Sun, Sand and *Stylisma*:

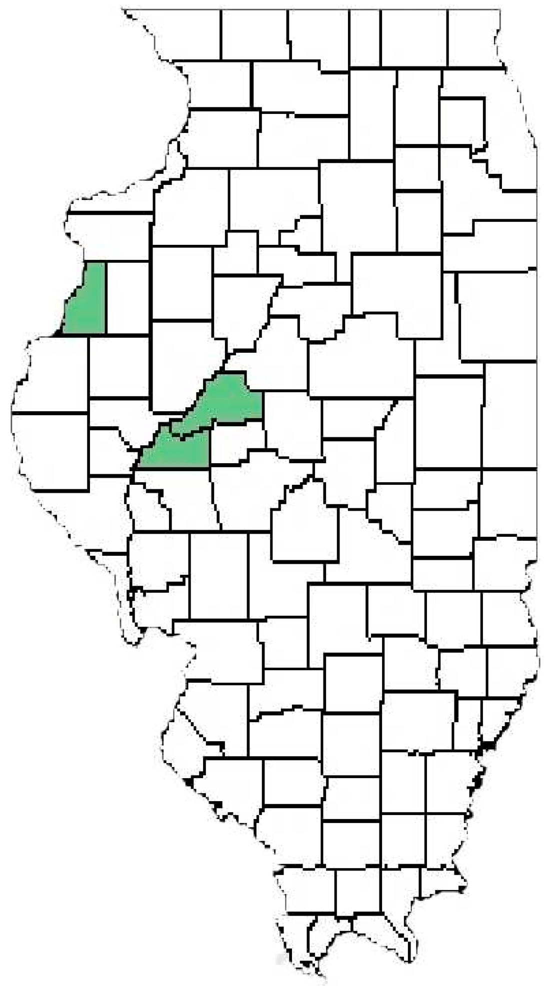
INFLUENCE OF LIGHT AND SUCROSE ON *IN VITRO* DEVELOPMENT OF SHOOTS IN *STYLISMA PICKERINGII* VAR. *PATTERSONI*

Brent E. Wachholder[1], Angela J. Kerber[1,2], Henry R. Owen[1], and Janice M. Coons[1]

[1] Eastern Illinois University, Charleston, IL 61920

[2] University of Illinois at Urbana-Champaign, Urbana, IL 61801

TOP: *Stylisma pickeringii* var. *pattersoni* growing in the wild. ABOVE: *S. pickeringii* coyledons emerging from sand. BELOW: The range of *S. pickeringii* in Illinois (Cass, Henderson, and Mason counties).



Abstract

Stylisma pickeringii (Torr.) Gray var. *pattersoni* (Fern. & Schub.) Myint (Convolvulaceae) is an Illinois-endangered perennial vine. Seedlings of *S. pickeringii* show a distinctive growth pattern. The cotyledons and hypocotyls become necrotic and wither after reaching the surface, while lateral shoots responsible for above-ground growth form 7-8 cm below the soil surface. The objectives of this study were to determine how light, photoperiod, and sucrose affect shoot initiation and growth. Plants were grown from seed in vitro on Murashige and Skoog medium with long-day (16 hours light/ 8 hours darkness) and short-day (8 hours light/ 16 hours darkness) photoperiods. Plants also were grown with long-day photoperiods or in continuous darkness, both treatments using media containing 0, 10, 20, or 30 g/l sucrose. Plants grown with long-day photoperiods had significantly increased lateral shoot initiation and lateral shoot length compared to plants grown with short-day photoperiods. Plants grown in darkness showed no lateral shoot development. Sucrose concentration had no significant effect on shoot growth in plants grown in the light. Higher sucrose concentrations significantly increased hypocotyl plus cotyledon length of plants grown in darkness. Hence, lateral shoot initiation and growth are light-dependent, but are not determined by sucrose concentration

Introduction

Stylisma pickeringii var. *pattersoni* is a rare perennial vine in the Convolvulaceae that is native to well drained, sandy open woods and dry prairies (Myint, 1966). *Stylisma pickeringii* var. *pattersoni* is endangered in Illinois and is represented by only five small populations in sand prairies near the Illinois and Mississippi rivers (Herkert and Ebinger, 2002).

Stylisma pickeringii var. *pattersoni* has thin, deep-penetrating, non-tuberous roots (Myint, 1966). The cotyledons of germinating seeds die back after they grow above the soil, and a thickened taproot develops before lateral shoots grow to the surface. These shoots were found to originate 7.2 cm below the soil surface (Todd *et al.*, 2001a). The objectives of this study were to determine how light, photoperiod, and sucrose affect shoot initiation and growth.

Materials and Methods

Seeds of *Stylisma pickeringii* var. *pattersoni* were obtained from a population on private land near Snicarte, Illinois, as part of a previous study (Todd *et al.*, 2002). Seeds were scarified in hydrochloric acid and disinfested in a solution of 15% bleach for 20 minutes, and rinsed in sterile distilled water (Todd *et al.*., 2002). Seeds then were placed into sterile 25mm x 150mm culture tubes containing tissue culture media composed of 20 ml MS inorganics (Murashige and Skoog, 1962), 9 g/l agar (Fisher) and 2 g/l glycine, 1 g/l nicotinic acid, 1 g/l pyridoxine-HCl, 10 g/l thiamine-HCl, and g/l 100 inositol, and the pH was adjusted to 5.7. The culture medium was divided into 4 treatments based on sucrose content (0, 10, 20, and 30 g/l). Forty-eight tubes of each culture medium were used (192 total). After the seeds were added, the tubes were divided into two treatments. Twenty-four tubes of each sucrose treatment were exposed to a 16 hour light/8 hour dark photoperiod (26 µmol/s/m^2), while the other 24 tubes were wrapped in aluminum foil and kept in darkness, except for brief exposures to low light levels (0.21 µmol/s/m^2) for data collection. Lengths of hypocotyl plus cotyledons and of subsequent lateral shoots were recorded for all treatments, and statistical tests were performed to evaluate the effects of sugar concentration and light availability on lateral shoot development.

A similar experimental protocol compared the effects of long- and short-day photoperiods on in-vitro *Stylisma* growth. Seeds were dusted for 24 hours with tetramethylthiuramdisulfide fungicide, soaked in 1% mercuric chloride for 20 minutes (HgCl^2), and 50% bleach solution for 20 minutes.

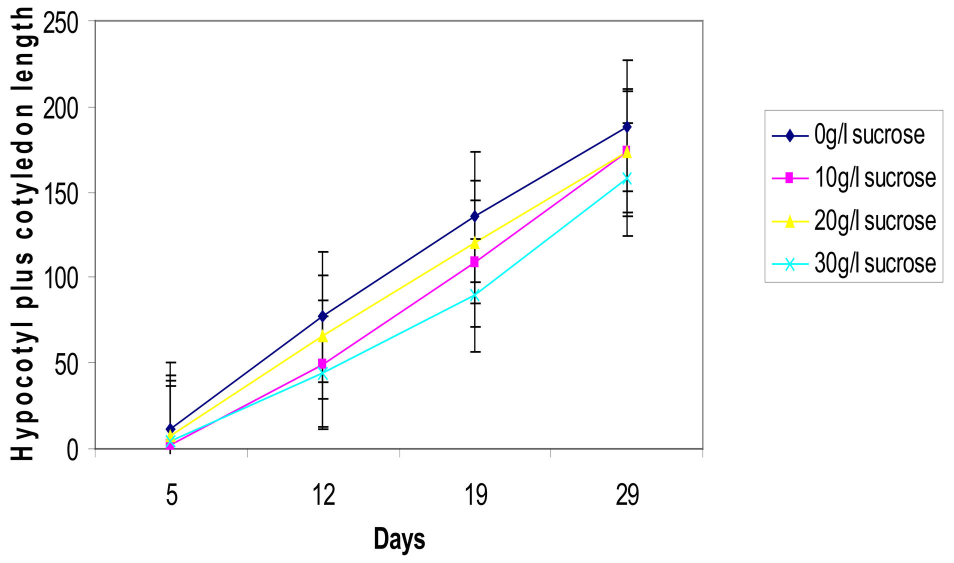


Fig. 1: Hypocotyl plus cotyledon lengths of *S. pickeringii* seedlings grown with long-days at four sucrose concentrations.

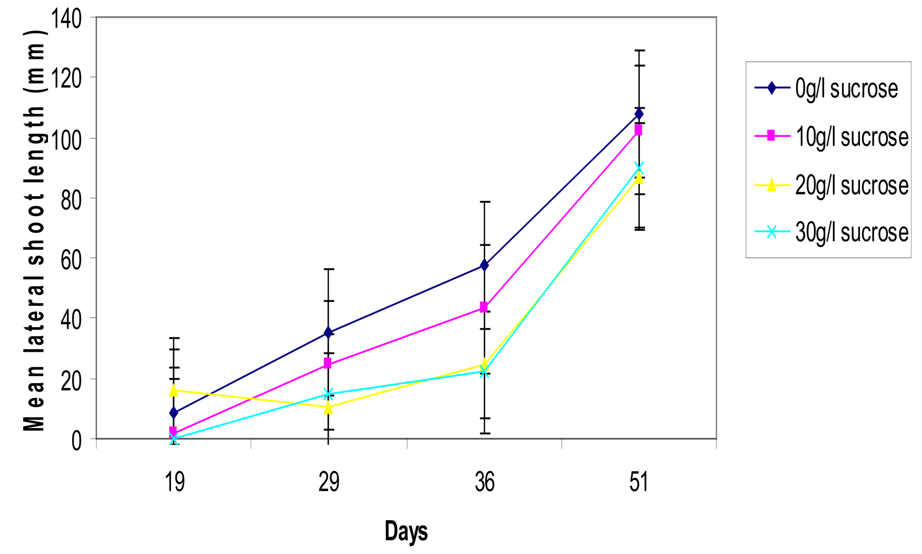


Fig. 3: Lateral shoot lengths of S. pickeringii seedlings grown with long-days at four sucrose concentrations.

Seeds were scarified by cutting the blunt end of the seed coat. Seed were placed in 64 tubes containing 20 ml MS organics plus 30g/l sucrose, 100mg/l inositol, and 8g/l agar, pH 5.8. Thirty-two tubes were placed in a growth chamber under short-day photoperiod of 8 hours light and 16 hours darkness, and 32 were grown under a long-day photoperiod of 16 hours light/8hours darkness. Four repetitions were performed with 32 tubes under each treatment, for a total of 128 tubes under short-day and 128 tubes under long-day treatments. Initiation of roots and both cotyledon and lateral shoot emergence and lengths were recorded every week for 10 weeks. Prior to statistical analyses, data were tested for normality in SAS; no transformations were needed. Two-way ANOVA and Fisher's least square mean were performed in SAS to test for significant differences between photoperiods in percentage emergence for roots, cotyledons, lateral shoots, and lateral shoot length. Linear regressions for lateral shoot percentage and length also were executed in Excel using means projected in SAS.

Results

In the first experimental protocol, there was a complete lack of lateral shoot development in all *S. pickeringii* plants grown in darkness. All plants kept in the light that germinated and were not discarded as contaminated (57%) had developed lateral shoots with a final mean length of 100 mm after 51 days of growth. All plants that germinated and were not discarded as contaminated that were raised in darkness (54%) did not develop lateral shoots. Regression analysis found no significant association (p=0.147) between hypocotyl plus cotyledon length and sucrose concentrations in plants grown under light treatment (Fig. 1). Regression analysis showed a strong (R^2 = 0.343) and highly significant (p= 0.000) positive relationship between hypocotyl plus cotyledon length and sucrose concentration in plants grown in darkness (Fig. 2). Regression analysis revealed no significant ass-ociation (p = 0.288) between sucrose concentration and lateral shoot length in plants grown under light (Fig. 3).

The second experimental protocol found a significant difference between germination percentages (p < 0.0001), lateral shoot initiation (p < 0.0001), and lateral shoot length (p = 0.024) of plants grown under long- and short-day photoperiods. There was no significant difference between groups in percentage of contaminated tubes, root development, or hypocotyl plus cotyledon axis development. Regression analysis of lateral shoot initiation percentages showed a greater slope for long-day (y = 13.767x – 22.573) than short-day (y = 10.954x – 22.601) photoperiods. Regression analysis also showed a greater increase in lateral shoot length under long-day (y = 15.625x – 214.14) than under short day (y = 13.675x – 230.53) photoperiods.

Discussion

Our data strongly suggests that *S. pickeringii* lateral shoot development is initiated by exposure of the cotyledons to light. This pattern of development is an advantageous adaptation to the sandy prairies bordering major rivers that are the primary habitat of *Stylisma pickeringii* var. *pattersoni*. Seeds buried under sand shifted by wind, dune movement, or flooding can postpone the energy consuming process of lateral shoot development until the hypocotyl plus cotyledon is at an appropriate depth, within 7-8 cm of the soil surface. The linkage between photoperiod and lateral-shoot production also would maximize lateral shoot development in summer and reduce development in spring and fall, giving the plant added protection against damage from spring flooding and cold fall and winter temperatures. The exact physiological mechanisms of lateral-shoot initiation in *Stylisma pickeringii* var. *pattersoni* remain unexplored, but experiments *in-vitro* show that lateral shoots are produced more quickly and with greater vigor under slightly elevated levels of cytokinin (0.1mg/l 6-benzylaminopurine) than in hormone-free media (Donnelly *et al.*, 2001). Perhaps exposure of the cotyledons to light causes increased cytokinin production or lowered auxin production (thus increasing relative cytokinin levels), resulting in the suppression of apical dominance in the initial shoot axis, senescence and death of the initial shoot and cotyledons, and production of lateral shoots from roots below the surface.

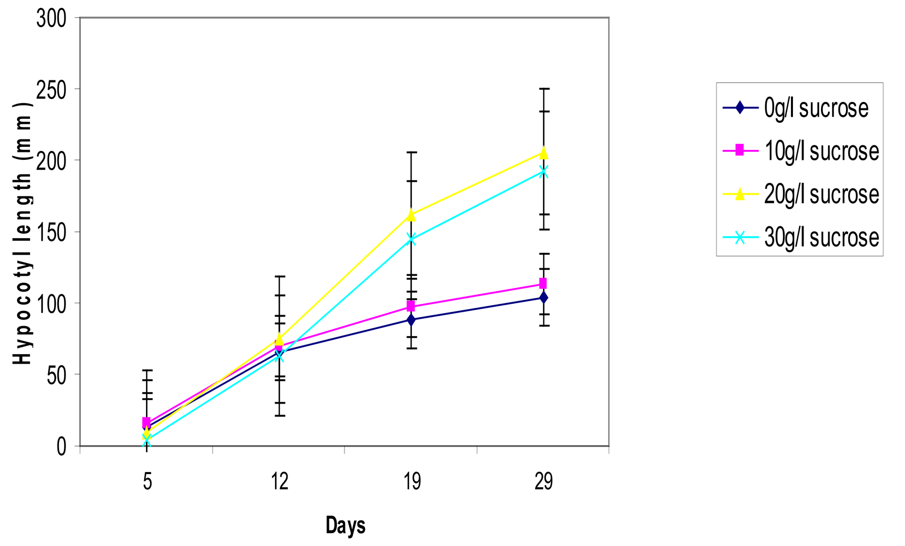


Fig. 2: Hypocotyl plus cotyledon lengths of S. pickeringii seedlings grown in continuous darkness.

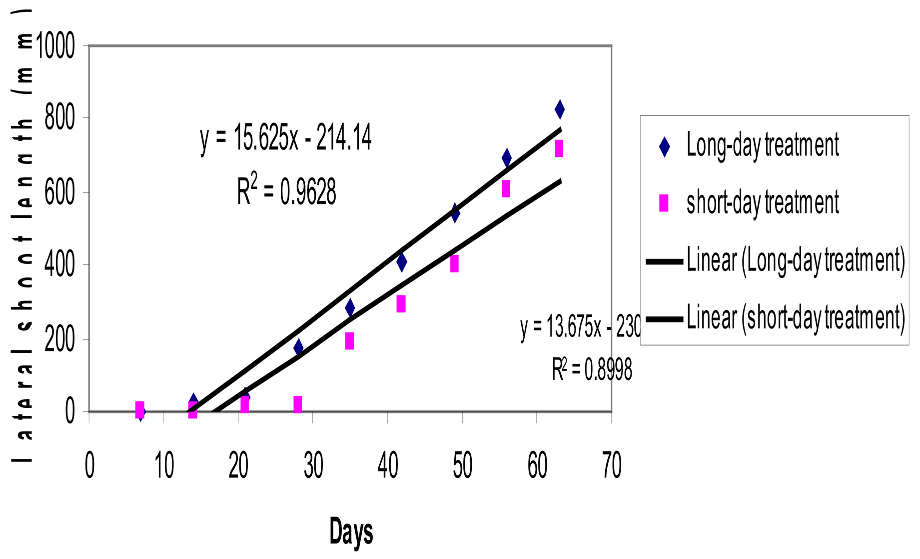


Fig. 4: Lateral shoot lengths for S. pickeringii seedlings grown with long and short days.

TOP: *In vitro* seedling of *S. pickeringii* showing hypocotyl (yellow arrow) and cotyledons (red arrows). ABOVE: Lateral shoots (arrow).

Acknowledgments

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