01-638W

Reproductive Potential of the Endangered Species *Stylisma pickeringii*

Henry R. Owen[1], Janice M. Coons[2], Brent L. Todd[1], Christina J. Heisler[1], Angela J. Kerber[1], and Kevin P. Donnelly[2]

[1] *Department of Biological Sciences, Eastern Illinois University, Charleston 61920*

[2] *Dept. of Natural Resources and Environmental Sciences, University of Illinois, Urbana 61801*

SUMMARY.—*Stylisma pickeringii*, Patterson bindweed (Convolvulaceae) is an Illinois-endangered sand prairies species. Management and reintroduction efforts are limited by a lack of knowledge about the reproductive biology of this plant. Consequently, management of Illinois sand prairies could be improved by providing information regarding endangered sand prairies species. The objective of this study is to investigate the requirements of *S. pickeringii* for seed germination and seedling development. To study seed biology, germination in relation to harvest year (1998, 1999, and 2000), seed color (yellow, tan, and maroon), and scarification was studied. The effect of seed age and harvest date on seed color also was studied. To study seedling development, the emergence and morphology of seedlings were observed. More yellow seeds were produced than tan and maroon in all harvest years. Scarification affects germination of *S. pickeringii* seeds. In 1998 and 1999, more yellow seeds germinated than tan and maroon seeds. More yellow seeds germinated in 2000 (100%) than in the previous harvest years. In 1999, a new seed color (green) was observed, but only at the earliest collection date. Early on, most seeds were green, but the numbers declined until none were observed 9 weeks after flowering. The number of yellow seeds increased between 3 and 9 weeks after flowering. Maroon seeds did not show up until 9 weeks after flowering. In 2000 tan seeds were dominant only 4 weeks after flowering; yellow seeds were dominant 7 weeks after flowering. Maroon seeds were observed both 4 and 7 weeks after flowering. There are considerably more yellow seeds than other colors at both dates. Most seedlings were observed in the disturbed site in early July. Measurements of component parts (root tip to side branch, side branch, main branch, maroon area, and cotyledon) are 11.7, 6.1, 7.2, 1.7, and 2.1 cm, respectively. Seedling density of undisturbed and disturbed sites ranged from 0-0.16 seedlings/m^2 and 0.08-1.84 seedlings/m^2, respectively. More yellow seeds develop and have higher germination than tan and maroon seeds. The seed coat inhibits germination as scarification increases germination. Effects of seed age and harvest date on seed color were inconclusive. Green seeds are most likely immature. *S. pickeringii* develops an extensive taproot before developing its shoots; its shoot branches originate 7.2 cm below the soil surface. The mature plant develops from side shoots that arise below the soil surface. In plant tissue culture, cytokinins increase shoot initiation. Thus, an additional study was conducted to determine how cytokinins affect *Stylisma* shoot development. Side shoot development was shown to increase with 1.0 mg/l benzylaminopurine (BAP) after 3-6 weeks *in vitro*. The peak vegetative growth period for *S. pickeringii* is in June and July, when photoperiods are long, and so an additional study was conducted to determine if long photoperiods stimulate lateral shoot formation. More growth of lateral shoots was observed for plants grown in tissue culture under a 16 h photoperiod than an 8 h photoperiod (47% vs. 27%). Plants grown under the longer photoperiod demonstrated lateral shoot growth at an average of 5.5 cm during the six week growing period, whereas plants grown under the shorter photoperiod had an average lateral shoot growth of only 4.0 cm. These studies have provided knowledge about the seed and seedling biology of *S. pickeringii*, which will be useful in the management of this species.

INTRODUCTION

Although Illinois sand prairies are limited throughout the state, many unique plant species are found in the habitat. Management of Illinois sand prairies could be improved by providing information regarding endangered sand prairie species. *Stylisma pickeringii*, Patterson bindweed (Convolvulaceae), is an Illinois-endangered sand prairie species found along the Illinois and Mississippi Rivers in three Illinois counties—Cass, Henderson, and Mason (Herkert, 1991). Currently all known populations of *S. pickeringii* are located on privately owned land, thus subjecting it to possible extirpation. Typically, *S. pickeringii*, a perennial prostrate or diffusely spreading herb grows in the south-central United States (Herkert, 1991). Management and reintroduction efforts are limited by a lack of knowledge about the reproductive biology of *S. pickeringii*. The objective of this study is to investigate the requirements of *S. pickeringii* for seed germination and seedling development.

MATERIALS AND METHODS

To study seed biology, germination in relation to harvest year, seed color, and scarification was studied. Seeds of *S. pickeringii* are of three colors—yellow, tan, and maroon (Heisler *et al.*, 1999). The effect of seed age and harvest date on seed color was studied. To study seedling development, the emergence and morphology of seedlings were studied. In Mason County, the *S. pickeringii* population is divided into two sites that are treated differently—one subjected to occasional disturbance due to its location in an agricultural field, the other relatively undisturbed.

Seeds

Germination.—Fruits of *S. pickeringii* were collected during the summers of 1998, 1999, and 2000 near Snicarte (Mason Co.), Illinois. Seeds of all three colors were scarified. Following scarification 5 seeds/dish, 4 dishes/treatment were dusted with the fungicide Thiram (tetramethylthiram disulfide, 50%) placed on two sheets of filter paper (Whatman #1) in 90 mm glass petri plates with 5 ml distilled water in plastic tubs (to reduce evaporation) and placed in an environmental control chamber at 25 C, 16 h photoperiod with a mean light intensity of 51 $\mu Em^{-2}s^{-1}$. Seeds were considered germinated when the radicle emerged from the seed at least 2 mm. Germination of seeds harvested in different harvest years (1998, 1999, and 2000), three colors (yellow, tan, and maroon), and scarified/unscarified seeds was compared.

Seed color.—In 1999 and 2000 *S. pickeringii* flowers were tagged and the seeds collected 3, 6, or 9 weeks after flowering (1999) or 4 or 7 weeks after flowering (2000). The seed colors from each year and time after flowering were recorded. In 1999 seeds were harvested on two different dates. The seed colors were recorded and the seed color on each harvest date compared.

Seedlings

Seedling density.—In 2000 a 50 m transect was placed in each of the two sites (disturbed and undisturbed). Three times throughout the growing season the number of seedlings observed at each meter in ½ x ½ m quadrats placed various distances away from the transect (determined by a random numbers table) was recorded. The number of seedlings/m^2 was compared in each site.

Seedling size.—Ten seedlings were removed from the sandy soil and the component parts (root tip to side branch, side branch, main branch, maroon area, and cotyledon) measured.

In Vitro Cultures

Photoperiod studies – For the photoperiod studies, two growth chambers were used, one set at a 16 h light/8 h dark photoperiod, the other at 8 h light/16 h dark photoperiod. The experiment was replicated after switching the respective photoperiods in the two chambers to eliminate a potential chamber effect.

Cytokinin Studies – Seeds were scarified in concentrated sulfuric acid for 2 h, and rinsed with deionized water. Next, seeds were disinfested in 15% bleach (0.8% sodium hypochlorite solution) for 20 minutes, followed by three sterile water rinses before being placed *in vitro*. Seeds were cultured on Murashige & Skoog basal medium containing 0, 0.1 mg/l, 1.0 mg/l, or 10.0 mg/l benzylaminopurine (BAP) for twelve weeks in a culture room (20 um/m^2/sec) at 20C).

RESULTS

More yellow seeds were produced than tan or maroon in all harvest years (Figure 1). Scarification affects germination of *S. pickeringii* seeds (Todd *et al.*, 2001). In 1998 and 1999, more yellow seeds germinated than tan or maroon seeds. More yellow seeds germinated in 2000 (100%) than in the previous harvest years; there is no data for tan or maroon seeds harvested in 2000 (Figure 2). In 1999 a new seed color (green) was observed. Early in the growing season most seeds were green, but the numbers declined until none were observed 9 weeks after flowering. The number of yellow seeds increased between 3 and 9 weeks after flowering. Maroon seeds did not show up until 9 weeks after flowering (Figure 3). In 2000 tan seeds were dominant only 4 weeks after flowering; yellow seeds were dominant 7 weeks after flowering. Maroon seeds were observed both 4 and 7 weeks after flowering. There are considerably more yellow seeds than other colors at both collection dates (Figure 4). Seed color percentages were not influenced by harvest date (Figure 5). Most seedlings were observed in the disturbed site and in early July (Figure 6). Measurements of component parts (root tip to side branch, side branch, main branch, maroon area, and cotyledon) are 11.7, 6.1, 7.2, 1.7, and 2.1 cm, respectively (Table 1). Seedling density ranged from 0-0.16 seedlings/m^2 in the undisturbed site and 0.08-1.84 seedlings/m^2 in the disturbed site (Table 2). Lateral shoot development under long (16 h light) and short (8 h light) photoperiods indicates that long photoperiods, characteristic of June and July in Illinois, stimulate increased lateral shoot development (Figures 7 and 8). The effect of cytokinin concentration on the number of side shoots initiated *in vitro* indicates that 1.0 mg/l BAP stimulates lateral shoot formation (Figure 9).

CONCLUSIONS

Yellow seed germination is significantly higher than that of tan and maroon seeds. More yellow seeds develop than tan and maroon. The seed coat inhibits germination as scarification increases germination (Todd *et al.*, 2001). Effects of seed age and harvest date on seed color were inconclusive. Green seeds were most likely immature seeds. *S. pickeringii* develops an extensive taproot before developing its shoots. Its shoot branches originate 7.2 cm below the soil surface. Lateral shoot formation is stimulated by longer photoperiods and after cytokinin treatment. This research has provided knowledge about the seed and seedling biology of *S. pickeringii*, which will be useful in the propagation of this species and could provide additional knowledge for the management of Illinois sand prairies.

Acknowledgments.—The authors wish to thank the Illinois Department of Natural Resources (IDNR), Bill McClain of IDNR, Eastern Illinois University (EIU) Honors Program, Council on Faculty Research (EIU), Special Undergraduate Research Experience (UIUC), Jim Davidsmeyer, landowner, and Ed Kramer, landowner.

LITERATURE CITED

Herkert, J.R. 1991. Endangered and threatened species of Illinois: status and distribution, volume 1-plants. Illinois Endangered Species Protection Board, Springfield, IL. 158 p.

Heisler, C.J., M.L. Rycerz, J.M. Coons, H.R. Owen, and W.E. McClain. 1999. Seed color and mechanical scarification affect germination of Paterson bindweed (*Stylisma pickeringii*). *Trans. Ill. State Acad. Sci.*, 90:71.

Todd, B.L., J.M Coons, and H.R. Owen. 2001. Scarification technique affects germination of *Stylisma pickeringii* (Patterson bindweed), an Illinois endangered plant. Submitted to *American Midland Naturalist*.

BUDGET

Travel	$605.29
Commodities	$ 46.71
Meals	$ 48.00
Student Help	$300.00
Total	$1000.00

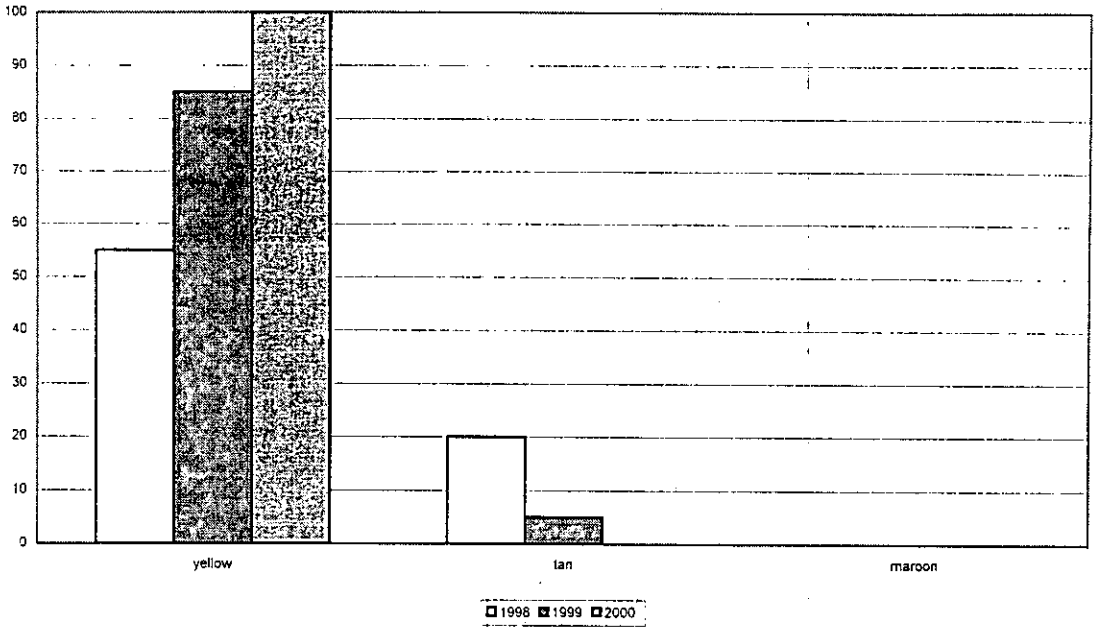

Figure 1: Percent germination of three colors of scarified *S. pickeringii* seeds harvested in 1998, 1999, and 2000.

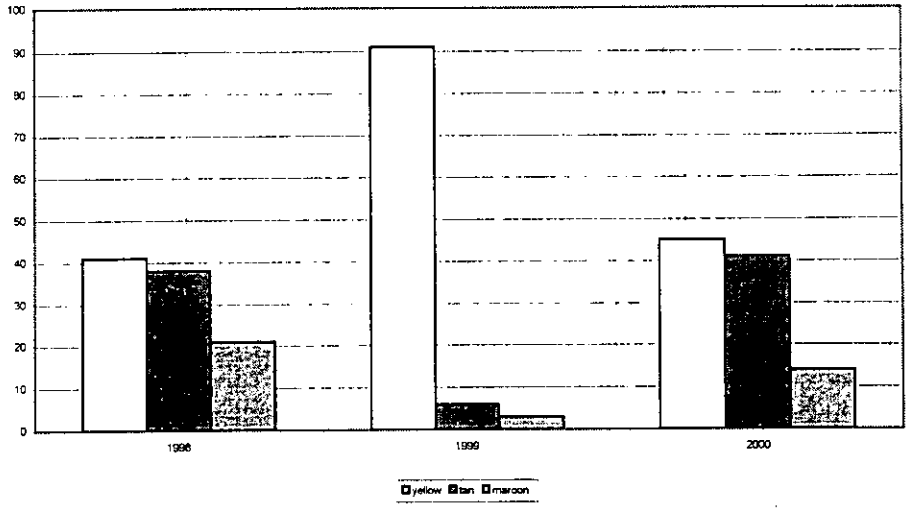

Figure 2: Percent of three colors of *S. pickeringii* seeds harvested in 1998, 1999, or 2000.

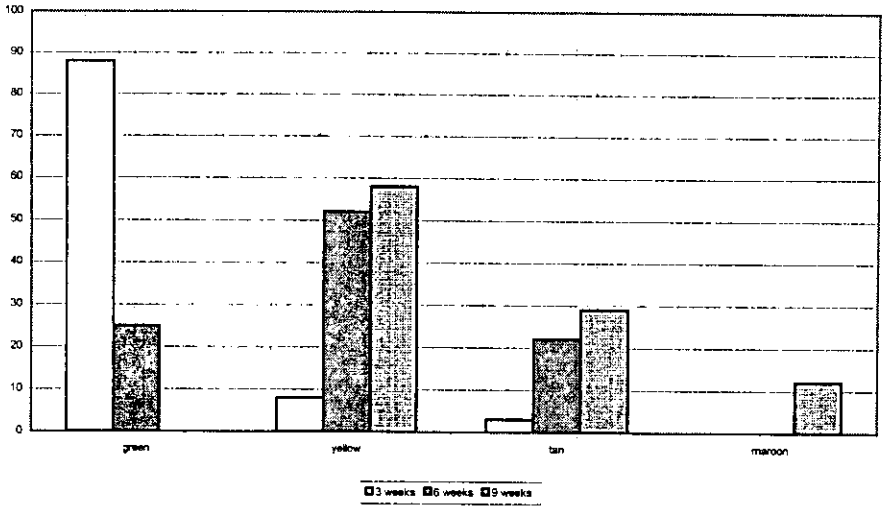

Figure 3: Percent color of *S. pickeringii* seeds 3, 6, or 9 weeks after flowering in 1999.

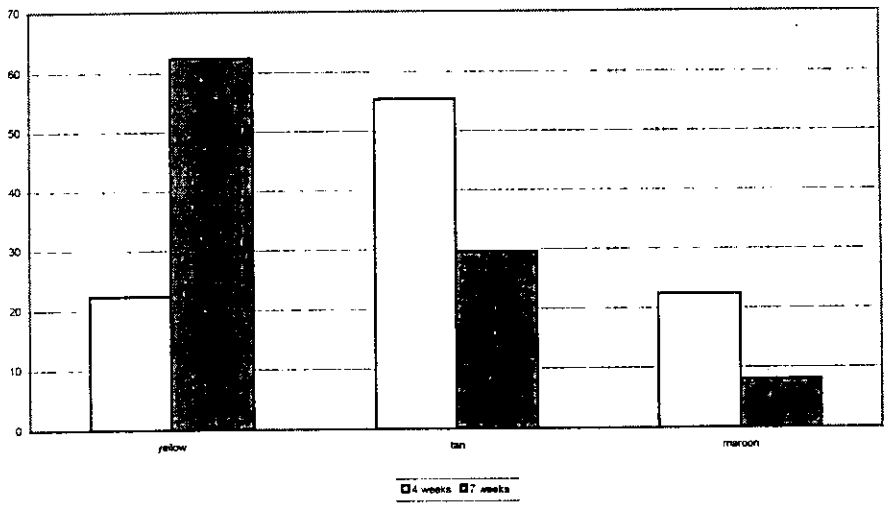

Figure 4: Percent color of *S. pickeringii* seeds 4 or 7 weeks after flowering in 2000.

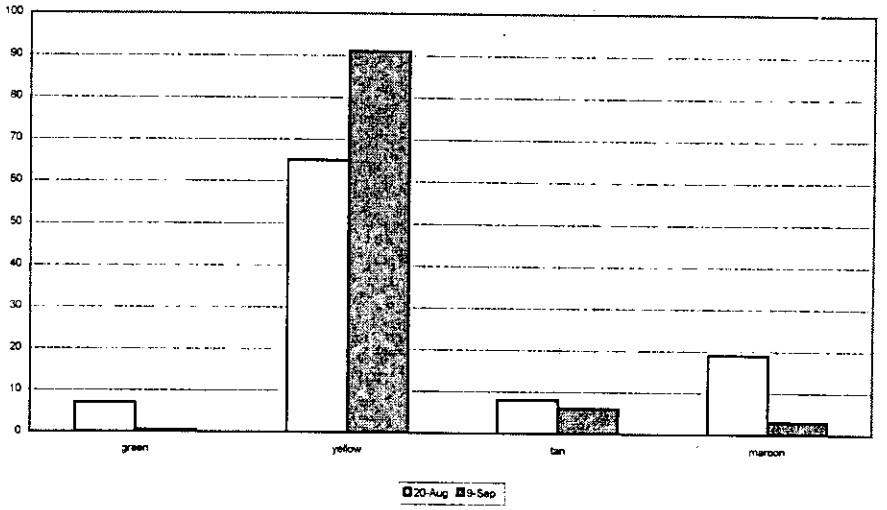

Figure 5: Seed color percentages on different harvest dates, 1999.

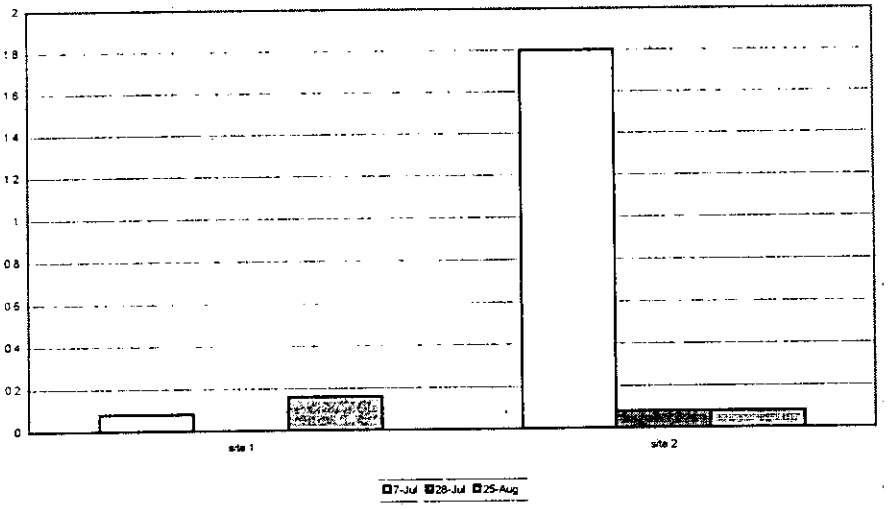

Figure 6: Seedling density (plants/m^2) over summer 2000.

Table 1: Average length (cm) of component parts of 10 *Stylisma pickeringii* seedlings.

Component part	Average length (cm)
Root tip to side branch	11.7
Side branch	6.1
Main branch	7.2
Maroon area	1.7
Cotyledon	2.1

Table 2: Number of seedlings/m^2 of *Stylisma pickeringii* in two different sites (disturbed and undisturbed) throughout the summer of 2000.

Date	Site	Seedlings/m^2
7 July 2000	Disturbed	1.84
28 July 2000	Disturbed	0.08
25 August 2000	Disturbed	0.08
7 July 2000	Undisturbed	0.08
28 July 2000	Undisturbed	0.00
25 August 2000	Undisturbed	0.16

Figure 7: Lateral shoot development of *S. pickeringii* 16 hour (LD) and 8 hour (SD) photoperiods, first replication.

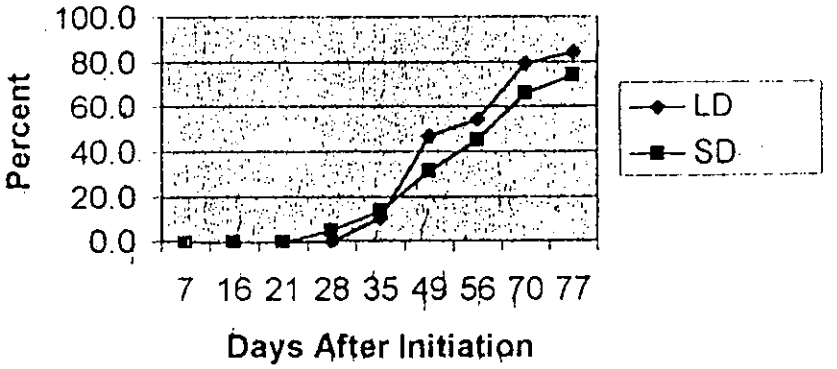

Figure 8: Lateral shoot development of *S. pickeringii* 16 hour (LD) and 8 hour (SD) photoperiods, second replication.

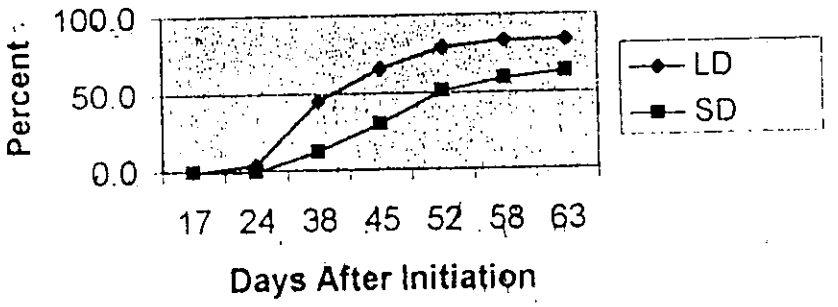

Figure 9: Effect of benzylaminopurine (BAP) concentration on lateral shoot formation of *S. pickeringii*.