Propagation

Gene Blythe

Section Editor

Effects of Auxins, Propagule Size, and Wounding on Adventitious Root Formation of *Echinopsis rodotricha* K. Schum. (Cactaceae) Cuttings

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Index Words: clonal propagation, rooting, indolebutyric acid, suberization, cactus, quick dip.

Significance to Industry: The plant propagation industry stands benefit from the establishment of new and efficient commercial methods of propagation, especially for species with ornamental potential or high value. Vegetative propagation of *Echinopsis rhodotricha* can be performed through rooting stem sprouts. However, the establishment of propagation protocols for large-scale production of this cactus needs scientific study to determine the factors controlling the process of adventitious root formation (ARF) to further improve and increase efficiency. Therefore, the objective of this research was to determine the effects of propagule size, various concentrations of K-IBA, and wounding of propagules to optimize ARF and overall plant growth during propagation. We found from this study that *Echinopsis rodotricha* is an easy-to-root plant species because ARF can take place without any auxin solution; however, the window of time for producing a response is reduced and an enhancement in root number and total root growth are produced by exposure to K-IBA at a range of concentrations. However, elevated concentrations (10,000 mg/L^{-1}) produced the highest values and positively affected other experimental variables tested. A wounding pre-treatment to the cuttings had no effect on most variables tested. With this study, we set up a reliable and efficient clonal propagation system for this cactus species, which could have commercial application for mass production and sustainable schemes.

Nature of Work: *Echinopsis rodotricha* is also classified as *Echinocactus forbesii, Echinopsis forbesii*, and *Echinopsis chacoana*. This Cactaceae member is native to South America in a region including Northern Argentine, Bolivia, South Brazil, Uruguay and Paraguay (1, 2, 3). *Echinopsis rodotricha* is a columnar cactus with a green body that branches into numerous stems, each of which reach 5-6 cm in diameter and up to 2 m tall. The stems have 8 to 12 ribs with yellow areoles including 1 to 3 central golden spines 12 mm long. White flowers emerge in late spring, have a nocturnal habit, and reach up to 20 cm long and 15 cm in diameter (3, 4). This cactus grows in altitudes

ranging from 0 to 500 m in grasslands, forests, and open sites on sandy soils with shrubs and small plants (3). The Maka natives use *Echinopsis rhodotricha* for medicinal purposes to treat chicken pox and measles (3). *Echinopsis rodotricha* has been dispersed around the world and has become popular because of its high adaptability to different climates, soil conditions, and rapid growth rate. As an ornamental, it is commonly cultivated as a pot or rockery plant (4).

We conducted a series of experiments to study the effects of several factors affecting ARF of *Echinopsis rodotricha* cuttings. Initially, we ran an experiment with a factorial design including 12 treatments with a combination of three levels of cutting size [small (10 mm in diameter), medium (20 mm in diameter), and large (more than 40 mm in diameter)] and four concentrations of indole-3-butyric acid potassium salt (K-IBA) [0, 1000, 3000, and 10000 mg/L^{-1}]. For this experiment we used a previously sterilized artificial substrate prepared with peat moss (Premier®), sand (1:1 v:v), and 1% lime. Each treatment was represented by 20 cuttings (replications).

Secondly, we set up a simple experiment with a completely randomized design to test the effect of propagule wounding on ARF. The two treatments tested included non-wounded (control) detached sprouts from stock plants and wounded sprouts in which 1 cm or about 25% of the basal portion was dissected to increase the surface cut. Each treatment was represented by 60 cuttings (replications).

For these experiments, the propagules or sprouts were obtained from 3-year-old healthy plants. The propagules of each experiment were detached from stock plants and suberized for 8 days under shaded conditions prior to transplant or application of any treatment. The stock plants and experimental cultures were grown in a greenhouse with maximum photosynthetic photon flux density (PPFD) of 1,000 µmol/m^2/s^{-1} at plant height, an average of day/night temperature of 27/20 ± 3° C, irrigation supplied as needed, and fertilization provided once a month (100 ppm N) with Peters Professional 20-20-20 (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA). The propagules for all experiments were planted in plastic germination trays (5-kg capacity). The K-IBA solutions were prepared with water and 0.1% of Tween-20 was added. We used the quick-dip technique to apply the auxin treatments, with the base of cuttings (10 mm) submerged for 60 sec and immediately planted. Experimental variables included root number, total root length (mm), average root length (mm), plant height (increase in cutting height during the period of experimentation) (mm), plant diameter (increase in cutting diameter during the time of experimentation) (mm), and root dry weight (DW) (g). All data obtained from each experiment were subjected to an analysis of variance and a mean separation test (Duncan, α = 0.05) (XLSTAT, 2014).

Results and Discussion: Propagule size and concentration of K-IBA significantly affected root and plant growth; however, even the control treatment was able to induce ARF. In regards to propagule size, large cuttings produced the highest values in all data measuring root growth: root number (11.6), root dry weight (1.65 g), and total root length (117.5 cm) as compared to the medium and small size cuttings (Table 1). The

plant growth variables showed the same trend with records for stem diameter (19.1 mm) and plant height (44.4 mm) (Table 1).

Increasing concentrations of K-IBA resulted in higher values for most root growth variables. In particular, 10,000 ppm K-IBA produced the highest values of root DW (1.14 g), root number (10.7), and total root length (99.3 cm) as compared with other treatments. The control produced the lowest values of root dry weight (0.75 g), root number (6.3), and total root length (75.4 cm).

A similar trend was obtained for the plant height data; however, 3,000 ppm (39.8 mm) resulted in greater height than 10,000 ppm (35.8 mm); however, this difference was not significant. In contrast, 10,000 ppm of K-IBA treatment produced the lowest root length (10.0 cm) in comparison to 3,000 ppm (10.6 cm), 1,000 ppm (11.3 cm), and the control (12.0 cm). A similar situation was obtained in plant diameter (Table 1). In general, the control treatment was able to induce ARF regardless the size of the propagule.
Data from the second experiment in which we tested the wounding of cuttings showed that most variables related to root growth (including root number, total root length, average root length and stem diameter) showed no significant differences when compared with the control treatment. In contrast, root dry weight was the only variable showing significance differences. Plant height was comparable among treatments (Table 2).

Acknowledgements: Authors express appreciation for the economic support provided by the Universidad De La Salle Bajío through the Office of the Research Council and CINVESTAV-IPN at Irapuato, Gto., Mexico for allowing the use of greenhouses and other facilities to run this study.

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Table 1. Effect of propagule size and K-IBA concentration on adventitious root formation and growth of *Echinopsis rodotricha* K. Schum. cuttings after 8 months of greenhouse culture.

Propagule size	K-IBA (mg/L^{-1})	Root dry weight (g)	Roots (no.)	Total root length (mm)	Root length (mm)	Stem diameter (mm)	Plant height (mm)
Small	0	0.33	4.65	49.58	10.85	15.64	25.56
Small	1,000	0.29	5.00	54.36	10.80	14.31	24.32
Small	3,000	0.53	5.90	66.34	11.42	16.59	32.98
Small	10,000	0.58	7.55	83.29	11.54	10.49	27.20
Medium	0	0.56	5.70	70.05	12.47	17.90	23.00
Medium	1,000	0.68	7.60	86.11	11.78	16.81	30.89
Medium	3,000	0.75	7.30	76.88	10.10	20.31	37.60
Medium	10,000	1.02	10.30	93.10	9.22	18.98	34.73
Large	0	1.37	8.50	106.70	12.77	19.86	34.91
Large	1,000	1.55	11.90	124.73	11.17	17.20	48.21
Large	3,000	1.79	11.80	117.15	10.33	22.60	48.87
Large	10,000	1.91	14.30	121.41	9.26	16.69	45.53
Significance:							
Propagule Size		***	***	***	NS	***	***
K-IBA level		***	***	***	***	***	***
Propagule Size × K-IBA		NS	NS	NS	***	NS°	NS

NS = Non significant; *, **, *** = Significant at 0.05, 0.01, or 0.001, respectively (n= 20).

Table 2. Effect of wounding on adventitious root formation and plant growth of *Echinopsis rodotricha* K. Schum. cuttings after 8 months of greenhouse culture.

Treatment	Root dry weight (g)	Roots (no.)	Total root length (mm)	Root length (mm)	Stem diameter (mm)	Plant height (mm)
Wounded	1.04	8.62	86.27	10.80	17.90	34.34
Non-wounded	0.84	8.13	88.68	11.13	16.66	34.63
Significance	***	NS	NS	NS	NS	NS

NS = Non significant; *, **, *** = Significant at 0.05, 0.01, or 0.001, respectively (n= 60).

Camellia sasanqua Cuttings Responds to Hormones and Magnetized Water

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Index words: *Camellia sasanqua*, magnetized water, rooting hormones, stem cuttings

Significance to Industry: *Camellia sasanqua* Thunb. (Christmas or yuletide camellia), native to China and Japan, is a popular ornamental plant and has been widely planted around the world. It offers an aesthetic growth habit, fine texture, glossy evergreen foliage, and many fragrant pink flowers. To better propagate this beautiful plant for market demand, hardwood stem cuttings were collected in April 2014 and treated with rooting hormones and magnetized water. Hardwood stem cuttings of *C. sasanqua* rooted well and hormones significantly affected rooting percentage and quality. With increasing hormone concentration, rooting rate and root volume increased using both a K-IBA quick dip and Hormondin powder (talc). For 8,000 mg•L^{-1} treatments, liquid (K-IBA) resulted in significantly better rooting (100%) and root volume (41.8 cm^3) than did a double dip in 5,000 mg·L^{-1} K-IBA + 3,000 mg·L^{-1} talc (93.8%, 26.5 cm^3) and Hormodin powder (87.5%, 21.8 cm^3). Cuttings treated with magnetized water and K-IBA at 3,000 mg·L^{-1} showed the greatest root volume (43.2 cm^3) and a high rooting rate (93.8%). Without magnetized water, cuttings treated with 8,000 mg·L^{-1} K-IBA had the highest rooting rate of 100% and root volume of 30.8 cm^3, which was 11.02 cm^3 less than that of magnetized water. Magnetized water did promote the growth of roots, but not root initiation. Cuttings at 10 cm long rooted better (100%) than 20-cm cuttings (89.0%) using a double dip, but also rooted within 2 months instead of 4 months. Hardwood cuttings of *C. sasanqua* should be collected in April at 10cm long, treated by liquid K-IBA at 3,000 or 8,000 mg·L^{-1} for enhancing root initiation. Magnetized water is highly recommended after root initiation, but not before.

Nature of Work: During the winter season, the lustrous dark green leaves and heavy flowering of *Camellia sasanqua* make this plant very attractive (1). Growers like the plant not only for its popularity, but also wide adaptation, sun tolerance, and cold hardiness (compared with *Camellia japonica*) (2). The species and its many cultivars are very versatile and can be used for hedges or screens; espaliered on walls or over arches; trained as standards; grown in containers; or employed as dense feature shrubs at garden entrances or in mixed shrubbery (3).

To better propagate this plant and improve production quality, stem cuttings of *Camellia sasanqua* was collected on April 10, 2014 and treated with both liquid and powder rooting hormones (Table 1). Cuttings were 10 or 20 cm long, depending on the

treatment (Table 2). All cuttings were inserted into 32-cell flats with rooting medium of perlite and perlite mix (1:1 by vol.) and placed under a mist system. Misting frequency was 20 seconds every 10 minutes for the first 2 weeks, and then reduced to 20 seconds every 20 minutes from 7:00 AM to 9:00 PM daily. Magnetized water was obtained by soaking a 500-g magnet in a 5-gallon bucket of water for more than 24 hours, then using the water to topdress cuttings once every other day.

A randomized complete block design was employed and each treatment was replicated 4 times (4 blocks/replicates) with 8 subsamples per replicate. Rooting percentage and rootball volume were collected after 70 and 130 days, respectively. All data were analyzed using the GLM procedure of SAS (version 8.1; SAS Institute Inc., Cary, NC). Mean separation was carried out using LSD ($\alpha = 0.05$)s.

Results and Discussion: Growers can propagate *C. sasanqua* using hardwood stem cuttings right before new growth initiation in April and hormones can significantly improve rooting percentage and quality. Generally, hormone concentration at 1,000 $mg \cdot L^{-1}$ did not work effectively and 8,000 $mg \cdot L^{-1}$ is recommended (Table 1). Hormone application methods did not show significant differences. Using 8,000 $mg \cdot L^{-1}$ treatments, liquid K-IBA resulted in 100% rooting, while other treatments produsd 87.5% and 93.8% rooting. Root quality (as indicated by rootball volume) increased significantly as the hormone concentration went up. Once again, 8,000 $mg \cdot L^{-1}$ liquid K-IBA treatment yielded a significantly higher rootball volume (30.80 cm^3) than did other treatments (ranging from 0.37 to 6.98 cm^3).

Magnetic water affected plant growth and development. Magnetic fields are known to induce biochemical changes and could be used as a stimulator for plant growth. Irrigation with magnetic water increased seed germination and yield of broad bean (4). Cuttings treated with magnetized water and K-IBA at 3,000 $mg \cdot L^{-1}$ yielded the greatest rootball volume (43.2 cm^3) and higher rooting rate of 93.8%. Under tap water, cuttings treated with 8,000 $mg \cdot L^{-1}$ K-IBA had the highest rooting rate of 100% and root volume of 30.8 cm^3, which was 11.02 cm^3 lower than that of magnetized water. With the same hormone treatment, all cuttings irrigated with magnetized water had significantly higher rootball volumes (Table 1). Magnetized water did promote the growth of roots, but not root initiation. Actually, irrigation with magnetized water significantly reduced the rooting percentage (Table 1 and 2).

A significant effect on stem cutting length (10 vs 20 cm) was observed in this experiment (Table 2). Short (10-cm) cuttings produced greater rootball volumes and a higher rooting percentage, especially using magnetized water. Furthermore, short cuttings initiated roots significantly faster than the longer cuttings. Cuttings at 10 cm long not only rooted better (100%) than 20-cm cuttings (89.0%) using a double dip, but also rooted within 2 months instead of 4 months. Growers could double their capacity for rooting *Camellia sasanqua* stem cuttings at 10 cm long and produce a significantly better quality of rooted cuttings Since we did not record the type of cuttings (1-year or 2-year hardwood stems), further studies should address this issue.

Acknowledgement. This research was supported by The University of Georgia Research Foundation Project #2521RC297295.

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Table 1: Effect of hormone and magnetized water on rooting of *Camellia sasanqua* (20-cm-long cuttings and data taken after 130 days).

Treatment	Magnetized water		Non-magnetized water	
	Rooting (%)	Root volume (cm^3)	Rooting (%)	Root volume (cm^3)
Control	50.00 bc	1.49 cd	68.75 cde	0.37 c
1,000 $mg \cdot L^{-1}$ IBA (powder)	12.50 c	0.31 d	59.38 de	0.43 c
3,000 $mg \cdot L^{-1}$ IBA (powder)	68.75 ab	2.68 cd	50.00 e	0.95 c
8,000 $mg \cdot L^{-1}$ IBA (powder)	68.75 ab	21.83 bc	87.50 abc	4.31 bc
1,000 $mg \cdot L^{-1}$ K-IBA (liquid)	56.25 ab	1.55 cd	75.00 bcd	1.91 c
3,000 $mg \cdot L^{-1}$ K-IBA (liquid)	93.75 a	43.19 a	75.00 bcd	1.92 c
8,000 $mg \cdot L^{-1}$ K-IBA (liquid)	93.75 a	41.82 ab	100.00 a	30.8 a
5,000 $mg \cdot L^{-1}$ K-IBA (liquid) + 3K $mg \cdot L^{-1}$ IBA (powder)	71.85 ab	26.48 ab	93.75 ab	6.98 b

Table 2. Effect of cutting length and magnetized water on rooting of *Camellia sasanqua* using a 5,000 K-IBA liquid + 3,000 K-IBA powder double dip.

Cutting length (cm)	Days	Magnetized water		Non-magnetized water	
		Rooting (%)	Root volume (cm^3)	Rooting (%)	Root volume (cm^3)
10	70	98.44 a	56.57 a	95.31 a	47.35 a
20	70	0.00 c	0.00 c	0.00 b	0.00 c
20	130	71.85 b	26.48 b	93.75 a	6.98 b

Germination of Beach Primrose Seeds In Response to Light Exposure

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Index words: *Oenothera drummondii*, seed propagation.

Significance to Industry: This study with seeds of *Oenothera drummondii* Hook. examines the need for light for germination to occur. The information obtained from this study provides growers with the most successful means of germinating *O. drummondii* from seed and will assist breeders with germination of controlled crosses. Seed-propagated *O. drummondii* may reduce the chances of virus transmission during propagation and may fit more readily into plug growing operations than would vegetative propagules.

Nature of Work: Ensminger and Ikuma (1) reported that *Oenothera biennis* L. required light for germination. They report that maximal germination occurs in *O. biennis* under extended light conditions, stating that *Oenothera* seeds are photosensitive and phytochrome is the photoreceptor. They also noted that maximal or near maximal germination results from prolonged (36-48 h) exposure to red or white light at 24 °C. Ensminger and Ikuma (1) tested several temperatures, including 22 to 24 °C as used in the present experiment.

Seeds of *Oenothera drummondii* were germinated under controlled conditions with light and under light exclusion to determine if light exposure or darkness were needed for germination to occur. A sample of 200 freshly harvested seeds were separated evenly between eight petri dishes with 25 seeds per petri dish.

Four petri dishes were placed under white light consisting of four 60 watt compact fluorescent (CFL) bulbs mounted one foot above the bench. The lights were set on a timer to turn on at 8 am and turn off at midnight to provide 16 hours of direct light. The remaining four petri dishes were wrapped in aluminum foil and placed under a metal pan to ensure no light penetration. The seeds used in this treatment were freshly harvested from *O. drummondii* plants two days prior to initiation of the study. The seeds within each petri dish were placed on top of white filter papers moistened with distilled water to ensure maximum hydration of the seeds. Treatment began on 22 October 2014 and the water and germination status was checked on a daily basis. Germination was defined as the point when the radicle visibly began to protrude from the seed. Germination of the seeds were determined using a dissecting microscope and documented pictorially utilizing a compound microscope (Fig. 1). The study was

replicated in time on February 7, 2015, using the same methods and materials as in the previous study. The one change in this study was the length of time the seeds were allowed to dry out. The second replication consisted of seeds which had been dried at room temperature (approx. 24 °C) for 60 days.

Results and Discussion: The seeds began to germinate on day nine when the first germinated seed was discovered in the light treatment (Fig. 1). Germination was rapid with the first seed to germinate showing no visible signs on day eight of germination and on day nine had germinated, producing two cotyledon leaves. After the first seed germinated in the light treatment, other seeds continued to germinate in the light treatment for the next thirteen days (Fig. 2). On the twelfth day of the study, germination of one seed was discovered in the dark treatment. The seed that germinated was pale in color (almost translucent) with no visible leaves and had a very elongated stem. After germination began in the light treatment, more seeds followed on a daily basis with a peak of nine seeds in one day. Germination was slow and erratic with seeds placed under light exclusion (Fig. 2). The study was continued a full eight weeks to ensure all seeds had adequate time for all viable seeds to germinate.

Interestingly, results differed with stored seed from the first replication using fresh seed (Fig. 3). Unlike the previous replication, seeds in the dark treatment began germination simultaneously with the light treatment; however, with stored seed, the dark-germinated seeds emerged much faster, showing the highest germination in both treatments on day five. While light exposure resulted in the greatest percent germination with fresh seeds (light 91%, dark 59%), stored seeds germinated at similar levels in light and dark conditions (light 90%, dark 87%) (Fig. 2 and 3). Our data suggests that fresh and stored seeds may have differential germination requirements. Greiner and Kohl (2) reported for *Oenothera* that seed quality, age, and/or strain can affect germination capacity and rate substantially up to the point of extreme delays. They also noted that large differences can be observed even between different sowings of the same seed lot. Although light may not be required for germination of *O. drummondii* seed, it was not harmful and was advantageous with fresh seed, suggesting be germinated under light conditions.

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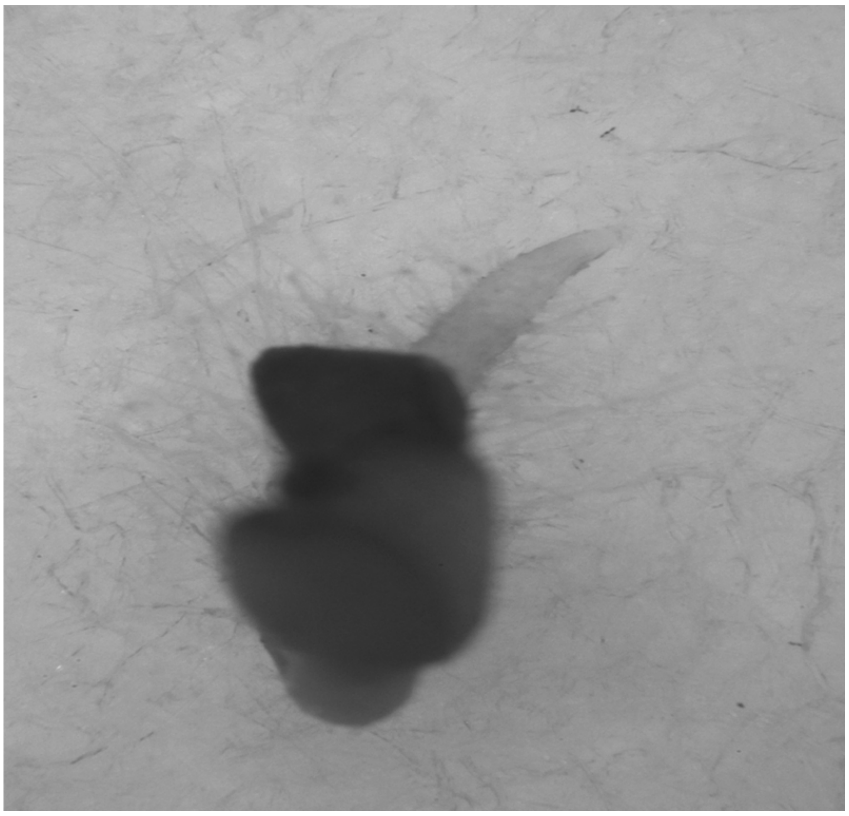

Fig. 1. Radicle emergence and germination of seeds of *Oenothera drummondii* as seen using a Canon Eos Rebel T3i camera (Melville, NY) mounted to an Olympus compound microscope (Shinjuku, Tokyo). Radicle emergence can be seen from the base of the seed. Root hairs are present on the radicle.

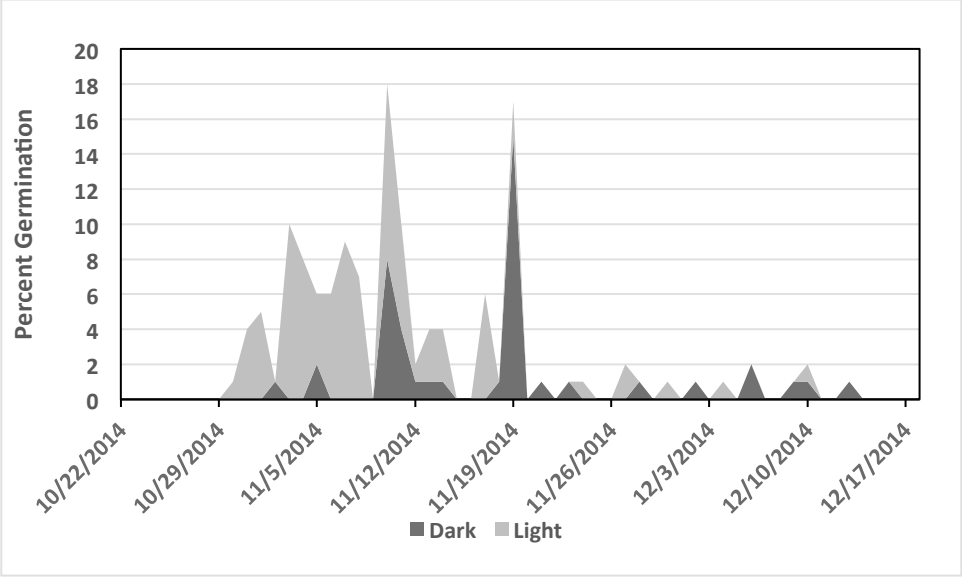

Fig. 2. Germination of *Oenothera drummondii* over time using fresh seeds.

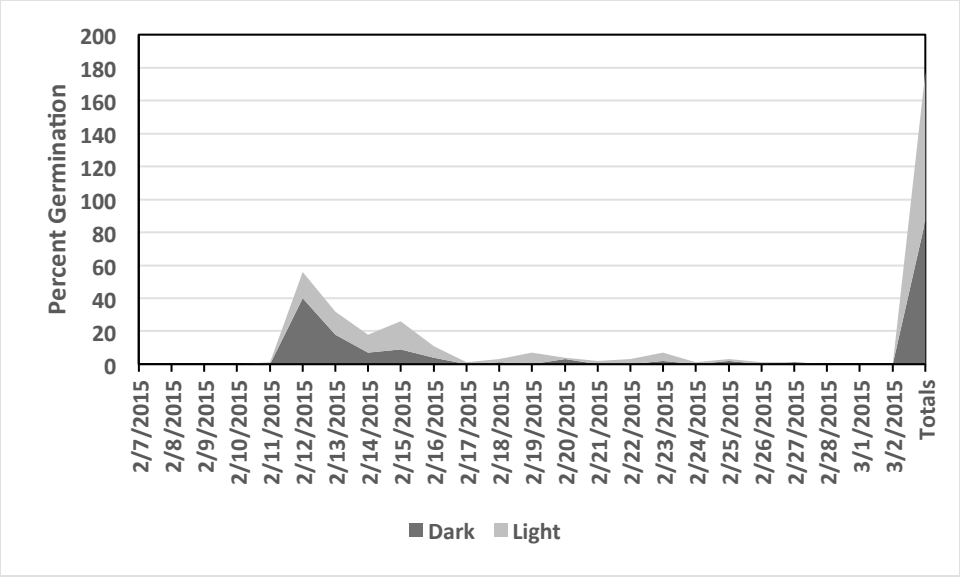

Fig. 3. Germination of *Oenothera drummondii* over time using stored seeds.