). Estimated number of *S. pickeringii* var. *pattersonii* seeds/colony sampled with soil scrapes is shown in Table 3. Seed color was not significantly different between colonies (Table 4).

Seed longevity in cold storage. Germination with no scarification was greatest for seeds harvested in 2000 and imbibed in 2002 (2 years of cold storage) (Table 5). Seeds harvested in 2000 had the highest germination rates (Figure 1).

Seed longevity in the field. Germination was 0% for both buried and unburied seeds after 4 months of storage. Germination was 6 % for buried seeds and 0% for unburied seeds after 8 months of storage. Unburied seeds stored in seed storage cabinets for 4 and 8 months were significantly redder than buried seeds after 5 hours of TTC application (Table 6).

DISCUSSION

Seeds of *S. pickeringii* var. *pattersonii* are not found very deep in the soil seed bank with the majority of seeds found at depths of 0.0–15.0 cm. One explanation for this observation could be that the seeds germinate quickly in nature and do not remain as seeds long enough to work deeper into the soil. Support for quick germination in the field

is shown with a decrease in viability of seeds stored in native soil conditions relative to those in controlled conditions. Seed survival in the field decreases with time that could encourage seeds to germinate quickly when favorable conditions allow. Since the screen mesh would provide a seed with less direct contact to the soil than it would have naturally lying in the soil, survival could be even less for seeds lying directly in the soil, where seeds would be subjected to less aerated conditions. The disturbed colony at Mason County had significantly more seedlings than the undisturbed colony at Mason County (Todd, 2002). One also would expect the seed banks of the disturbed colony at Mason County to contain more *S. pickeringii* var. *pattersonii* seeds. However, no difference was found in the number of seeds found in cores 0.0–10.0 cm deep or scrapes from the first 7.5 cm. Rather than being related to the number of seeds at each colony, the difference in seedling numbers between colonies could be a result of less competition from species intolerant of disturbance. Also, more germinating seedlings at the disturbed colony could be due to more frequent occurrence of natural scarification (blowing sand) of the seed coat in areas of soil disturbance. Viability of seeds in the field decreases significantly after 4 and 8 months of storage when compared to seeds in cold storage, yet seeds were still viable. Seeds must remain viable in the field for greater than 8 months. Future studies could compare germination of seeds in field conditions in disturbed and undisturbed colonies over time. Although, the colonies of Henderson and Mason County appear to differ in size (Todd, 2002), no difference was seen in the number of *S. pickeringii* var. *pattersonii* seeds/m^2 at depths of 0.0 –10.0 cm (soil cores) or in the first 7.5 cm (soil scrapes) of the seed banks. Therefore, populations in Henderson and Mason Counties store similar numbers of seeds at generally the same level in the soil.

Decrease in percent viability in buried seeds could be due to conditions unfavorable to seed storage such as fluctuating moisture and varying temperatures. These conditions favor the growth of fungi and bacteria. A few seeds had visible signs showing that buried seeds also were susceptible to predation by insects that were small enough to fit through the holes in the screen. Even seeds in cold storage, where humidity and temperature is constant and low enough to suppress the growth of fungi and microorganisms, show a gradual decrease in viability with time. Seeds in this study were only stored in the field for 8 months. Seeds stored in native and cold storage for longer than 8 months should be tested to determine if *S. pickeringii* var. *pattersonii* seeds remain viable for longer terms.

Table 1. *Stylisma pickeringii* var. *pattersonii* seeds found in each increment at depths of 0.0–30.0 cm for preliminary cores from disturbed and undisturbed colonies in Mason County sampled on December 18, 2001.

Depth	0.0–2.5 cm	2.5–5.0 cm	5.0–10.0 cm	10.0–15.0 cm	15.0–20.0 cm	20.0–25.0 cm	25.0–30.0 cm
Disturbed Colony	0.6 ± 0.3[v] a[w]	0.1 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	(67%)[x]	(11%)	(11%)	(11%)	(0%)	(0%)	(0%)
	849[y] seeds/m^2	142 seeds/m^2	142 seeds/m^2	142 seeds/m^2	0 seeds/m^2	0 seeds/m^2	0 seeds/m^2
	9,084,300 seeds/colony	1,519,400 seeds/colony	1,519,400 seeds/colony	1,519,400 seeds/colony	0 seeds/colony	0 seeds/colony	0 seeds/colony
Undisturbed Colony	0.6 ± 0.4 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	(86%)	(14%)	(0%)	(0%)	(0%)	(0%)	(0%)
	849 seeds/m^2	142 seeds/m^2	0 seeds/m^2	0 seeds/m^2	0 seeds/m^2	0 seeds/m^2	0 seeds/m^2
	37,780,500 seeds/colony	6,319,000 seeds/colony	0 seeds/colony	0 seeds/colony	0 seeds/colony	0 seeds/colony	0 seeds/colony

[v]Means are expressed ± standard error.

[w]Mean separation within column (between disturbed and undisturbed colonies) based on Duncan's multiple range test at 5%, n=10. Means followed by different letters are significantly different between disturbed and undisturbed colonies.

[x]Numbers in parentheses indicate percent of total seeds found at each depth in each colony.

[y]Numbers indicate estimates for number of seeds/m^2 (area of soil core 0.000703 m^2) found at each depth in each colony.

[z]Numbers indicate estimates for number of seeds/colony found at each depth in each disturbed (10,700 m^2) and undisturbed (44,500 m^2) (Todd, 2002).

Table 2. *Stylisma pickeringii* var. *pattersonii* seeds found at depths of 0.0-10.0 cm for soil cores from disturbed and undisturbed colonies in Mason County sampled on May 7, 2002 and Henderson County sampled on July 9, 2002.

County	Colony	Seeds/core	Estimated number of seeds/m^2	Estimated number of seeds/colony
Mason	Disturbed	0.3 ± 0.1^{w} a^{x}	426^{y}	$4{,}558{,}200^{z}$
Mason	Undisturbed	0.3 ± 0.1 a	426	18,957,000
Henderson	--	0.4 ± 0.1 a	568	--

[w]Means are expressed ± standard error.

[x]Mean separation within column based on Duncan's multiple range test at 5%, n=20. Means followed by different letters are significantly different.

[y]Numbers indicate estimates for number of seeds/m^2 (area of soil core 0.000703 m^2) in each colony.

[z]Numbers indicate estimates for number of seeds/colony found at each depth in each disturbed (10,700 m^2) and undisturbed (44,500 m^2) (Todd, 2002). Area of Henderson County colony is unknown.

Table 3. *Stylisma pickeringii* var. *pattersonii* seeds found in soil scrapes from disturbed and undisturbed colonies in Mason County sampled on May 7, 2002, July 10, 2002, August 21, 2002, September 9, 2002, and April 8, 2003 and Henderson County sampled on July 9, 2002.

County	Colony	Seeds/soil scrape	Estimated number of seeds/m^2	Estimated number of seeds/colony
Mason	Disturbed	23 ± 3^{w} a^{x}	2,300 seeds/m^{2y}	$24{,}610{,}000^{z}$
Mason	Undisturbed	20 ± 3 a	2,000 seeds/m^2	89,000,000
Henderson	--	13 ± 2 a	1,300 seeds/m^2	--

[w]Means are expressed ± standard error. Means were determined by averaging number of total seeds collected across all collection dates for each colony.

[x]Mean separation within column based on Duncan's multiple range test at 5%, n=50 for Mason County and n=10 for Henderson County. Means followed by different letters are significantly different.

[y]Numbers indicate estimates for number of seeds/m^2 found in scrapes 100 cm^2 in area and 7.5 cm deep at each colony.

[z]Numbers indicate estimates for number of seeds/colony found at each depth in each disturbed (10,700 m^2) and undisturbed (44,500 m^2) (Todd, 2002). Area of Henderson County colony is unknown.

Table 4. Color of *Stylisma pickeringii* var. *pattersonii* seeds found in soil scrapes from disturbed and undisturbed colonies in Mason County sampled on May 7, 2002, July 10, 2002, August 21, 2002, September 9, 2002, and April 8, 2003 and from colonies in Henderson County sampled on July 9, 2002.

County	Colony	Yellow	Tan	Maroon
Mason	Disturbed	88 ± 22[x] a[y] (38%)[z]	123 ± 39 a (52%)	24 ± 7 a (10%)
Mason	Undisturbed	22 ± 10 a (15%)	99 ± 26 a (65%)	31 ± 14 a (20%)
Henderson	--	34 a (28%)	56 a (47%)	30 a (25%)

[x]Means are expressed ± standard error. Means were determined by averaging total number of seeds of each seed color across all collection dates for each colony. Henderson County had only one collection date, and therefore numbers indicate total number of seeds for each color. Henderson County numbers represent a total not an average, and a standard error can not be calculated.

[y]Mean separation within column (between colonies) based on Duncan's multiple range test at 5%, n=5 for Mason County colonies and n=1 for Henderson County. Means followed by different letters are significantly different between colonies.

[z]Numbers in parentheses indicate percent of total seeds found in all scrapes at each colony.

Table 5. Percent germination of scarified and unscarified *Stylisma pickeringii* var. *pattersonii* seeds collected in different years and stored in cold storage until germinated in 2002.

Year collected	Scarification treatment	Germination (%)
2000	Scarified	95 ± 5[y] a[z]
2002	Scarified	90 ± 10 a b
1999	Scarified	65 ± 10 b c
2001	Scarified	60 ± 10 c d
2000	Unscarified	35 ± 10 d e
2001	Unscarified	20 ± 14 e f
1999	Unscarified	5 ± 5 f
2002	Unscarified	0 ± 0 f

[y]Means are expressed ± standard error.
[z]Mean separation within column based on Duncan's multiple range test at 5%, n=4. Means followed by different letters are significantly different.

Table 6. Viability of *Stylisma pickeringii* var. *pattersonii* seeds after 4 and 8 months of cold storage or in field conditions shown as redness of seed after application of 1% TTC.

Seed treatment	Redness[x] 4 months of storage	Redness 8 months of storage
Cold storage	1.9 ± 0.2[y] a[z]	3.5 ± 0.2 a
Field conditions	0.7 ± 0.1 b	1.8 ± 0.3 b

[x]Degrees of redness: 0–1 no color change, 1–2 pink, 2–3 dark pink, 3–4 partially red, 4–5 completely red.
[y]Means are expressed ± standard error.
[z]Mean separation within column based on Duncan's multiple range test at 5%. n=30 for seeds tested after 4 months of storage (cold and field storage) and after 8 months of cold storage. n=28 for seeds tested after 8 months of field storage. Means followed by different letters are significantly different.

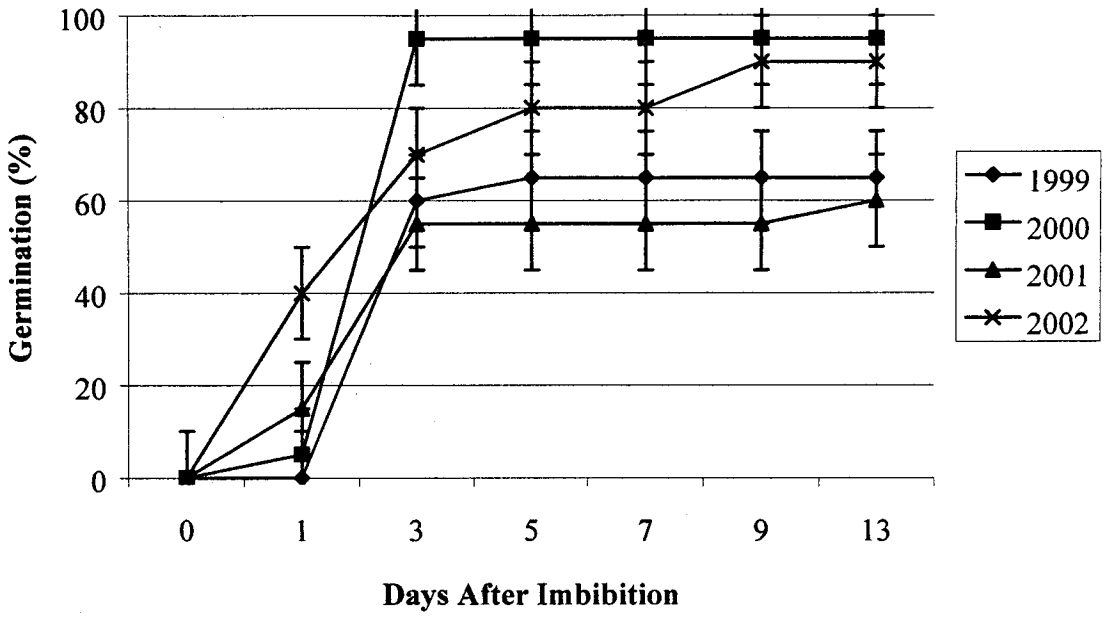

Figure 1. Germination rate of *Stylisma pickeringii* var. *pattersonii* seeds harvested in 1999, 2000, 2001, and 2002 with imbibition beginning on October 22, 2002. Means calculated ± SE.

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Seedbanks and Plant Develolopment of *Stylisma pickeringii*, an Endangered Plant in Illinois

(Plant Development—Objective 3)

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ABSTRACT

Stylisma pickeringii var. *pattersonii* is an endangered Illinois sand prairie species currently found in only three Illinois counties—Cass, Henderson, and Mason. Seedling establishment in sand habitats can be difficult, but *S. pickeringii* var. *pattersonii* has adapted to survive in these harsh conditions. Relatively little is known about how seedlings of *S. pickeringii* var. *pattersonii* establish. One objective of this study was to determine if native soil, greenhouse mixes, and supplemental light conditions contribute to the successful establishment of *S. pickeringii* var. *pattersonii* seedlings in the greenhouse. Other objectives were to determine the effect of photoperiod on side shoot development in soil, to determine how disturbance affects re-introduced seeds, to identify the stages of seedling development, and to quantify movement of seed and transition zone before shoot initiation. For native soil, *S. pickeringii* var. *pattersonii* seeds were scarified and germinated with 100% Universal mix (U), 100% sterilized native sand (Plainfield sand 1 to 7 percent slope) (SN), 100% unsterilized native sand (UN), sterilized native sand (Plainfield sand 1 to 7 percent slope):Universal mix(1:1) (SN:U), or unsterilized native sand sand:Universal mix(1:1) (UN:U). Percent emergence and side shoot length were recorded. For supplemental light, seeds were scarified and grown with 100% Sunshine mix and High Intensity Discharge (HID) light (L), sand:Sunshine mix(1:1) and

HID light (S:S,L), 100% Sunshine mix and no HID light (N), or sand:Sunshine mix(1:1) and no HID light (S:S,N). Percent cover and stem height were recorded. For photoperiod effect on side shoot development, seeds were scarified and planted in Cone-tainers (4 cm diameter and 20 cm deep) containing Sunshine Mix placed in long days (16-hour day) and short days (8-hour day). Seedling emergence, number of seedlings with side shoots, and length of side shoots were recorded weekly for 26 weeks. For disturbance and seedling establishment, plantings were made in the disturbed and undisturbed populations of *S. pickeringii* var. *pattersonii* near Snicarte, Illinois. Plots (1 m^2) were situated within each of the disturbed and undisturbed populations and contained sections both planted and left unplanted with *S. pickeringii* var. *pattersonii* seeds. Number of seedlings, number of side shoots, number of flowers, and percent cover of *S. pickeringii* var. *pattersonii* were recorded within each 1 m^2 plot. For seedling development and movement of seed, seeds of *S. pickeringii* var. *pattersonii* were placed in test tubes containing medium with 2 g/L Gelrite, 4.3 g/L Murashige and Skoog (JRH Biosciences, Lenexa, KS) salts, 10 ml/L rose vitamins, 0.1 g/L myoinositol, and 30 g/L sucrose with pH between 5.7 and 5.8. A horizontal line was drawn at the base of the seed. Seeds were germinated in continuous light at 5 $\mu mol\ s^{-1}\ m^{-2}$ at 25°C. Seeds were monitored every 2 days for seed movement and changes in development. Movement of the base of the seed relative to the line was measured, and distinct stages in development were noted. Distinct stages of seedling development were preserved in Formalin-Acetic acid-Alcohol (FAA) fixative (50 cc ethanol [95%], 5 cc glacial acetic acid, 10 cc of formalin [37% formaldehyde], 35 cc deionized water). For movement of transition zone, seeds were placed in the same media and germinated in the same conditions as the previous tissue culture experiment.

Tubes were marked with a horizontal line where their transition zone appeared. Movement of the base of the transition zone relative to the line was measured once a week for 4 weeks, then once after 2 additional weeks, and finally once after 6 additional weeks. Percent emergence was not affected significantly by substrate. Side shoot length was greatest for plants in U, UN, and SN:U with 8.0, 7.8, and 7.3 cm, respectively. Plants in S:S,L produced the largest percent cover (48%). Longest stems were from plants in S:S,L (28.4 cm) and L (25.2 cm). HID irradiance benefited growth. Responses to media were mixed. Short day plants produced significantly more plants with side shoots. Disturbance studies were inconclusive. Seventy-eight percent of seeds moved a distance of -0.02 ± 0.04 cm perpendicular to the initial horizontal line drawn at the base of the seed. Fifty-two percent of plants developing transition zones showed movement in these zones with overall movement being -1.0 ± 0.1 cm.

INTRODUCTION

Stylisma pickeringii var. *pattersonii* is an endangered Illinois sand prairie species currently found in only three Illinois counties—Cass, Henderson, and Mason (Herkert and Ebinger, 2002). *Stylisma pickeringii* var. *pattersonii* is found in the Midwest on well-drained soils, dry prairies, sandy open woods, rarely moist sandy soils of inland plains from Illinois and Iowa south to Mississippi and Texas (Myint, 1966). The native habitat of this plant is the sand prairie which is a unique community occurring on remnant sand deposits (Gleason, 1910). The sand prairie is a xeric environment, where the uppermost centimeters of sand usually have little or no available moisture (Baldwin and Maun, 1983; Hart and Gleason, 1907; Tolstead, 1942). Many prairie plants share a number of common morphological adaptations that have developed to reduce stress caused by low

moisture levels (Moore, 1999). Seedling establishment in sand habitats can be difficult due to problems associated with lack of moisture, shifting sands, and shallow seed depth (Bach, 1998; Chen and Maun, 1999; Martinez and Moreno-Casasola, 1993; Maun, 1981; Zhang and Maun, 1990).

The importance of native soil conditions, such as the presence of mycorrhizae or other microorganisms, in the establishment of plants has been demonstrated in both laboratory and field studies (Allen, 1991; Brundrett, 1991). However, a lack of mycorrhizal response in prairie species grown in soil from Sand Ridge State Forest (Mason County, IL) was found, which is attributed to the greater plant-available P level of this soil (Anderson *et al.*, 1994). The native soil at *S. pickeringii* var. *pattersonii* colonies in Mason County share the same soil series as the Sand Ridge State Forest—the Plainfield Bloomfield. These soils are excessively drained, rapidly permeable soils on dunes, stream terraces and uplands (Calsyn, 1995).

Despite its ability to survive harsh growing conditions in the field, *Stylisma pickeringii* var. *pattersonii* has been difficult to establish in greenhouse conditions. Plants grown in the greenhouse will survive to produce only cotyledons and possibly a few shoots before the leaves brown and the plant dies. Trial studies have explored the establishment requirements of *S. pickeringii* var. *pattersonii*. Seedlings in the greenhouse remain alive when watered once a day or twice a week with 30 ml water (Heisler, 1999). Growing media with 3:1 greenhouse mix:torpedo sand and 100 percent greenhouse mix is most beneficial for seedling root formation when compared to growing media containing 100 percent torpedo sand, 3:1 torpedo sand:greenhouse mix, 1:1 greenhouse mix: torpedo sand (DuFrain, 1999). Successful establishment protocols would allow controlled studies

of its reproductive biology and also provide plants for reintroduction into the native landscape.

The seedlings of *S. pickeringii* var. *pattersonii* have a special morphology that could be considered a survival strategy linked to successful establishment. After the emergence of roots with germination, cotyledons appear above ground. Once roots are well developed, a lateral side shoot initiates approximately 7.2 cm below the soil surface (Todd *et al.*, 2001). No additional growth occurs from the above ground stem axis bearing the cotyledons. The cotyledons become necrotic and above ground growth continues as the side shoot reaches the soil surface (Todd *et al.*, 2001). The stimulus that initiates these side shoots is unclear. Drawings of field bindweed (*Convolvulus* sp.) seedlings show similar side shoot morphology, but little was found to explain the stimulus of this below ground growth (Kummer, 1951). In tissue culture, photoperiod affected lateral side shoot development. Percentage of plants that developed side shoots was greater with long day than short day conditions, and side shoot height was greater in long day than short day conditions (Kerber *et al.,* 2000).

Established populations of *S. pickeringii* var. *pattersonii* show preference for disturbance. *Stylisma pickeringii* var. *pattersonii* grows in the sand prairies of Illinois, which have a history of shifting sand and unstable growing conditions (Gleason, 1910). Two of the healthiest colonies of *S. pickeringii* var. *pattersonii* are in Mason County near Snicarte, Illinois. One colony (10,700 m^2) is prone to frequent disturbance by discing (most recently in 1999) or chemical treatment (pendimethalin, trade name: Prowl). The second colony (44,500 m^2) is an undisturbed area exposed to less ground disturbance. Average number of seedlings/m^2 observed in the disturbed area was significantly higher

than in the undisturbed area. It was estimated that 7,200 and 3,560 seedlings emerged in the disturbed and undisturbed areas, respectively, in 2000 (Todd, 2002; Todd *et al.*, 2001). *S. pickeringii* var. *pickeringii* shares a preference for frequently disturbed habitats. It is an early successional species that germinates after a disturbance that adversely affects competitors. Hot water enhances seed germination, and fire could produce the same results in nature (Schuyler, 1990). *S. pickeringii* var. *pickeringii* grows in the New Jersey Pine Barrens and the North Carolina Sandhills, where fire has considerable influence on the flora (Garren, 1943; Little, 1979). Further, in Fort Bragg, North Carolina, *S. pickeringii* var. *pickeringii* is found around paratrooper drop zones, that are subjected to frequent soil-disturbing activities such as roller-chopping and bush-hogging (Fort Bragg Endangered Species Branch, 2001).

Seeds of *S. pickeringii* var. *pattersonii* may have adaptations to ensure germination in the proper environment. The seed coat inhibits germination, and this dormancy is best overcome with a sand paper shake or acid scarification (Heisler *et al.*, 1999; Todd, 2002). In tissue culture, seeds of *S. pickeringii* var. *pattersonii* were submerged half way into growing medium. When germination occurred, seeds would sink or the radicle would pull them further into the medium until completely covered (Kerber *et al.*, 2002). This response was merely observed but not quantified. The cause or mechanism for this movement has yet to be identified.

The objective of this study was to determine if native soil, greenhouse mixes, and supplemental light conditions contribute to the successful establishment of *S. pickeringii* var. *pattersonii* seedlings in the greenhouse. Other objectives were to determine the effect of photoperiod on side shoot development in soil, to determine how disturbance affects

re-introduced seeds, to identify the stages of seedling development, and to quantify movement of seed and transition zone before shoot initiation.

MATERIALS AND METHODS

Native soil. Seeds were collected from the largest native population in Mason County on August 25, 2000. Seeds were placed in coin envelopes after collection and stored at 4°C and 40–50% relative humidity. On July 11, 2002, seeds were scarified in concentrated sulfuric acid (18.0 M H_2SO_4) for 2 hours and then rinsed with distilled water for 2 minutes. Five seeds each were planted in round pots (22 cm diam. x 37 cm depth) with 100% Universal mix (U), 100% sterilized native sand from *S. pickeringii* var. *pattersonii* populations in Mason County, Illinois (SN), 100% unsterilized native sand (UN), sterilized native sand:Universal mix(1:1) (SN:U), and unsterilized native sand:Universal mix(1:1) (UN:U) with 6 replications of each soil mixture for a total of 30 pots. Universal mix was SunGro (Seneca, Illinois) SB300 containing 5–50% composted pine bark, Canadian sphagnum peat moss, vermiculite, perlite, dolomitic limestone, gypsum, starter nutrient charge, and wetting agent. Native soil was Plainfield sand, 1 to 7 percent slope, of Mason County Illinois. Native soil was sterilized in an autoclave for 2.5 hours at 117,211 Pascal. Ambient irradiance with no supplemental lighting was used from July 2002 to January 2003. Average air temperature of the greenhouse was 21°C. Plants were watered 3 times a week in sunny weather and 2 times a week in cloudy weather. Plants were fertilized with 20-10-20 at 250 mg/l N every other week. Percent emergence, side shoot length, and color ranking were recorded weekly for 9 weeks and then monthly until the end of the experiment at 26 weeks.

Supplemental light. Seeds collected from the largest native population in Mason County in 1999 and 2000 were scarified in June 2001 with concentrated sulfuric acid (18.0 M H_2SO_4) for two hours, rinsed with dionized water, and planted in Cone-tainers (4 cm diam. x 20 cm depth) containing 100% Sunshine mix or coarse silica sand:Sunshine mix (1:1). Sunshine mix was SunGro (Seneca, Illinois) LC1 containing 70-80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, starter nutrient charge, and wetting agent. Plants were transplanted into round pots (22 cm diam. x 37 cm depth) with the same media in October 2001. In July 2002 plants of equal size and cover were selected. Ten were placed under HID and 10 remained in ambient greenhouse light conditions. HID irradiance was from 3 Philips metal halide 1,000-Watt bulbs spaced approximately 1 meter apart and 3 meters above the plants. Each treatment of 100% Sunshine mix and HID light (L), sand:Sunshine mix (1:1) and HID light (S:S,L), 100% Sunshine mix and no HID light (N), or sand:Sunshine mix (1:1) and no HID light (S:S,N) was replicated 5 times for a total of 20 pots containing one plant each. Average air temperature until plants were placed under HID lights was 26°C. Starting on August, 12, 2002, percent cover, number of stems, and shoot length were recorded once a month for 3 months until October 25, 2002. Ambient irradiance from July to October 2002 were used for plants not under HID. For plants not under HID, average monthly air temperature from July to October 2002 was 31°C (maximum) and 21°C (minimum) with an average soil temperature of 26°C at 2.5 cm depth on October 10, 2002. Supplemental HID irradiance emitted 147 $\mu mol\ s^{-1}\ m^{-2}$ for 16 hours from 6:00 am–10:00 pm each day from July to October 2002. For plants under HID light, average monthly air temperature from July to October 2002 was 34°C (maximum) and 16°C (minimum) with an average

soil temperature of 29°C on October 10, 2002. Plants were watered 3 times a week in sunny weather and 2 times a week in cloudy weather. Plants were fertilized with 20-10-20 at 250 mg/l N every other week.

Photoperiod effect on side shoot development. On October 31, 2001, seeds collected on September 7, 2001 were scarified with concentrated sulfuric acid (18.0 M H_2SO_4) for 2 hours and then rinsed with distilled water for 2 minutes. Seeds were dusted with the fungicide Thiram (tetramethylthiuram disulfide) and planted 2.5 cm deep in Sunshine Mix (LC1 SunGro containing 70-80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, starter nutrient charge, and wetting agent) contained in a Cone-tainer (4 cm diam. and 20 cm depth). Half of the Cone-tainers were placed in long days (16-hour day) and half were placed in short days (8-hour day). Fourteen seeds were placed in each of fourteen Cone-tainers, to comprise one replication. Five replications were in each day length. The photoperiod experiment began when natural daylength was over 10 hours (sunrise at 6:21 am and sunset was at 4:51 pm). The long day treatment was provided by supplemental lighting from 60-Watt incandescent lamps strung every 0.3 meters along the bench and one meter above the plants. Lamps were turned on at 5:30 am and off at 9:30 pm daily with an electric time clock. Short days were provided by pulling an opaque black shade cloth over the bench at 4:00 pm and back off the next day at 8:00 am. Average irradiance for the short day bench ranged from 42 $\mu mol\ s^{-1}\ m^{-2}$ on a cloudy day (October 5, 2001) to 434 $\mu mol\ s^{-1}\ m^{-2}$ on a sunny day (November 16, 2002) and for the long day bench from 48 to 252 $\mu mol\ s^{-1}\ m^{-2}$ on the cloudy and sunny days respectively. Average monthly temperatures were 24°C (maximum) and 15°C (minimum). Soil temperature on November 7, 2001 at 2.5 cm depth was 24°C and 25°C

for long and short days, respectively. Plants were watered 3 times a week in sunny weather and 2 times a week in cloudy weather. Plants were fertilized with 20-10-20 at 250 mg/l N every other week. Seedling emergence, number of seedlings with side shoots, and length of side shoots were recorded weekly for 26 weeks. Percentages for emergence and for side shoots were calculated. Data were analyzed with ANOVA using Microsoft Excel (Microsoft Corporation, 2000) and SAS 8.2 (SAS Institute, Inc., 2001). Means are expressed plus or minus standard error.

Disturbance and seedling establishment. On May 7, 2002, plantings were made in the disturbed and undisturbed populations of *S. pickeringii* var. *pattersonii* near Snicarte, Illinois. Plots (1 m^2) were situated within each of the disturbed and undisturbed populations. Half of the plots were planted with 25 *S. pickeringii* var. *pattersonii* seeds/ m^2 and the other half was left unplanted. Treatments were replicated 4 times at each population and were located randomly throughout the replications. Plots were visited on July 10, 2002 and August 21, 2002, when number of seedlings, number of side shoots, number of flowers, and percent cover of *S. pickeringii* var. *pattersonii* were recorded within each 1 m^2 plot.

Seedling development and movement of seed. Fifty-two seeds of *S. pickeringii* var. *pattersonii* collected in fall 2001 were scarified in concentrated sulfuric acid (18.0 M H_2SO_4) for 2 hours and rinsed for 2 minutes in distilled water. Seeds then were surface sterilized by being soaked in 15% bleach (6% sodium hypochlorite) for 20 minutes and rinsed 3 times with sterile water for 30 seconds, 5 minutes, and 5 minutes. Seeds were placed in 25 x 150 mm culture tubes just below the medium orientated with the tapered end up. A horizontal line was drawn on the culture tube to indicate the location of the

base of the seed. The Gelrite medium contained 2 g/L Gelrite, 4.3 g/L Murashige and Skoog salts (JRH Biosciences, Lenexa, KS), 10 ml/L rose vitamins, 0.1 g/L myoinositol, and 30 g/L sucrose. pH was between 5.7 and 5.8. Seeds were germinated in continuous fluorescent light at 5 μmol s^{-1} m^{-2} at 25°C. Contamination was 56%, and germination was 91% of the uncontaminated seeds. Seeds were monitored every 2 days for seed movement and changes in development. Movement of the base of the seed relative to the line was measured, and distinct stages in development were noted. Distinct stages of seedling development were preserved in Formalin-Acetic acid-Alcohol (FAA) fixative (50 cc ethanol [95%], 5 cc glacial acetic acid, 10 cc of formalin [37% formaldehyde], 35 cc deionized water) (Ruzin, 1999).

Movement of transition zone. Based on the stages of seedling development determined in the initial tissue culture experiment, another experiment was designed to measure the movement of the transition zone through the media. The transition zone was defined as the ring around the hypcotyl from where side shoot growth initiated (Photo 1—*see photo in separate file on this CD*). Twenty-five seeds of *S. pickeringii* var. *pattersonii* collected in fall 2001 were acid scarified and surface sterilized with the same protocol as the previous tissue culture experiment. Seeds had the same orientation and placement into the same media as in the previous tissue culture study. Seeds were germinated in 24 hours of light at 5 μmol s^{-1} m^{-2} at 25°C. Contamination was 24%, and germination was 95% of the uncontaminated tubes. Eighteen uncontaminated tubes were marked with a horizontal line where their transition zone appeared. Movement of the base of the transition zone relative to the line was measured once a week for 4 weeks, then once after 2 additional weeks, and finally once after 6 additional weeks.

In all studies, data were analyzed with ANOVA using Microsoft Excel (Microsoft Corporation, 2000) and SAS 8.2 (SAS Institute, Inc., 2001). Mean separations were determined with Duncan's multiple range test at 5% level. Means and percentages were calculated plus or minus standard error.

RESULTS

Native soil. Percent emergence was not affected significantly by media (Table 1). Percent emergence increased steadily for about 20 days and then leveled off (Figure 1). Side shoot length was greatest for plants in U, UN, and SN:U and increased steadily with time (Table 1 and Figure 2). Ranked on a scale from 1 (brown) to 4 (dark green), plants grown in UN were darkest green (Table 1). Figure 3 shows the ranking of seedling color with time. Most seedlings became browner with time.

Supplemental light. Plants in S:S, L produced the largest percent cover and largest number of stems (Table 2). Plants under supplemental lighting had greater increases in percent cover over time (Figure 4). Longest shoots were from plants in S:S, L and L (Table 2). Three plants in the HID lights (2 S:S, L plants and 1 L plant) flowered during the course of this experiment.

Photoperiod on side shoot development. There were no significant differences in overall percent emergence by photoperiod. Both long day and short day seedlings had 50% emergence. Percent emergence with time is shown in Figure 5. Side shoots appeared after day 71 for both long day and short day plants (Figure 6). Short day plants produce significantly more plants with side shoots than long day plants (Table 3). Percentage of plants developing side shoots over time is shown in Figure 6. Short days also had significantly longer side shoots than long day plants (Table 3). An overall increase in side

shoot length for plants in short days occurred while long day side shoots began to turn brown and shrink after day 135 (Figure 7).

Disturbance on seeds. Number of seedlings, number of side shoots, number of flowers, and percent cover for colonies on disturbed and undisturbed sites that were planted and unplanted with seed are shown in Table 4. No significant differences were seen for number of seedlings and number of side shoots between disturbed and undisturbed plots. Both planted and unplanted disturbed plots had significantly higher number of flowers than undisturbed plots. Percent cover was significantly greater for both planted and unplanted plots in disturbed areas when compared to plots in undisturbed areas.

Seedling development and movement of seed. Four distinct stages in seedling development were identified for *S. pickeringii* var. *pattersonii.* At Stage I the seed germinates with radicle emerging at least 2 mm. Germination was 91% with 52% of seeds germinating by day 5. Germination with time is shown in Figure 8. At Stage II, a small ring less than a millimeter thick encircles the radicle. This ring is the transition zone. All transition zones appeared by day 25. At Stage III the cotyledons appear. Eighty-one percent of the germinated seeds developed cotyledons, which were seen before all of the transition zones appeared, on day 22. At Stage IV the side shoot develops from the transition zone. Eighty-five percent of the germinated seeds developed side shoots. Percentages of plants that developed side shoots with time are shown in Figure 9. Length of side shoots with time is shown in Figure 10. Seventy-eight percent of seeds moved a distance of -0.02 ± 0.04 cm perpendicular to the initial horizontal line drawn at the base of the seed.

Movement of transition zone. Fifty-two percent of plants developing transition zones showed movement in these zones. The overall movement of transition zones averaged over time was -1.0 ± 0.1 cm perpendicular to the initial horizontal line drawn at the first appearance of the transition zone. Movement of the transition zone averaged with time is shown in Figure 11.

DISCUSSION

Future culture of *S. pickeringii* var. *pattersonii* in the greenhouse should include Universal Mix in 1:1 combination with sand. Emergence will not be affected. This combination will benefit side shoot length and benefit the overall appearance of the plant, allowing the plant to remain greener. The plants should be grown under supplemental HID lighting to increase percent cover and promote flowering. Native soil is Plainfield sand that is 97% sand, highly permeable, and has a low water holding capacity (Calsyn, 1995). Due to their inability to hold water, sandy soils also contain very few nutrients. A 1:1 combination of Universal Mix and sand is beneficial to growth because it creates a growing media that is well drained yet contains different sized particles to aerate and store nutrients. Plants were able to establish in both sterile and unsterlile mixtures of native sand. This observation supports the findings of Anderson *et al.* (1994) that prairie plants grown in this soil type lack a mycorrhizal response. Seedling establishment of *S. pickeringii* var. *pattersonii* does not rely on soil microbes. HID lighting extends the hours of photosynthetic irradiance available to the plant and could increase net photosynthetic growth. Flowering is most likely related to developmental stage, and plants could be reaching adequate developmental stage faster with the enhanced light conditions.

It should be noted that due to limited greenhouse resources the replications in the photoperiod experiment were considered to be the pots and not the benches. An experimental design with replicated benches may allow for more partitioning of the error due to light and temperature differences on the bench.

Future studies should investigate other possible parameters that might increase growth in *S. pickeringii* var. *pattersonii* plants in the greenhouse. Temperature, fertilizer, and age to maturity are all factors that could affect the establishment and flowering of *S. pickeringii* var. *pattersonii.*

Photoperiod had a significant effect on percent emergence or the development of side shoots. Percentage of plants developing side shoots and side shoot length were promoted by short day conditions. This finding contradicts previous experiments by Kerber *et al.* (2002) in tissue culture where long days promoted side shoot length. It is unknown when seeds germinate and produce side shoots in the field. The seeds have a dormancy, which might require prolonged exposure to blowing sand in order to break the seed coat. Seeds dehiscing in the fall may require time to be scarified by the elements. By spring (short days), seeds may be effectively scarified and able to germinate when moisture and temperature conditions are favorable. If seedlings do emerge and produce side shoots in the shorter days of spring, then plants in the greenhouse could be increasing side shoot length during short days just like plants in the field might. Plants could also be growing slowly and not produce a side shoot until the short days of late fall. A side shoot that initiated below ground in late fall would be protected underground for the winter months. Side shoot development could be affected by a combination of

growing conditions, including moisture, photoperiod, photosynthetic rate, and temperature.

Seedling establishment studies in the field must be repeated with more adequate planting plots chosen. The selection of planting plots was chosen in May before the plants developed shoots, so it was unknown whether the plots were located in areas where *Stylisma pickeringii* var. *pattersonii* was abundant. As a result, the plots in the disturbed area were located in an area where *S. pickeringii* var. *pattersonii* was abundant and the undisturbed plot was located where *S. pickeringii* var. *pattersonii* was not abundant. Results for number of flowers and percent cover of *S. pickeringii* var. *pattersonii* show that the plots were not equally replicated in terms of pre-existing *S. pickeringii* var. *pattersonii* plants. In future studies, plots should be designated the previous season to ensure adequate replication of pre-existing *S. pickeringii* var. *pattersonii* plants.

Experiments on seedling development and movement of seeds were intended as only a trial to determine the stages of seedling development. Other than drawings of seedlings with cotyledons, particular stages of seedling development in *S. pickeringii* var. *pattersonii* had not been fully identified. Given the xeric native habitat of *S. pickeringii* var. *pattersonii*, one would expect this plant to possess adaptations in order to exploit all available moisture for germination and seedling development. In the field, side shoots consistently originate below the soil surface. Any seed or transition zone movement that provided the plant with more available moisture presumably would be diminished in tissue culture where germination and seedling development conditions are ideal. In addition to providing moisture, this adaptation also may help with stability in shifting

sand. This study has provided preserved specimens for future histological studies that might determine whether the transition zone is pushed down by the elongation of the cotyledons or pulled down by the radicle.

Table 1. Percent emergence (at end of study), side shoot length (over time), and color rating (over time) of *Stylisma pickeringii* var. *pattersonii* in different growing media.

Growing media[w]	Emergence (%)	Side shoot length (cm)	Color rating[x]
U	83.3 ± 13.1[y] a[z]	8.0 ± 0.6 a	2.5 ± 0.2 c
UN	73.3 ± 8.2 a	7.8 ± 0.5 a	3.4 ± 0.2 a
SN:U	73.3 ± 9.8 a	7.3 ± 0.4 a	3.2 ± 0.2 a b
UN:U	63.3 ± 10.7 a	6.8 ± 0.3 a b	2.9 ± 0.2 a b c
SN	57.6 ± 13.1 a	5.6 ± 0.5 b	2.6 ± 0.3 b c

[w]Growing Media: U=100% Universal mix, UN=100 % unsterilized native sand, SN:U=sterilized native sand:Universal mix (1:1), UN:U=unsterilized native sand:Universal mix (1:1), and SN=100% sterilized native sand.

[x]Color Ranking of seedlings: 4=Dark Green, 3=Green to Yellow, 2=Entirely Yellow, 1=Brown or Dead.

[y]Means are expressed ± standard error.

[z]Mean separation within column based on Duncan's multiple range test at 5%, n=30. Means followed by different letters are significantly different.

Table 2. Averages over time for percent cover, number of stems, and stem length of *Stylisma pickeringii* var. *pattersonii* plants in different growing media and lighting conditions in fall 2002.

Growing Media	Light	Cover (%)	Number of stems	Shoot length (cm)
(S:S, L) Sand:Sunshine Mix (1:1)	HID	48 ± 10[y] a[z]	12.1 ± 2.5 a	28.4 ± 4.4 a
(L) 100% Sunshine Mix	HID	25 ± 3 b	5.6 ± 1.2 b	25.2 ± 1.9 a
(S:S, N) Sand:Sunshine Mix (1:1)	No HID	5 ± 1 c	2.6 ± 0.5 b c	10.6 ± 2.0 b
(N) 100% Sunshine Mix	No HID	1 ± 0 c	0.6 ± 0.2 c	2.0 ± 0.7 c

[y]Means are expressed ± standard error.

[z]Mean separation within column based on Duncan's multiple range test at 5%, n=15. Means followed by different letters are significantly different.

Table 3. Averages over time for percent of *Stylisma pickeringii* var. *pattersonii* plants developing side shoots and side shoot length under short day (8-hour day) and long day (16-hour day) conditions in the greenhouse.

Photoperiod	% of plants developing side shoots	Side shoot length (cm)
Short day	70 ± 4[y] a[z]	3.4 ± 0.3 a
Long day	50 ± 3 b	2.5 ± 0.2 b

[y]Means are expressed ± standard error.
[z]Mean separation within column based on Duncan's multiple range test at 5%, n=50. Means followed by different letters are significantly different.

Table 4. Number of seedlings, number of side shoots, number of flowers, and percent cover of *Stylisma pickeringii* var. *pattersonii* (averaged for July 10, and August 21, 2002 sampling dates) within 1 m^2 plots in disturbed and undisturbed colonies that were planted and unplanted with *S. pickeringii* var. *pattersonii* seed on May 7, 2002.

Site	Planting	Number of seedlings	Number of side shoots	Number of flowers	Cover (%)
Disturbed	Planted	3.4 ± 2.7[y] a[z]	0.0 ± 0.0 a	64.0 ± 28.6 a	33.8 ± 7.0 a
Disturbed	Unplanted	2.4 ± 1.3 a	0.0 ± 0.0 a	65.9 ± 29.5 a	28.8 ± 5.0 a
Undisturbed	Planted	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Undisturbed	Unplanted	0.1 ± 0.1 a	0.1 ± 0.1 a	3.0 ± 2.0 b	0.5 ± 0.2 b

[y]Means are expressed ± standard error.
[z]Mean separation within column based on Duncan's multiple range test at 5%, n=45. Means followed by different letters are significantly different

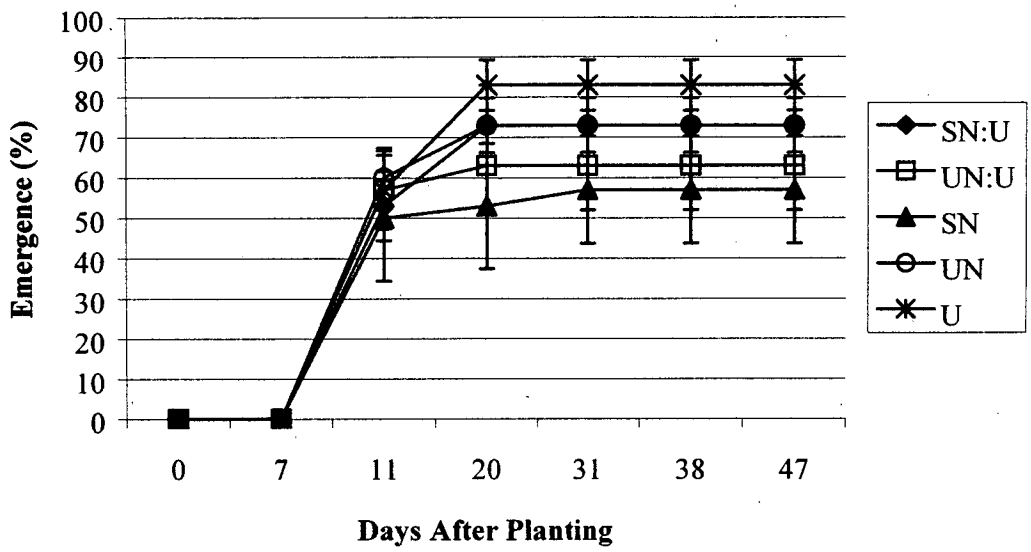

Figure 1. Emergence of *Stylisma pickeringii* var. *pattersonii* seedlings in different growing media—SN:U=sterilized native sand:Universal mix (1:1), UN:U=unsterilized native sand: Universal mix (1:1), SN=100% sterilized native sand, UN=100% unsterilized native sand, U=100% Universal mix. Means calculated ± SE.

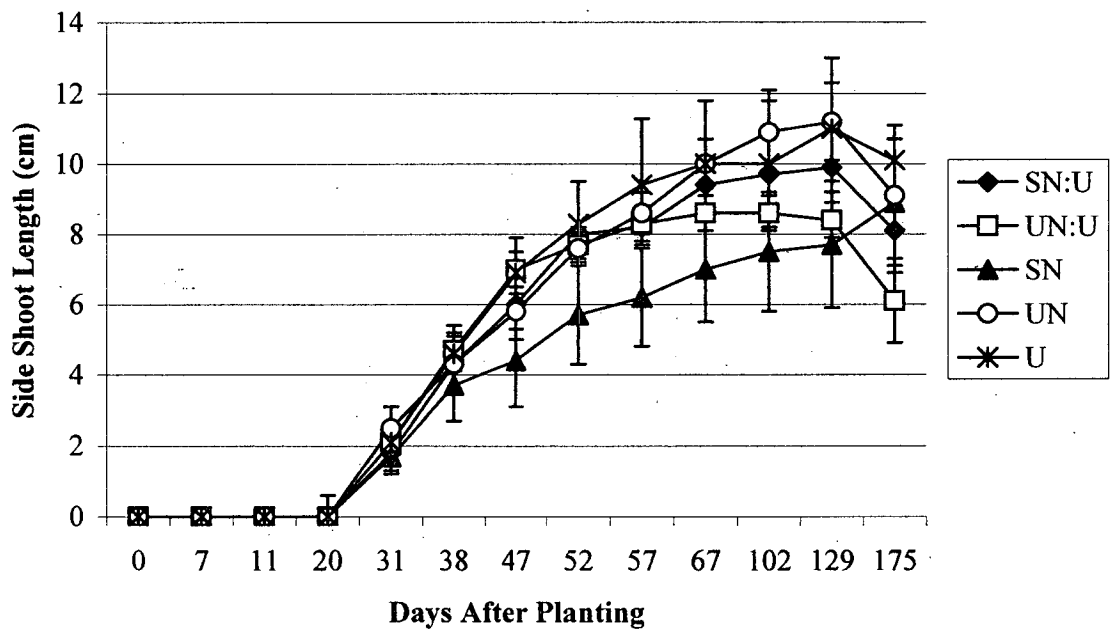

Figure 2. Side shoot length of *Stylisma pickeringii* var. *pattersonii* in different growing media—SN:U=sterilized native sand:Universal mix (1:1), UN:U=unsterilized native sand: Universal mix (1:1), SN=100% sterilized native sand, UN=100% unsterilized native sand, U=100% Universal mix. Means calculated ± SE.

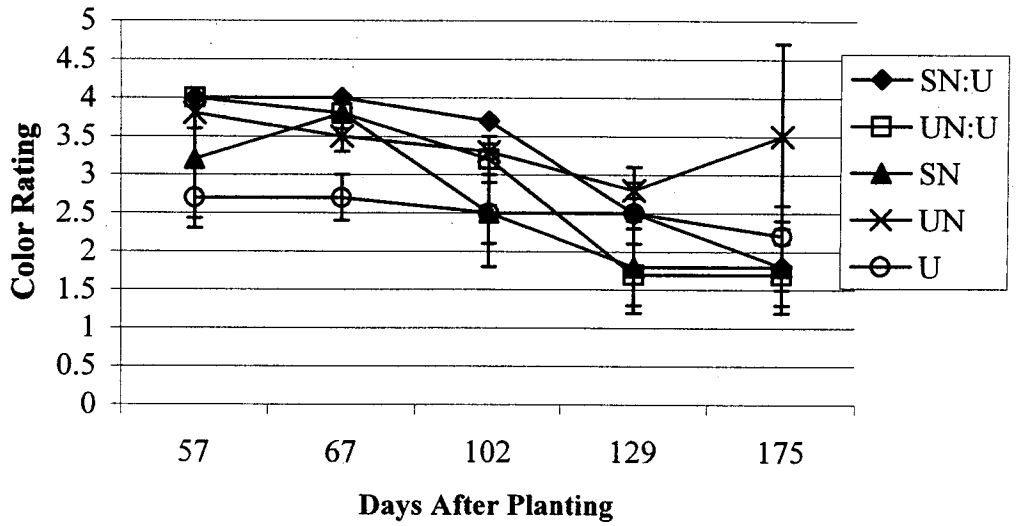

Figure 3. Change in color ranking of *Stylisma pickeringii* var. *pattersonii* seedlings in different growing media—SN:U=sterilized native sand:Universal mix (1:1), UN:U=unsterilized native sand: Universal mix (1:1), SN=100% sterilized native sand, UN=100% unsterilized native sand, U=100% Universal mix. Color Ranking of seedlings: 4=Dark Green, 3=Green/Yellow, 2=Yellow, 1=Brown/Dead. Means calculated ± SE.

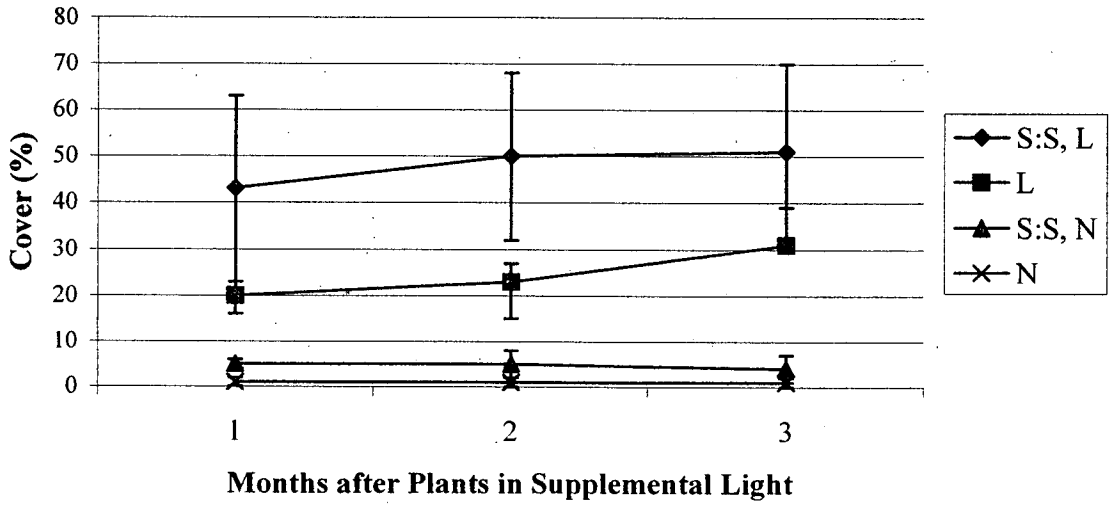

Figure 4. Percent cover with time for *Stylisma pickeringii* var. *pattersonii* plants in different media and lighting conditions—S:S, L= 1:1 sand:soilless mix, HID lights; L= 100% soilless mix, HID lights; S:S, N= 1:1 sand: soilless mix, no HID; N= 100% soilless mix, no HID. Means calculated ± SE.

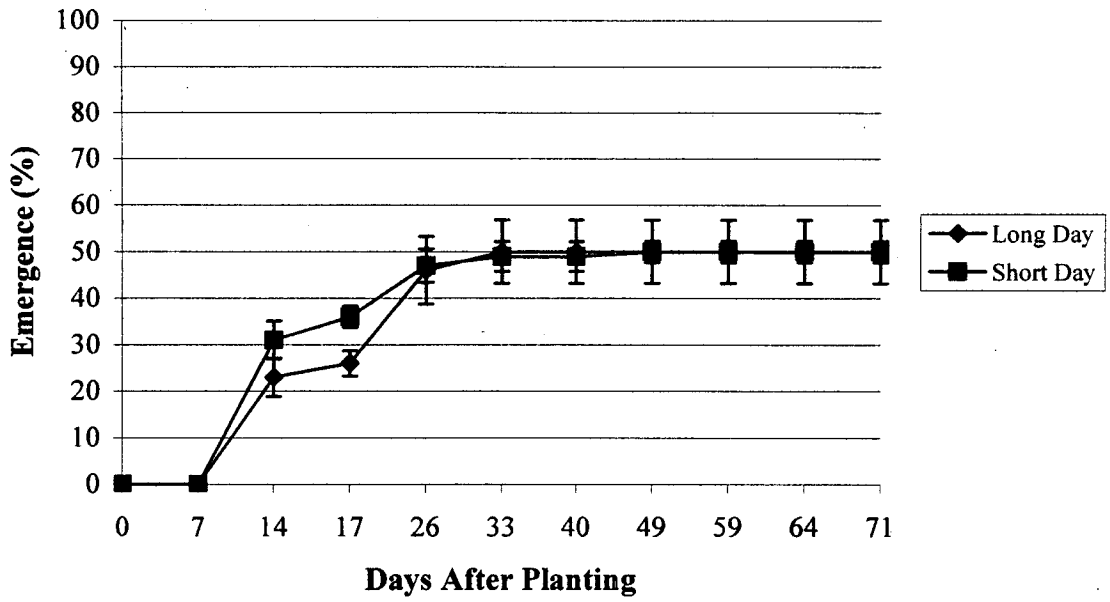

Figure 5. Emergence rate of *Stylisma pickeringii* var. *pattersonii* seeds in long day (16-hour day) and short day (8-hour day) conditions in the greenhouse. Means calculated ± SE.

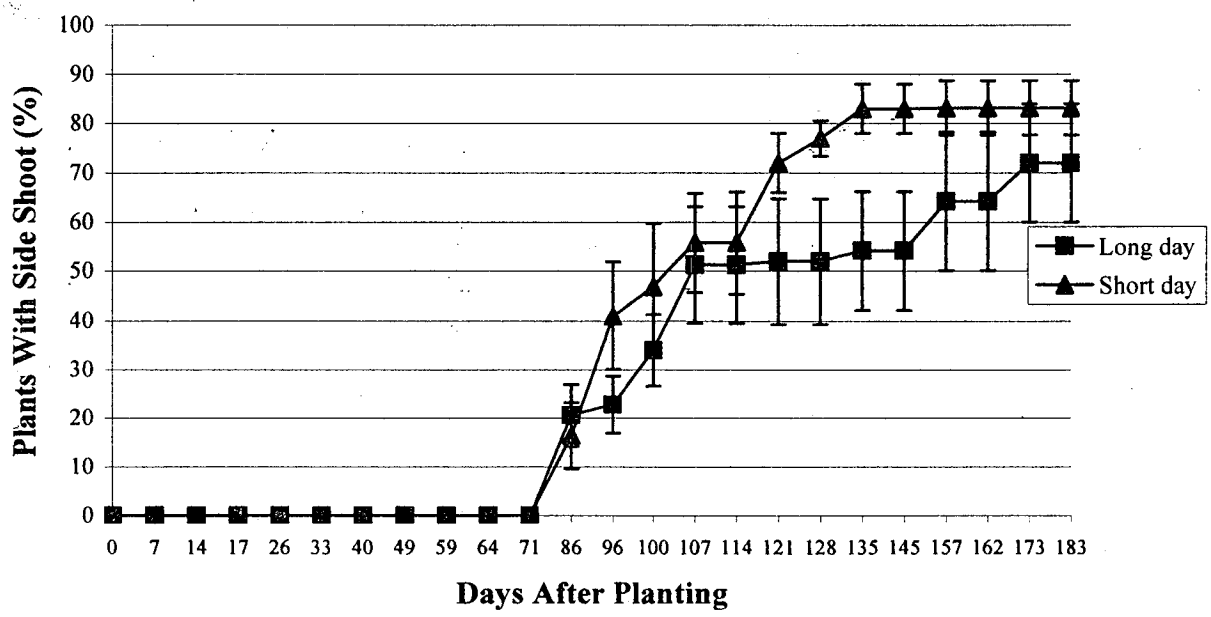

Figure 6. Percentage of *Stylisma pickeringii* var. *pattersonii* plants that developed side shoots 0 to 183 days after planting in long day (16-hour day) and short day (8-hour day) conditions in the greenhouse. Means calculated ± SE.

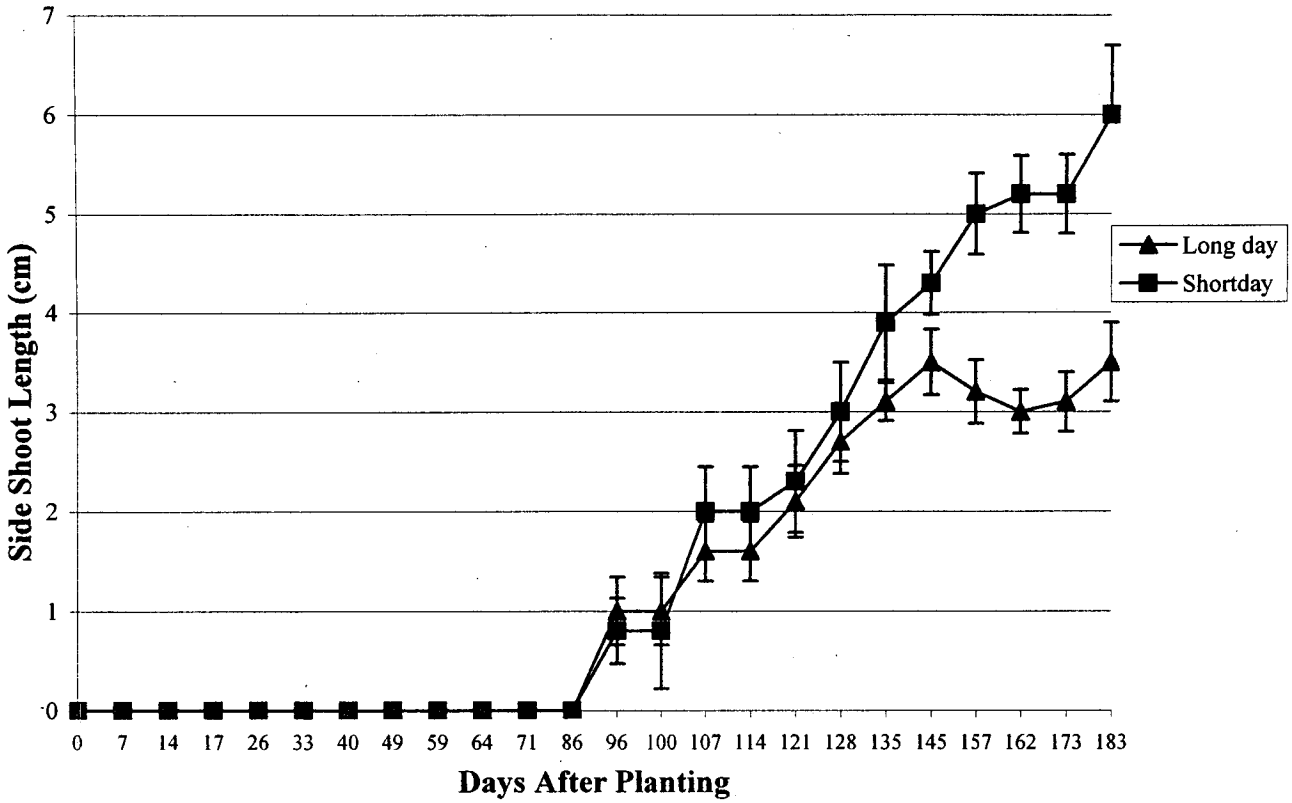

Figure 7. Length of *Stylisma pickeringii* var. *pattersonii* side shoot at 0 to 183 days after planting in long day (16-hour day) and short day (8-hour day) conditions in the greenhouse. Means calculated ± SE.

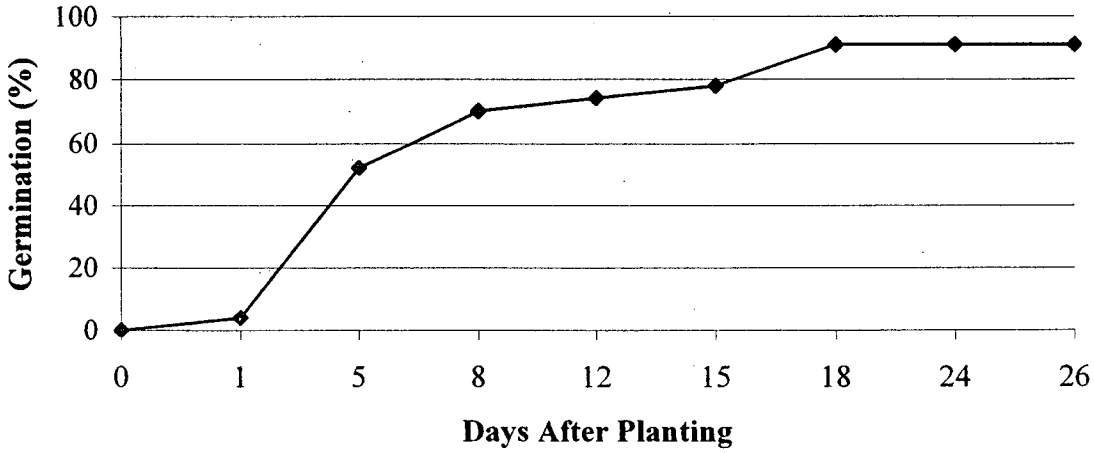

Figure 8. Germination of *Stylisma pickeringii* var. *pattersonii* seeds in tissue culture used to monitor seedling development and movement of seed.

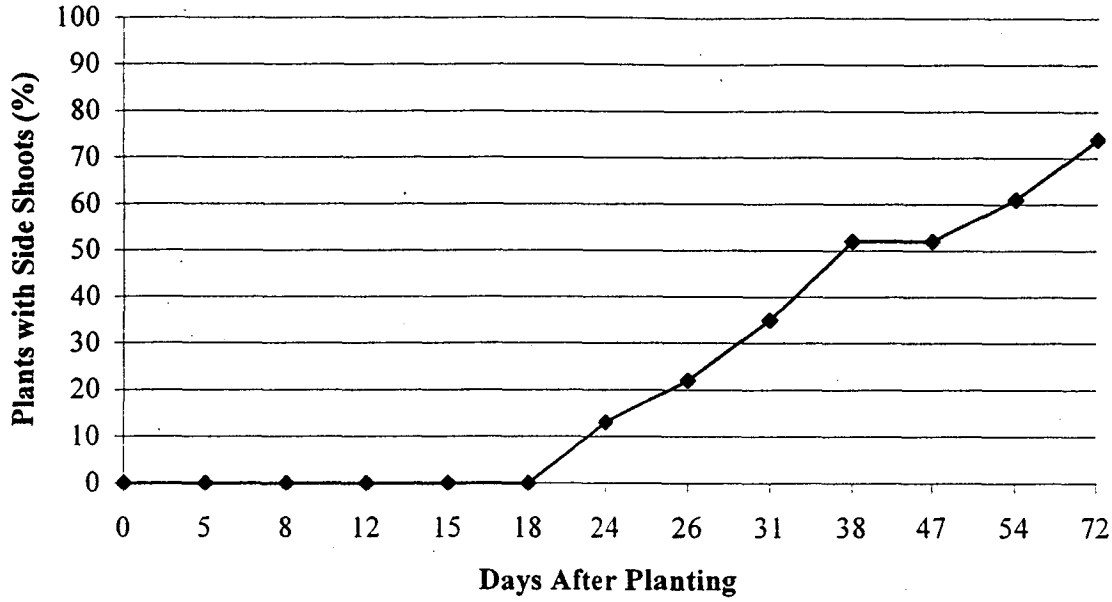

Figure 9. Percentage of *Stylisma pickeringii* var. *pattersonii* plants to develop side shoots 0 to 72 days after planting in tissue culture.

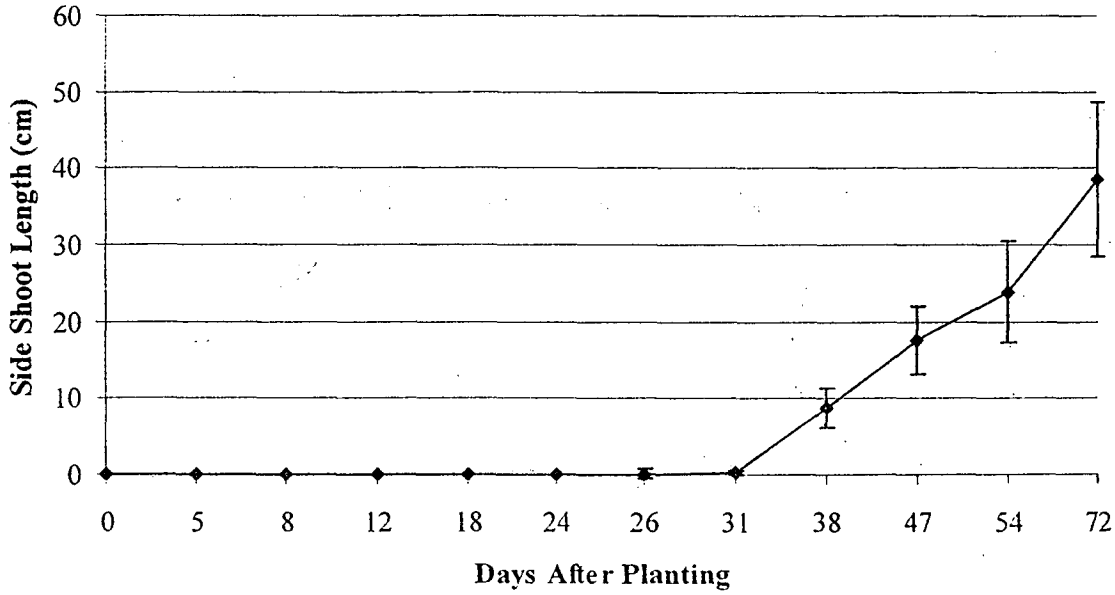

Figure 10. Length of *Stylisma pickeringii* var. *pattersonii* side shoot at 0 to 72 days after planting in tissue culture. Means calculated ± SE.

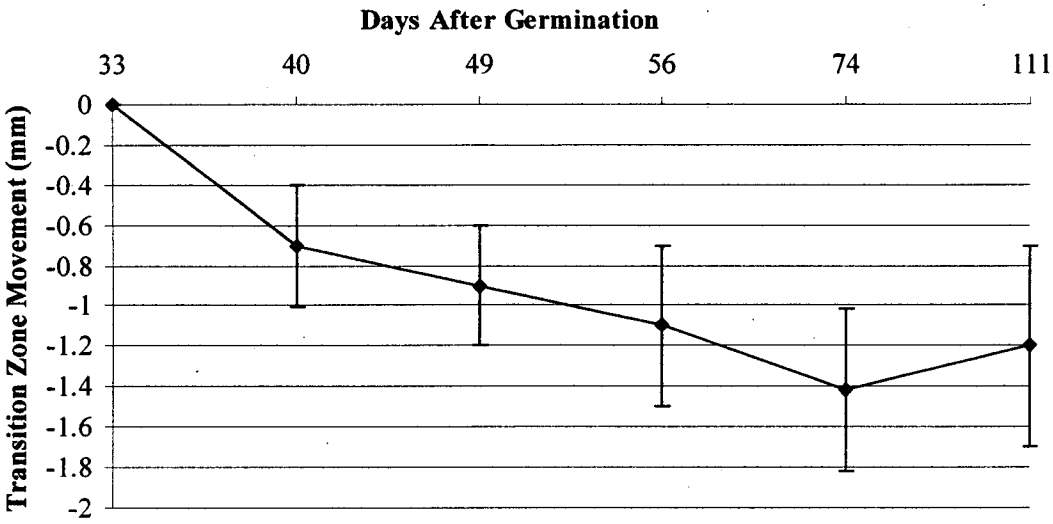

Figure 11. Transition zone movement in *Stylisma pickeringii* var. *pattersonii* from 33 to 111 days after planting in tissue culture. Means calculated ± SE.

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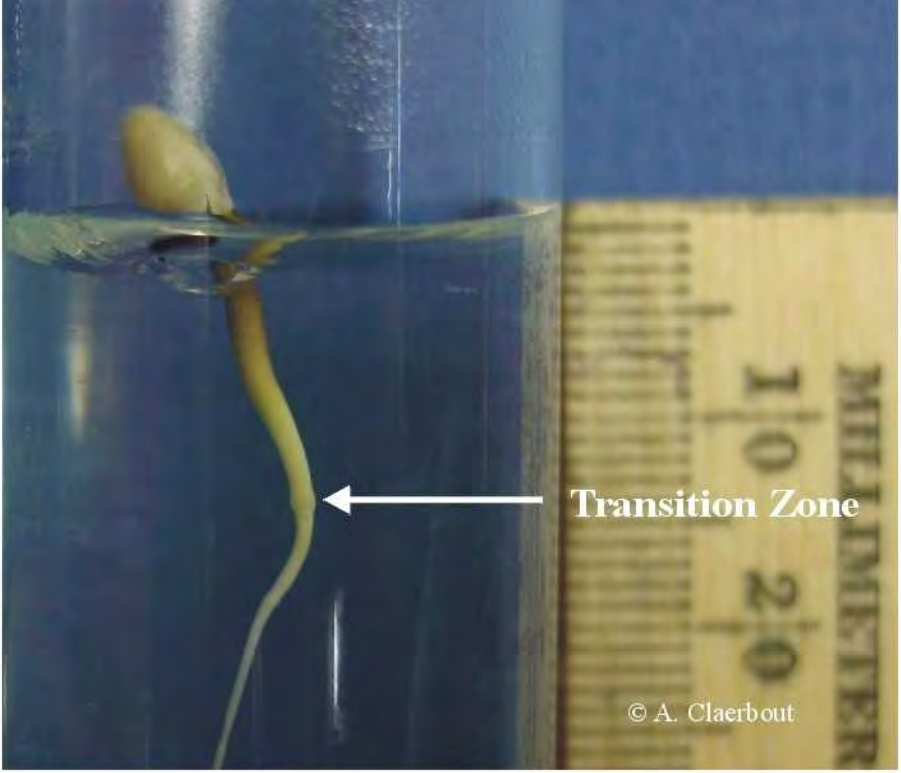

Photo 1. Transition zone in a seedling of *Stylisma pickeringii* var. *pattersonii* grown in tissue culture.