FINAL REPORT

Reproductive Limitations of Seeds and Seedlings in *Stylisma pickeringii* (Patterson Bindweed)

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Introduction

Stylisma pickeringii is an endangered plant found in Mason County, Illinois. Until recently (Heisler et al., 1999; Sojka et al., 1999), documented information was limited to descriptive information and reports of its existence. This research project involved site inventories and collection of plant materials, with the following objectives:

1. To determine if seeds with different colors develop from flowers that bloom at different times during the season.
2. To investigate techniques for breaking seed dormancy.
3. To study the morphology of lateral shoot development
4. To investigate ways of producing vigorous seedlings that develop lateral branches.

Materials and Methods

Seed Color: Five visits were made to three populations near Snicarte, Illinois (a recently disturbed area adjacent to a farmer's field, a roadside ditch, and a relatively undisturbed field near a gravel pit). On June 8th, initial population observations were made to estimate the onset of its flowering. Flowers were tagged on three separate dates during flowering (July 9th - 50 flowers, July 30th - 50 flowers, and August 20th - 25 flowers). Twenty-five fruits from each of the first tag dates were collected on August 20th. Remaining fruits were collected on September 9th. These fruits provided seeds that were 3, 6, and 9 weeks from flowering, and thus differed in seed age. In a second experiment, stems with fruit were collected on August 20th and September 9th. Seeds were removed from fruit on each third of the stems (upper, middle and lower) corresponding to youngest to oldest seed. Four seed colors were observed: green, yellow, tan, and maroon.

Seed Scarification: Four seed-scarification treatments were undertaken to determine the best technique to increase germination percentages: concentrated sulfuric acid (15, 30, 45, 60, 120, and 180 minutes); shaking with sandpaper (60 grit/500 rpm) for 18, 48, and 72 hours; shaking with course sand (18, 48, 72 hours); and sonication in water (2 or 4 hours). A final experiment was conducted comparing the 120-minute sulfuric acid, 48-hour sandpaper shake, 72-hour sand shake, and 4-hour sonication, and basal cut of the seed coat with a razor blade.

Plant Morphology: Two plants were dug from the soil and measurements were taken to determine the degree of stem tissue that extended below the soil surface. One of these plants was taken to the H.F. Thut Greenhouse at E.I.U. and grown in its natural soil. The second plant was used as starting material for attempts to vegetatively propagate the plant from nodal cuttings.

Seedling Production: Seeds were surface-sterilized and germinated *in vitro*, utilizing a technique developed previously (Sojka et al., 1999). Nodal cuttings from these plants were taken and placed on culture media containing either 0.2 mg/l or 2.0 mg/l of one of three 3 auxins (IBA, NAA, or 2,4-D) to stimulate root initiation. In a second experiment, seeds were scarified by shaking with sandpaper for 48 hours and germinated in greenhouse soil mix in 3" pots. The plants were

watered with solutions of either 1.0 or 5.0 mg/l of one of three cytokinins (BAP, KIN, or 2iP) to stimulate shoot initiation.

Results and Discussion

Seed Color: The number of green seed decreased with the age of the seeds, whereas the number of yellow and tan seed increased with seed age. Maroon seed showed no consistent pattern. Green seed decreased from upper to lower stem sections and yellow seed increased from upper to lower stem sections. This result suggest that yellow seed are older than green seed. Tan and maroon seed showed no consistent pattern. The amount of yellow seed increased with harvest date, whereas the amount of green and maroon seed decreased with harvest date, indicating that yellow seed may be older than green seed. See Figures 1-4.

Seed Scarification: Acid scarification for 120 minutes yielded the highest germination (80%) when compared to the other acid scarification times (0-5%). The 72-hour sand shake had a germination of 55%, which was significantly higher than the other sand-shake treatments. Seeds shaken in a jar lined with sandpaper had the highest germination (85% at 48 hours and 75% at 72 hours). Seeds sonicated for 2 or 4 hours had 0% and 10% germination. See Tables 1-4.

Plant Morphology: Plants removed from the soil indicated that the stem tissue extended 8 to 15 cm below the soil surface. A well-developed taproot system was evident, which extended at least ½ meter into the soil. The plant which was transported to the EIU greenhouse and potted in ½ native soil/ ½ greenhouse mix (BX ProMix) did not survive. Taller pots (11 cm wide and 35 cm high) have been purchased for future attempts at growing mature plants in the greenhouse.

Seedling Production: Nodal cuttings cultured *in vitro* in media containing no auxin or 0.2 mg/l IBA resulted in a healthy (*i.e.* greenest) condition of the leaves; however, no root initiation was observed for any of the treatments. Seedlings germinated in pots and irrigated with 1.0 mg/l 2iP or 5 mg/l BAP initiated the highest number of sideshoots (25% and 20%, respectively). Control seedlings did not initiate any sideshoots. Additional studies are planned to examin the influence of cytokinin treatments on multiple shoot initiation.

References

Heisler, C.J., J.M. Coons, and H.R. Owen. 2000. Age and harvest time affect color of *Stylisma pickeringii* seeds. Trans. Illinois Acad. Sci. 93:52.

Heisler, C.J., M.L. Rycerz, J.M. Coons, and H.R. Owen. 1999. Seed color and mechanical scarification affect germination of Patterson Bindweed (*Stylisma pickeringii*). Trans. Illinois Acad. Sci. 90:71.

Sojka, M.M., C.L. LaZier, H.R. Owen, and J.M. Coons. 1999. Surface sterilization treatments for *in vitro* germination of *Stylisma pickeringii*. Trans. Illinois Acad. Sci. 90:42.

Todd, B.L., J.M. Coons, and H.R. Owen. 2000. Scarification of *Stylisma pickeringii* (Patterson Bindweed) seeds using different techniques. Trans. Illinois Acad. Sci. 93:81.

Budget

Travel (5 trips)	$418.07
Commodities	$277.93
Total	$996.00

Figure 1. Seed Color Percentages at Three Week Intervals After Flowering

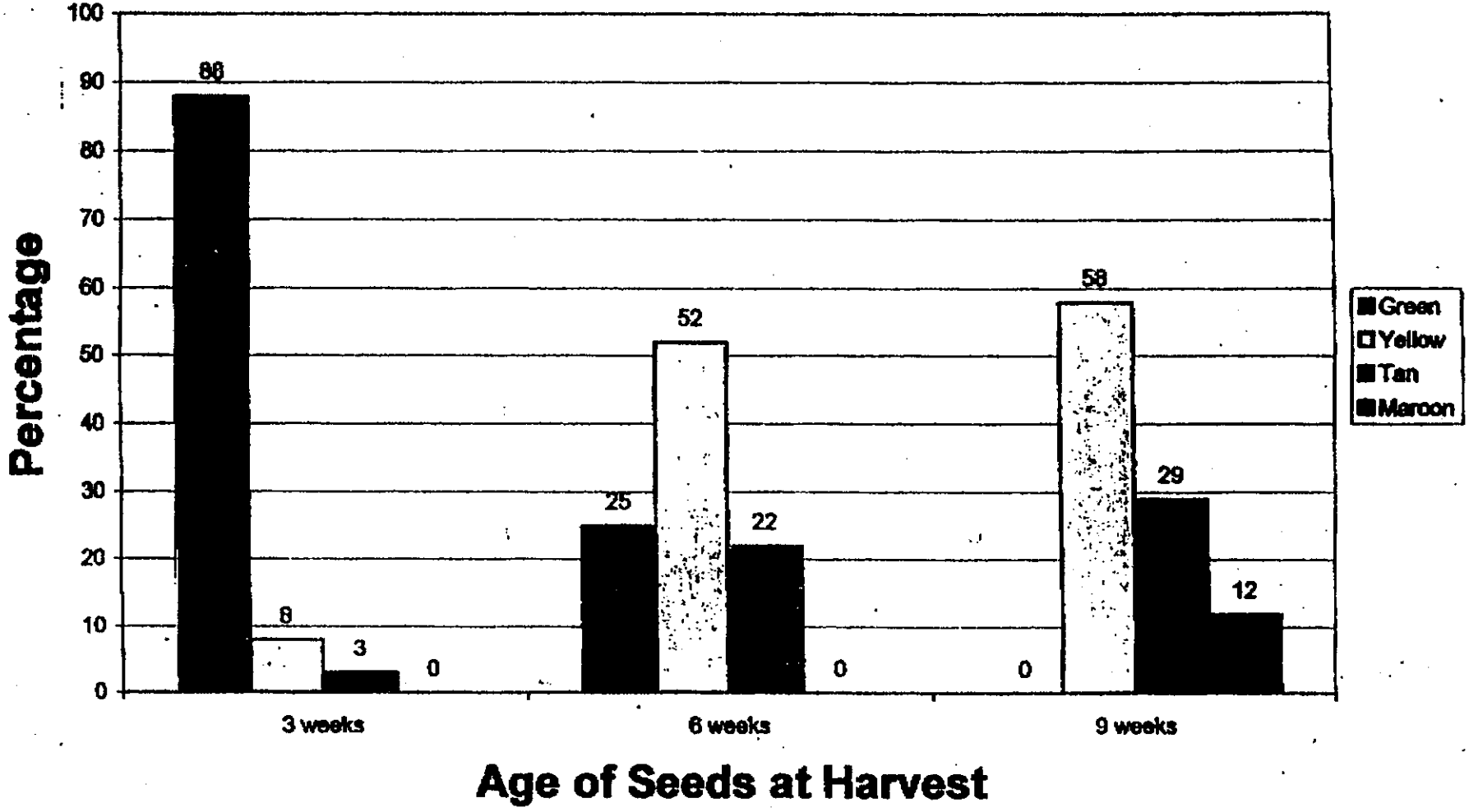

Figure 2. Seed Color Percentages on Different Parts of Stems (Aug.20)

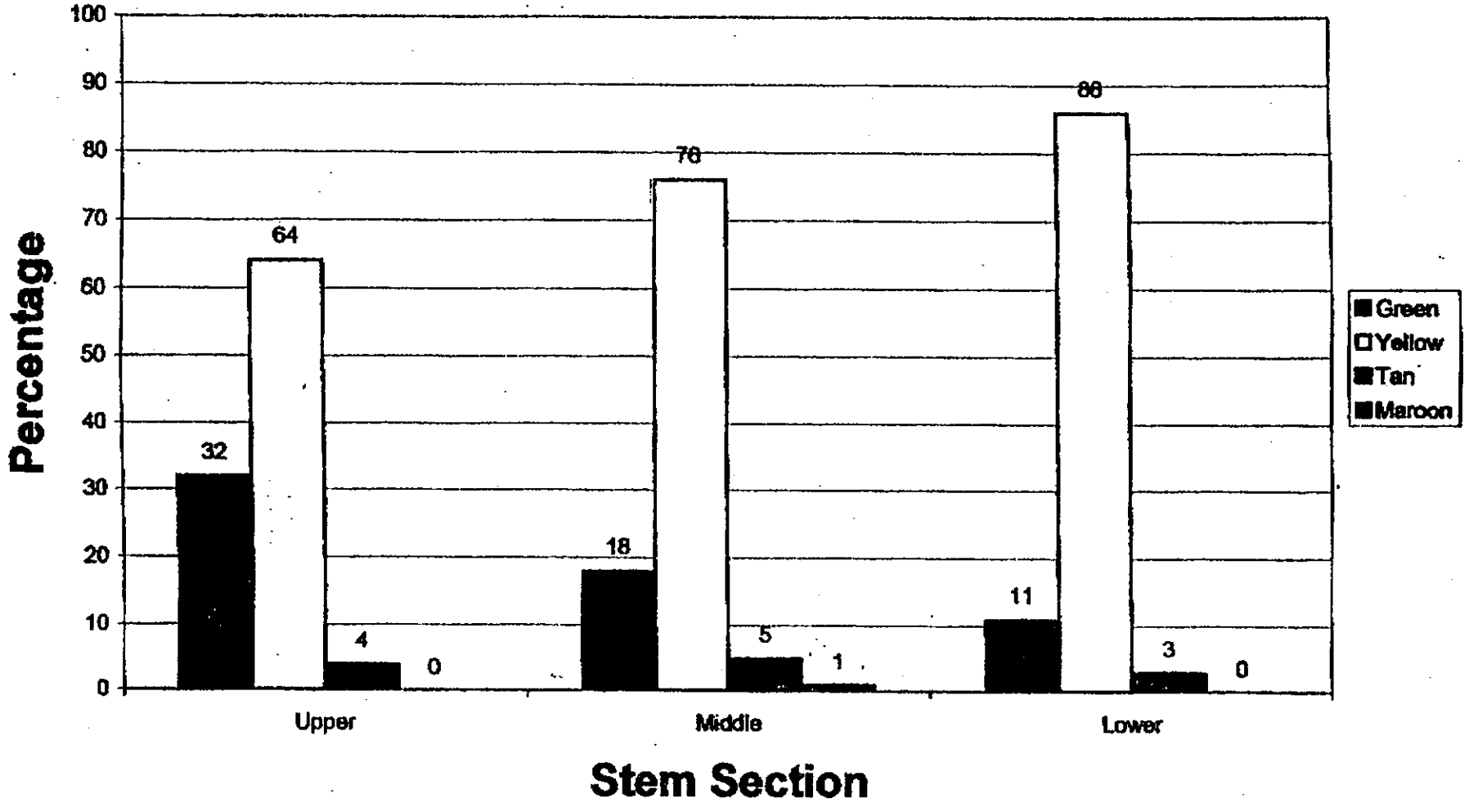

Figure 3. Seed Color Percentages on Different Parts of Stems (Sept. 9)

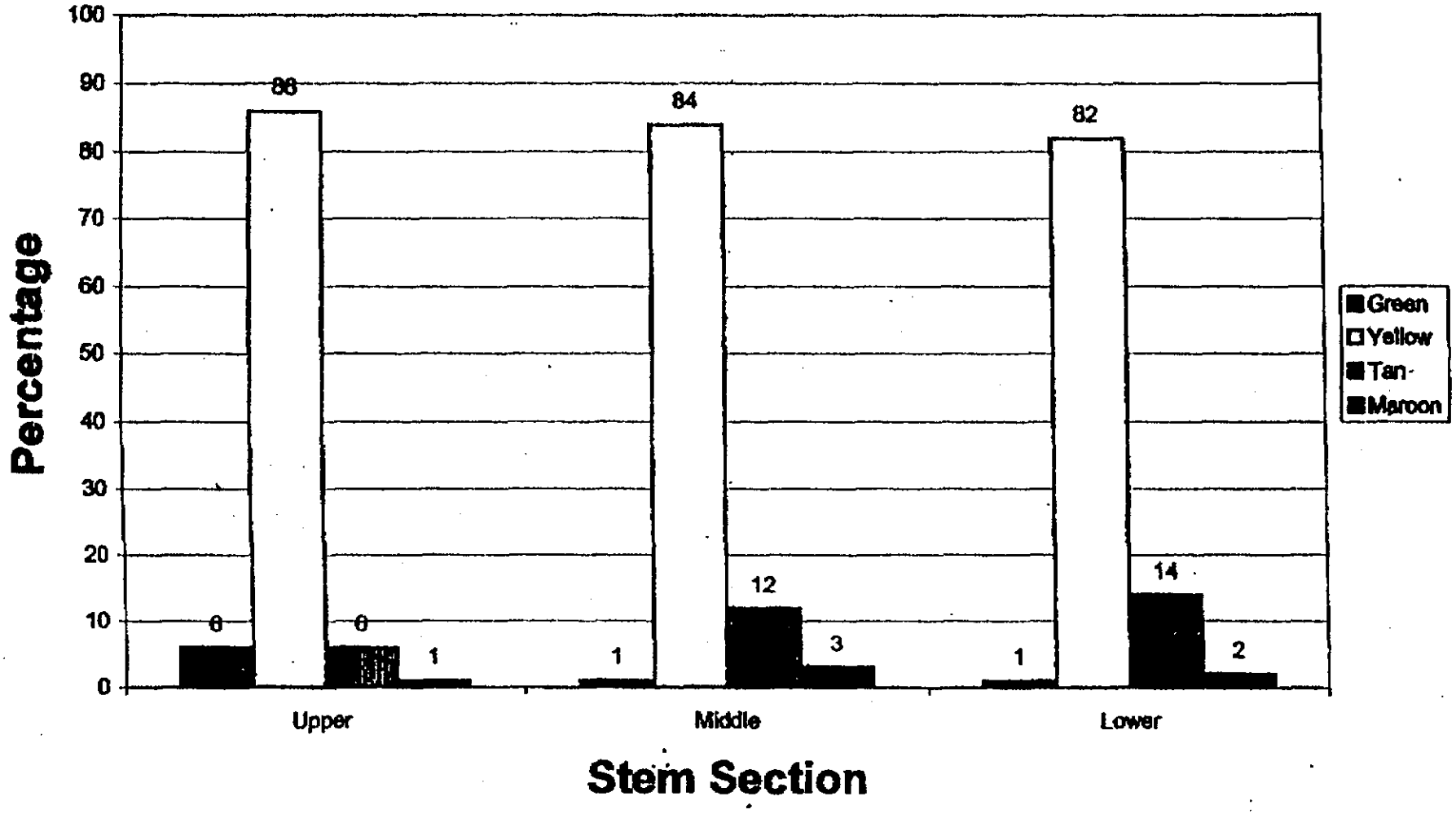

Figure 4. Seed Color Percentages on Different Harvest Dates

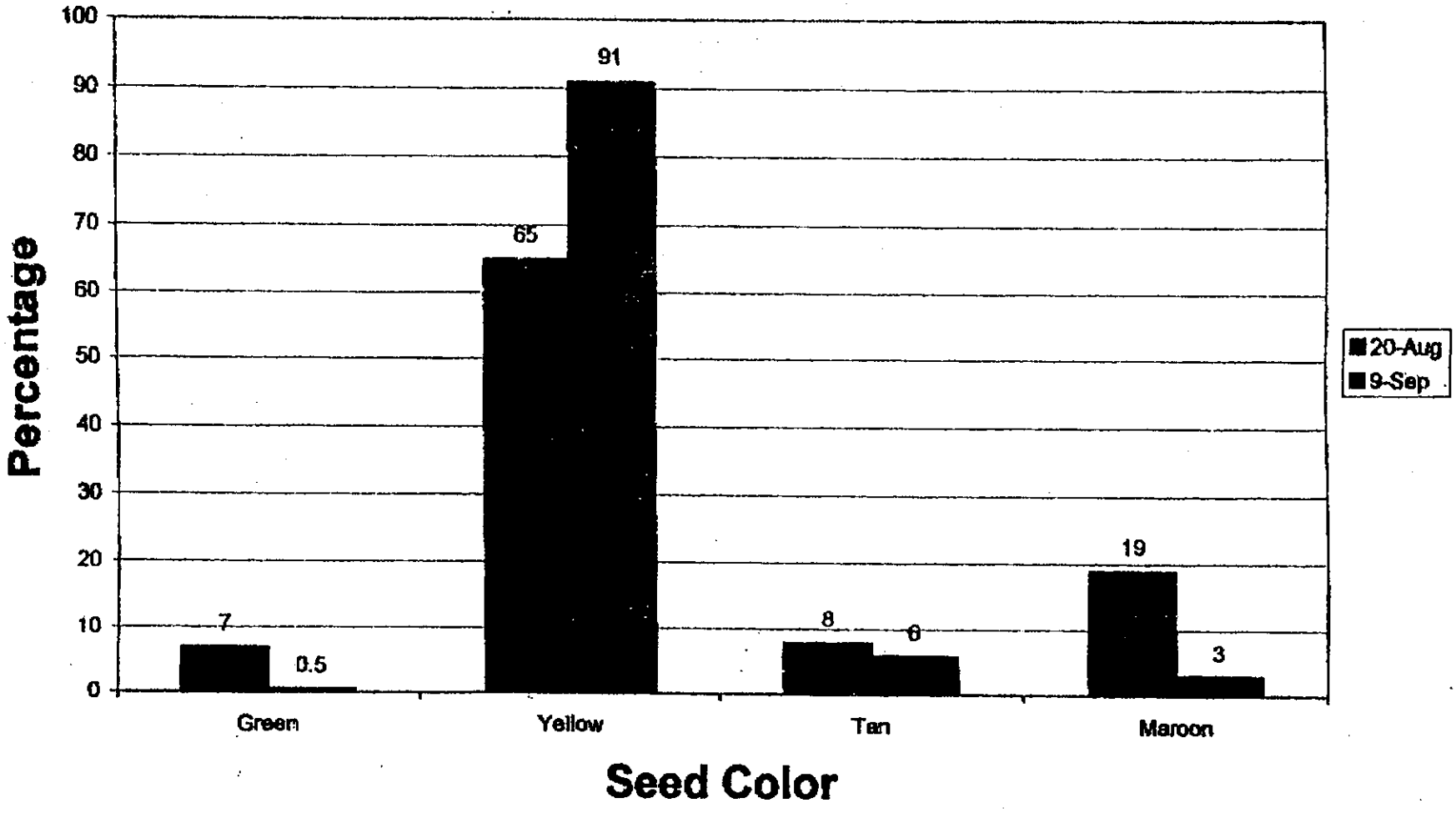

Table 1: EFFECTS OF SULFURIC ACID (H_2SO_4) ON GERMINATION OF PATTERSON BINDWEED SEEDS (FALL 1998 SEED)

Time in Acid (min.)	Germination (%)	
0	5	b[z]
15	0	b
30	5	b
45	0	b
60	5	b
120	80	a
180	0	b

Table 2: EFFECTS OF AUTOMATIC SHAKING IN SAND OR IN SANDPAPER-LINED JARS ON GERMINATION OF PATTERSON BINDWEED SEEDS (FALL 1999 SEED)

Time in Shaker (hr.)	Germination (%)			
	Sand		Sandpaper	
0	0	b[z]	0	c[z]
18	0	b	25	b
48	5	b	85	a
72	55	a	75	a

Table 3: EFFECTS OF SONICATION ON GERMINATION OF PATTERSON BINDWEED SEEDS (FALL 1999 SEED)

Time in Sonicator (hr.)	Germination (%)	
0	0	b[z]
2	0	b
4	10	a

Table 4: EFFECTS OF FIVE SCARIFICATION TECHNIQUES ON GERMINATION OF PATTERSON BINDWEED (FALL 1999 SEED)

Treatment	Germination (%)	
Control	0	b[z]
Basal cut	96	a
120-minute acid	84	a
48-hour sandpaper shake	92	a
72-hour sand shake	84	a
Sonicator	4	b

[z]Mean separation based on Duncan's multiple range at 5% level. Means followed by different letter are significantly different.