Propagation

Gene Blythe

Section Editor

Effects of K-IBA, Propagule Size, Substrate, and Wounding on Adventitious Root Formation of *Echinopsis spachiana* (Lemaire) H. Friedrich & G.D. Rowley (Cactaceae)

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

Significance to Industry: The nursery and plant propagation industries stand to benefit from the establishment of new and efficient commercial methods of plant reproduction, especially for species with ornamental potential. Vegetative propagation of *Echinopsis spachiana* can be performed through rooting stem cuttings; however, the establishment of propagation protocols for large-scale production of this cactus needs scientific study and data to further improve and increase efficiency. One aspect of this research was to study the effects of different factors on rooting, root quality, and performance of rooted cuttings. Therefore, the objective of this research was to determine the effects of the propagule size, various concentrations of K-IBA, type of substrate, and wounding of propagules to optimize adventitious root formation (ARF) and overall plant growth during propagation. We found that *Echinopsis spachiana* may be considered an easy-to-root species since it is able to induce ARF without K-IBA; however, the rooting process may be enhanced as a result of a wide range of concentrations. Higher concentrations, such as 10,000 mg/L^{-1}, produced the largest values in root number, root dry mass and size and positively affected other variables. The best substrate for ARF and root and plant growth was Mix B. Propagule wounding had no a significant effect on most variables tested. With this study, we developed a reliable and efficient clonal propagation system for *Echinopsis spachiana*, which could have commercial application for mass production.

Nature of Work: *Echinopsis spachiana*, which is known as golden torch cereus, is a member of the Cactaceae native from Northern Argentine in South America (1,2). It is a columnar cactus with a lime-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 10 to 15 ribs with yellow areoles including 1-3 central golden spines 12 mm long. The 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 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 ornamental, it is commonly cultivated as pot or rockery plant and can be used as rootstock for grafting cacti (5).

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

In the second trial, we ran a simple experiment with a completely randomized design to test the effect of the type of substrate. Mix A was prepared with peat moss (Premier®) and sand (1:1 v:v) plus 1% lime for mix A. Mix B was prepared with Sunshine potting soil (Sun Gro Horticulture, Canada), dry decaying residues of oak, perlite, vermiculite, and sandy-loam top soil (3:2:1:1:1). Both substrates were sterilized in an autoclave during 3 alternate days. Cuttings were treated with a solution of 10,000 mg/L^{-1} of K-IBA to induce ARF. Each treatment was represented by 40 cuttings (replications).

Finally, we set up a simple experiment with a completely randomized design to test the effects 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 (about 25%) of the basal portion was dissected to increase the surface cut. Each treatment was represented by 60 cuttings or replications.

For all experiments, the propagules or sprouts were obtained from healthy 3-year old plants. The propagules for each experiment were detached from stock plants and suberized for 8 days under shaded conditions prior to transplantation 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 level, an average of day/night temperature of 27/20 ± 3°C. Irrigation was 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 germination plastic trays (5-kg capacity). To prepare the K-IBA solutions, we added 0.1% of surfactant Tween-20 to each treatment. K-IBA was applied to cuttings with a quick-dip for 60 sec according to the treatment 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). Data were subjected to analysis of variance and mean separation with Duncan's test (α=0.05) (XLSTAT, 2014).

Results and Discussion: Eight months after establishing the first experiment, both propagule size and K-IBA level significantly affected the six experimental variables evaluated; however, there was statistical interaction for propagule size × K-IBA level for root dry weight, root number, and total root length. Despite this, the general trends in the root dry weight data showed that large cuttings produced higher values (2.26 g) than medium (1.01 g) and small (0.62 g) size propagules. In addition to this, 10,000 mg/L^{-1} of K-IBA produced the higher values (2.29 g) of root dry mass than the other concentrations evaluated (3,000 mg/L^{-1}: 1.50 g; 1,000 mg/L^{-1}: 0.89 g; 0 mg/L^{-1}: 0.573 g) (Table 1). The highest values of root number and total root length were obtained with medium size propagules (13.68 and 182.05 mm) followed by small (11.63 and 142.65 mm) and large size (10.23 and 171.07 mm) propagules. Higher values of root number and root length were obtained with increased levels of K-IBA (10,000 mg/L^{-1}: 15.55 and 237.69 mm) as compared to other concentrations (3,000 mg/L^{-1}: 13.95 and 187.18 mm; 1,000 mg/L^{-1}: 10.37 138.33 mm; 0 mg/L^{-1}: 7.517 and 97.83 mm) (Table 1). Large propagules produced higher values of average root length (16.84 mm) as compared to medium (13.07 mm) and small (12.22 mm) propagules with elevated concentrations of K-IBA producing the highest values of average root length (15.63 mm) compared with other concentrations (1,000 mg/L^{-1}: 13.841 mm; 3,000 mg/L^{-1}: 13.617 mm; control: 13.09 mg/L^{-1}) (Table 1). For stem diameter, large propagules produced significantly higher values (27.14 mm) compared to other treatments (medium: 22.36 mm and small: 17.45 mm). In regards to K-IBA level, the mean test resulted in two significantly different groups. The first group included 1,000 mg/L^{-1}: 23.09 mm, control: 22.94 mm, and 10,000 mg/L^{-1}: 22.375 mm, which was significantly higher than with 3,000 mg/L^{-1} (20.86 mm). Finally, greater plant height was obtained when large size propagules were used (17.00 mm) followed by the small size (15.21 mm) and medium size (13.74 mm). Increased values of plant height were obtained when cuttings were treated with K-IBA; however, a higher concentration was better (10,000 mg/L^{-1}: 16.97 mm; 1,000 mg/L^{-1}: 15.53 mm; 3,000 mg/L^{-1}: 15.33 mm), as compared with the control that produced significantly smaller plants (13.43 mm) (Table 1).

In the second experiment, the type of substrate affected most of the experimental variables tested; however, no effect was observed on the root dry weight. In general, mix B, which has better physiochemical traits than mix A (including water holding capacity, increased aeration, more balanced nutrient status, and better ion exchange capacity) highly benefited the *Echinopsis spachiana* cuttings during ARF. Statistical significance was obtained for the root number data when comparing mix B (11.55) versus mix A (10.30). Total root length and average root length data showed statistical significance between mix B (101.91 mm and 8.89 mm) and mix A (51.57mm and 4.98mm). Stem diameter and plant height data were statistically greater when plants were cultivated in mix B (18.73 mm and 15.88 mm) than mix A (15.77 mm and 13.16 mm) (Figure 1).

Data obtained in the last experiment in which we tested the wounding of cuttings showed no statistical differences in root dry weight, root number, total root length, average root length, and stem diameter data between wounded and non-wounded

cuttings. Plant height was the only variable showing statistical significance between treatments [wounded: 16.13 mm vs.14.51 mm of non-wounded cuttings] (Table 2).

Acknowledgements: The authors express their thanks for 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 spachiana* H. (Lemaire) H. Friedrich & G.D. Rowley cuttings after 8 months of greenhouse culture.

Propagule size	K-IBA level (mg/L-1)	Root dry weight (g)	Roots (no.)	Total root length (mm)	Root length(mm)	Stem diameter (mm)	Plant height (mm)
Large	0	0.83	7.60	119.73	15.73	26.62	19.80
Medium	0	0.52	6.55	79.54	12.18	23.82	13.10
Small	0	0.36	8.40	94.54	11.36	18.39	13.90
Large	1,000	1.60	8.60	151.61	17.70	28.76	17.80
Medium	1,000	0.71	13.85	156.43	11.39	22.42	14.15
Small	1,000	0.35	8.65	106.95	12.43	18.08	14.65
Large	3,000	2.63	10.90	163.69	15.33	25.38	17.10
Medium	3,000	1.01	16.80	239.96	14.44	20.77	14.40
Small	3,000	0.70	14.15	157.88	11.08	16.43	14.50
Large	10,000	3.98	13.80	249.26	18.60	27.81	19.80
Medium	10,000	1.80	17.55	252.59	14.29	22.44	13.30
Small	10,000	1.08	15.30	211.22	14.00	16.88	17.80
Significance:							
Propagule Size		***	***	***	***	***	***
K-IBA level		***	***	***	***	***	***
Propagule Size × K-IBA Level		***	***	***	***	NS°	NS

° NS= Non significant, *= Significant (p=0.05), **= Significant (p=0.01), ***= Significant (p=0.001); n= 20.

Table 2. Effect of wounding on adventitious root formation, root and plant growth of *Echinopsis spachiana* H. (Lemaire) H. Friedrich & G.D. Rowley cuttings after 8 months of greenhouse culture.

Source of Variation	Root dry weight (g)	Roots (no.)	Total root length (mm)	Root length (mm)	Stem diameter (mm)	Plant height (mm)
Wounding	1.35	12.10	170.80	14.36	22.33	16.13
Non Wounding	1.25	11.59	159.71	13.73	22.30	14.51
Significance:						
Treatment	NS	NS	NS	NS	NS	**

◦ NS= Non significant, *= Significant (0.05), **= Significant (0.01), ***= Significant (0.001). n= 60.

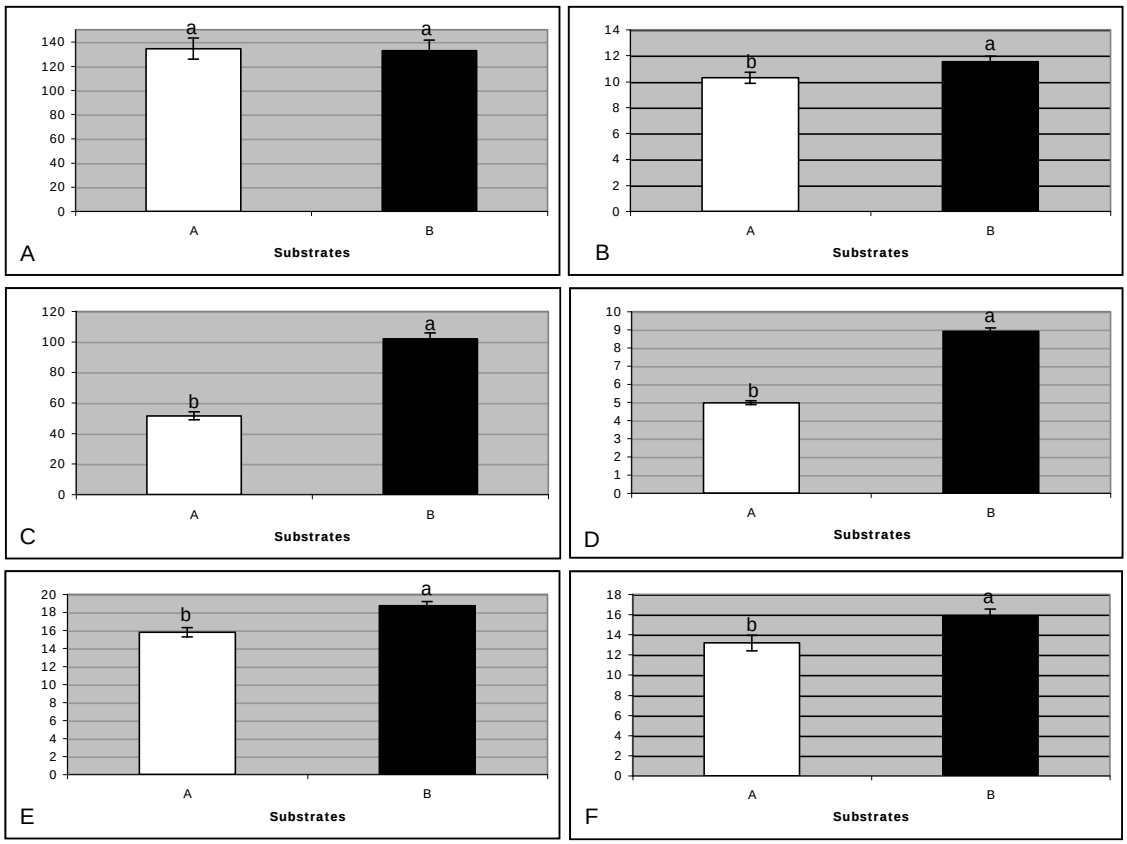

Figure 1. Effect of the type of substrates on adventitious root formation, root and plant growth of *Echinopsis spachiana* H. (Lemaire) H. Friedrich & G.D. Rowley cuttings after 4 months of greenhouse culture.

Morphogenetic Responses Induced by Cytokinins During Micropropagation of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae)

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Index Words: *Turbinicarpus*, tissue culture, *in vitro* propagation, large-scale propagation

Significance to Industry: This study was conducted to develop an *in vitro* regeneration system for *Turbinicarpus ysabelae* (Cactaceae), which is both an endemic and threatened Mexican cactus naturally growing in a small area near the town of Tula in the state of Tamaulipas (1) and an ornamental plant highly appreciated by collectors. Because natural populations have a tendency to disappear, the Mexican government recently classified this plant as a threatened species (NOM-059-ECOL-2010) (2). The establishment of a system of mass propagation for this plant species can benefit the nursery industry and help to rescue and restore native habitats. In general, we found that breaking dormancy of axillary meristem located in the areole can be achieved by varies cytokinins or a combination of NAA+BA at different concentrations to produce as many as 12 shoots per explant. Whole plants can easily be produced if the regenerated shoots are cultured on a medium free of growth regulators; however, the presence of K-IBA (1.2 and 2.4 mg/L^{-1}) significantly improved responses. Typically, *Turbinicarpus ysabelae* is a monopodic plant; however, through this protocol it is possible to produce plants with multiple shoots, which may be an attractive ornamental trait.

Nature of Work: To establish the micropropagation protocol for *Turbinicarpus ysabelae*, we performed several experiments involving standard stages of micropropagation. Before starting the experiments and because this plant is a threatened species, we focused on generating the explants (shoots) from aseptic germinated seeds. Because of this, the seeds were initially cleaned through immersion in an ethanol solution for 5 min, immersion in a Clorox solution (30% v/v) plus 0.1% of Tween-20 for 20 min, rinsed 5 times in deionized sterile water, and then initiated on a half-strength Murashige and Skoog (MS) (3) medium.

For initial culture (Stage 1), we ran a simple experiment with a randomized design to assess the effects of four cytokinins [6-BAP (6-Benzylaminopurine), 2ip (6-(γ,γ-Dimethylallylamino) purine, KN (kinetin), and TDZ (thidiazuron)] using five concentrations of each (BA at 0, 2, 4, 8, or 12 mg/L^{-1}; 2ip at 2, 4, 6, or 8 mg/L^{-1}; KN at 3, 6, 9, or 12 mg/L^{-1}; TDZ at 0.065, 0.125, 0.25, or 0.5 mg/L^{-1}) on breaking dormancy of axillary buds. For proliferation (Stage 2), we performed a series of experiments to optimize increase in shoot production. In a first trial, we tested the growth regulators and concentrations that produced better results during the initial cultures. In order to do this,

we set up a simple experiment with a randomized design composed of 9 treatments, which included three cytokinins and three concentrations (BA at 0, 4, or 8 mg/L^{-1}; 2ip at 0, 6, or 8 mg/L^{-1}; KN at 0, 9, or 12 mg/L^{-1}). In a second trial, we established a simple experiment with a randomized design including 6 treatments to evaluate additional concentrations of 2ip (10, 12, or 14 mg/L^{-1}) and KN (15, 18, or 21 mg/L^{-1}). Finally, we ran a third simple experiment with a randomized design to evaluate 15 treatments including BA (0, 6, 8, or 10 mg/L^{-1}), 2ip (0, 8, 10, or 12 mg/L^{-1}), KN (0, 12, 15, or 18 mg/L^{-1}) and the combinations NAA+BA (1:3, 1:4.5, or 1:6 mg/L^{-1}). After all this, we established another simple experiment with a randomized design to test the media concentration on shoot growth and elongation using two treatments: 100% or 50% MS salts and organic compounds. For rooting (Stage 3), four treatments (0, 0.6, 1.2 or 2.4 mg/L^{-1} of K-IBA) were used in a simple experiment with a randomized design to test their effect on adventitious root formation. For the Stage 4, 60 micropropagated plantlets of *Turbinicarpus ysabelae* were transplanted and acclimatized for 15 days on a bench with low light conditions [400 μmol/m^{-2}/s^{-1} photosynthetic photon flux density (PPFD)] prior the transfer to a greenhouse with 30% (UV filtration) with 800 μmol/m-2/s^{-1} of PPFD to evaluate plant performance and survival after one year under greenhouse culture. In all experiments, we used MS (4) culture media, which was prepared with pH adjusted to 5.8, agar at 7 g/L, and sucrose at 3%. Cultures were incubated in a room with a photoperiod of 16 h of light and 400 μmol/m^{-2}/s^{-1} PPFD. Response data included percentage of explants with callus production, number of shoots per explant, root number, total root length (mm), mean root length (mm), and percentage of plantlet survival. All data were subjected to ANOVA and Tukey's test ($\propto$= 0.05) for mean separation.

Results and Discussion: The process of seed germination started 13 days after inoculation and by day 60 we registered 87% total seed germination. At this time, the seedlings had reached between 7 to 10 mm in height and included between 20 to 25 areoles each. In the initial cultures (Stage 1 of micropropagation), we observed after 70 days of culture that all treatments had induced production of undifferentiated, rapidly growing callus, which notably turned red as they grew; however, differentiated organogenic structures (shoots) were also produced only in some treatments, especially those supplemented with BA, KN, and 2ip, regardless of the concentration. Both callogenesis and organogenesis were initiated at the base of the explant (shoot without roots from the seedlings); however, callus was produced from cells at the cut surface while shoots originated through activation of axillary meristems, which were located in the areole of each tubercle. Callus production varied according to type and concentration of cytokinin. TDZ (0.5 and 0.25 mg/L^{-1}), and 2ip and BA (4 and 8 mg/L^{-1}) were able to induce callus formation on all explants (100%), which was significantly higher to other treatments (which varied from 49 to 90%). In contrast to this, the control treatment that lacked any growth regulator produced callus on only 16.6% of the explants. In regards to shoot production, the highest numbers of shoots were produced by treatments including KN 12 mg/L^{-1} (3.7), BA 8 mg/L^{-1} (3.6), KN 9 mg/L^{-1} (3.5), and 2ip 8 mg/L^{-1} (3.2), which showed no statistical differences among each other, but were significantly higher than other treatments (Table 1). Our data showed a high, positive

correlation between concentration and shoot number for treatments including 2ip and KN; however, with the range of concentrations evaluated in this experiment, we did not reach the optimal peak for shoot number. In contrast to this, the optimal concentration of BA seemed to be 8 mg/L^{-1} because it produced an average of 3.6 shoots per explant, which was reduced to 2.0 shoots at an increased concentration (12 mg/L^{-1}) (Table 1).

Similar to what happened in the induction cultures, in Stage 2 the explants produced two general responses: proliferating undifferentiated callus and producing shoots. Shoots, in general, were produced using a wide range of treatments and concentrations. Interestingly, the propagation rate was significantly increased in comparison to that observed in the induction cultures. In the first experiment, we observed that callus was produced by all growth regulators (BA, 2ip or KN), regardless of the concentration; however, the percentages ranged from 41.7% to 100%. There were statistical significance among the treatments such that media supplemented with BA (4 and 8 mg/L^{-1}) and 2ip (6 and 8 mg/L^{-1}) produced the highest values (100%) as compared to other treatments. The best responses for shoot number were obtained using treatments supplemented with two different cytokinins and concentrations: KN (12 mg L^{-1}) and BA (8 mg/L^{-1}), which produced an average of 6 and 5 shoots per explant, followed by KN (9 mg L^{-1}), 2ip (8 mg/L^{-1}), and 2ip (6 mg/L^{-1}) with an average of 5.5, 4.9 and 4.5 shoots per explant, respectively; however, there were no statistically significant differences among these (Table 2).

In the second experiment, in which we tested additional concentrations of KN and 2ip to optimize shoot production, we again found callogenesis in all treatments (Table 3). The highest values were produced by treatments including 2ip (10, 12, and 14 mg/L^{-1}), which were comparable to the values for KN (15, 18, and 21 mg/L^{-1}) that produced 83.3, 66,7 66.7 %, respectively. Treatments including KN in the range of 15 to 21 mg/L^{-1} produced the highest values with 8.9, 6.8, and 6.8 shoots per explant, respectively. These values were, by far, considerably higher than the results obtained in previous experiments. Media supplemented with 2ip (10, 12, 14 mg/L^{-1}) produced lower responses (4, 5.3, and 5 shoots per explants, respectively) (Table 3). Treatments free of growth regulators did not produce shoots.

In a third experiment, we compared the best concentrations of BA, KN and 2ip with several concentrations of NAA+BA. Most treatments including growth regulators produced callogenesis as previously observed; however, for an unknown reason, the percentage with callus was considerably reduced in all treatments supplemented with cytokinins alone or did not produce any response as observed using KN (15 and 18 mg/L^{-1}). Means were grouped by Tukey's Test into 3 different groups in which NAA+BA (1:3, 1:4.5 mg/L^{-1}) produced the highest values (100%), which were comparable to results using NAA+BA (1:6, 1:8 mg/L^{-1}) with 80%. Results with other treatments ranged between 20% and 60 %. For shoot number, Tukey's test resulted in 5 different groups. The higher values were obtained with the combinations NAA+BA 1:8, 1:6, and 1:4.5 mg/L^{-1} (12.67, 11.92, and 11.5 shoots per explant); however, the values for KN (18 mg/L^{-1}) and 2ip (10mg/L^{-1}) (10.58 and 10.08, respectively) resulted in no statistical differences (Table 4). Responses obtained in this experiment were greater compared to

the other experiments. Results for other treatments were in the range of 3.32 to 9.92 shoots.

It was found that a subculture to promote shoot elongation allowed for the production of better and more uniform materials before rooting to regenerate whole plants. In an experiment to test the effects of media concentration on shoot growth, the two treatments (MS-100= 11.01 mm vs. MS-50%= 5.67 mm) were statistically significant (Fig. 1). Whole plantlets were easily obtained through adventitious root formation since rooting of the regenerated shoots was produced on half-strength MS medium free of auxins; however, if K-IBA was added to the culture medium at concentrations of 1.2 or 2.4 μM, the responses estimated as root number were significantly better (5.3 and 5.0, respectively) per explant with longer roots (6.4 mm and 5.0 mm, respectively) (Table 5). Total root length and average root length were comparable among treatments (Table 5). Survival in soil and greenhouse conditions of the in vitro generated plants was 92% after a year of transplantation to *ex vitro* conditions.

Acknowledgements: The authors want to thank the Universidad De La Salle Bajío through the Office of the Research Council for economic support.

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Table 1. Effect of different cytokinins and concentrations on callus production and shoot number during culture induction of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) after 70 days of culture.

Cytokinin	Concentration (mg/L^{-1})	Callus production (%)	Shoots (no.)
BA	0	16.7 c	0.0 b
BA	2	91.7 ab	1.0 ab
BA	4	83.3 ab	2.3 ab
BA	8	100.0 a	3.6 a
BA	12	91.7 ab	2.0 ab
2ip	0	16.7 c	0.0 b
2ip	2	91.7 ab	2.3 ab
2ip	4	100.0 a	1.0 ab
2ip	6	91.7 ab	2.8 ab
2ip	8	91.7 ab	3.2 a
KN	0	16.7 c	0.0 b
KN	3	41.7 bc	0.0 b
KN	6	66.7 abc	1.7 ab
KN	9	91.7 ab	3.5 a
KN	12	91.7 ab	3.7 a
TDZ	0	16.7 c	0.0 b
TDZ	0.65	75.0 ab	0.0 b
TDZ	0.125	55.6 abc	0.0 b
TDZ	0.25	100.0 a	0.0 b
TDZ	0.5	100.0 a	0.0 b
MSD value			

*Values with the same letter are statistically similar according to Tukey's Test ($\propto$= 0.05); n= 3.

Table 2. Effect of different cytokinins and concentration on callus production and shoot number during proliferation subcultures of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) after 70 days of culture.

Cytokinin	Concentration (mg/L^{-1})	Callus production (%)	Shoots (no.)
BA	0	41.7 b	0.0 c
BA	4	100.0 a	2.9 b
BA	8	100.0 a	5.9 a
2ip	0	41.7 b	0.0 c
2ip	6	100.0 a	4.5 ab
2ip	8	100.0 a	4.9 ab
KN	0	41.7 b	0.0 c
KN	9	83.3a	5.5 ab
KN	12	66.7 ab	6.0 a
MSD value		4.76	4.76

*Values with the same letter are statistically equal according to the Tukey's Test ($\propto$= 0.05); n= 4.

Table 3. Effect of different cytokinins and concentration on callus production and shoot number during proliferation subcultures of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) after 70 days of culture.

Cytokinin	Concentration (mg/L^{-1})	Callus production (%)	Shoots (no.)
2ip	0	41.7 b	0.0 c
2ip	10	100.0 a	4.0 b
2ip	12	100.0 a	5.3 b
2ip	14	100.0 a	5.0 b
KN	0	41.7 b	0.0 c
KN	15	83.3a	8.9 a*
KN	18	66.7 ab	6.2 ab
KN	21	66.7 ab	6.8 ab
MSD value		4.68	4.68

*Values with the same letter are statistically equal according to the Tukey's Test ($\propto$= 0.05); n= 4.

Table 4. Effect of different cytokinins, combinations, and concentration on callus production and shoot number during proliferation subcultures of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) explants with shoot apex after 70 days of culture.

Cytokinin	Concentration (mg/L^{-1})	Callus production (%)	Shoots (no.)
BA	0	0.0 c	0.00 e
BA	6	20.0 bc	3.92 de
BA	8	20.0 bc	9.92 abc
BA	10	20.0 bc	10.08 abc
2ip	0	0.0 c	0.00 e
2ip	8	60.0 abc	5.92 cd
2ip	10	40.0 abc	9.67 abc
2ip	12	80.0 ab	4.58 d
KN	0	0.0 c	0.00 e
KN	12	20.0 bc	4.92 d
KN	15	0.0 c	7.33 bcd
KN	18	0.0 c	10.58 abc
NAA:BA	1:3	100.0 a	7.25 bcd
NAA:BA	1:4.5	100.0 a	11.5 ab
NAA:BA	1:6	80.0 ab	11.92 ab
NAA:BA	1:8	80.0 ab	12.67 a
MSD value		5.04	5.11

*Values with the same letter are statistically equal according to the Tukey's Test ($\propto$= 0.05). n= 4.

Table 5. Effect of K-IBA on adventitious root formation of regenerated shoots *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) after 50 days of culture.

Growth Regulator	Concentration (mg/L^{-1})	Root number	Total root length (mm)	Root length (mm)
K-IBA	0	1.83 b	14.67 a	4.86 a
K-IBA	0.6	3.17 ab	19.5 a	5.83 a
K-IBA	1.2	5.33 a	35.33 a	6.40 a
K-IBA	2.4	5.08 a	28.58 a	5.03 a
MSD value		4.26	4.20	4.20

*Values with the same letter are statistically similar according to the Tukey's Test ($\propto$= 0.05). n= 4 .

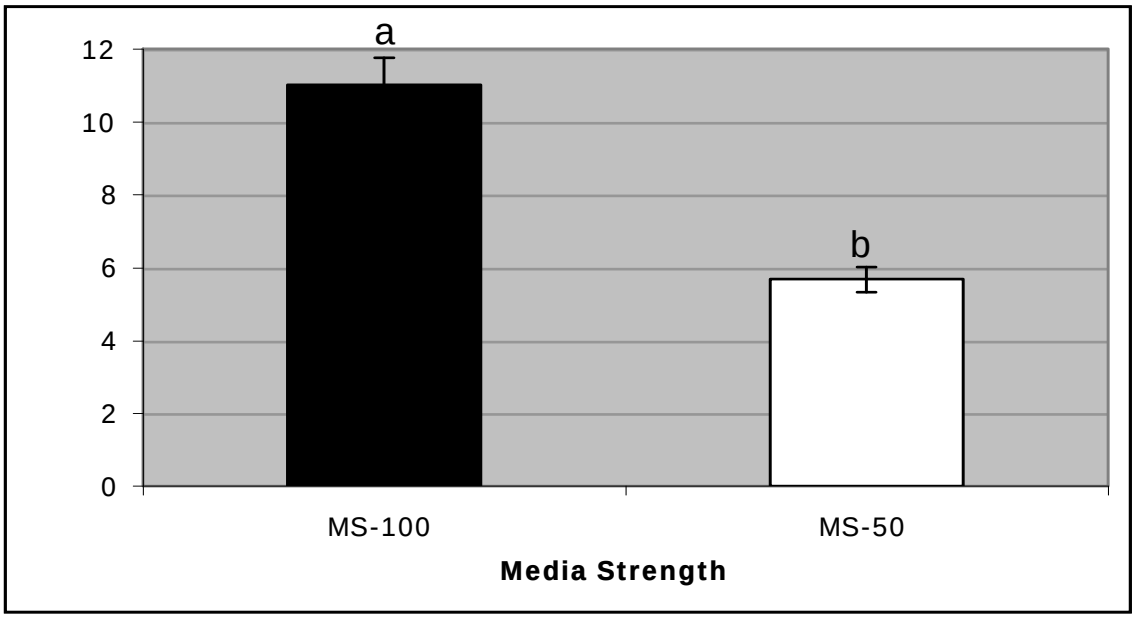

Fig. 1. Effect of media concentration on shoot growth of *Turbinicarpus ysabelae* (Werderm.) John & Riha (Cactaceae) after 40 days of culture; n= 16.

Chipped *Juniperus virginiana* as a Perlite Substitute in Stem Cutting Propagation

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Index words: alternative substrate, eastern redcedar, *Euonymus kiautschovicus, Forsythia ×intermedia, Hedera helix,* juniper, *Lantana camara,* perlite, *Solenostemon scutellarioides,* wood chips.

Significance to Industry: Eastern redcedar (*Juniperus virginiana* L.) trees occur as a weed species throughout the eastern half of the United States. Aged wood can be chipped and then hammermilled to produce a substrate component with aerating properties similar to perlite. Stem cuttings of 'Defiance' coleus [*Solenostemon scutellarioides* (L.) Codd], 'Anne Marie' English ivy (*Hedera helix* L.), forsythia (*Forsythia ×intermedia* Zab.), 'Irene' lantana (*Lantana camara* L.), and spreading euonymus (*Euonymus kiautschovicus* Loes.) were rooted in substrates containing eastern redcedar chips (ERC) that had been hammermilled to pass a 4.8-mm (0.19-in) screen. Cuttings of coleus, English ivy, and lantana rooted as well (≥95%) in the 100% ERC substrate as they did in a standard rooting substrate [3 perlite: 1 peat (v/v)]. Spreading euonymus also rooted well (≥95%) in all substrates, but root length decreased as ERC replaced perlite. Forsythia rooted poorly in all substrates (8% to 36%). Growers seeking an alternative to perlite should consider ERC as a rooting substrate component.

Nature of Work: Researchers have investigated many locally available alternatives for perlite, which is a dusty, eye and lung irritant. Eastern redcedar trees are frequently cleared from grassland and burned as "trash" wood. Processing coarsely chipped eastern redcedar through a hammermill yields material suitable as a horticultural substrate component. Work by Starr (5) demonstrated that ERC could replace peat moss without reducing propagation success from stem cuttings of several herbaceous ornamental species. The current study investigated the potential of ERC as a substitute for perlite in a general purpose, 3 perlite: 1 sphagnum peat moss (v/v) rooting substrate used for propagation of coleus, English ivy, forsythia, lantana, and spreading euonymus

On 6 Dec. 2013, chipped eastern redcedar (Queal Enterprises, Pratt, Kans.) was further processed through a hammermill (Model 30HMBL, C.S. Bell Co., Tiffin, Ohio) to pass a 0.19-in (4.8-mm) screen. These processed chips were used to prepare five substrates of increasing ERC content (0%, 25%, 50%, 75%, and 100% by vol.). All substrates contained 25% sphagnum peat moss, except the 100% ERC substrate. The remaining volume of each substrate was coarse perlite. Clean flats [15.75 in x 15.75 in x 5 in (40

cm x 40 cm x 12.7 cm] with a screen bottom were filled with substrate and placed under intermittent mist (8 sec. every 4 min. during daylight hours) in a glass greenhouse with natural photoperiod and constant temperature set at 84°F (29°C).

Woody cuttings of euonymus and forsythia were harvested 16 Dec. 2013 at Kansas State University, Manhattan, KS. Stems from most recent season's growth were cut above nodes to form 4- to 6-in (10- to 15-cm) cuttings. Leaves were stripped from dormant forsythia cuttings and from the basal one-half of euonymus cuttings. Herbaceous stem cuttings were purchased from a commercial unrooted cutting supplier (North Carolina Farms Inc., Indian Trail, NC.) and planted on 4 Jan. 2014. Single node stem cuttings of English ivy were 0.8 to 1.6 in (2 to 4 cm) in length and were trimmed just above a node at both ends. Lantana and coleus both arrived as 0.8- to 1.6-in (2- to 4-cm) stem tip cuttings.

The bottom 0.4 in (1 cm) of cuttings of each species (except coleus) was dipped 5 s in 1000 ppm (0.1%) potassium salt of indole-3-butyric acid (K-IBA) dissolved in distilled water. Coleus cuttings were not treated with auxin. Six cuttings of each species were inserted 0.4 to 0.8 in (1 to 2 cm) deep into each substrate, which was gently firmed around cuttings. Intermittent mist was initially set to maintain moist leaf surfaces, but was gradually reduced as herbaceous cuttings rooted. Coleus, forsythia, English ivy, lantana, and spreading euonymus cuttings were harvested and data collected after 25, 59, 32, 32, and 51 days, respectively. Data was collected at harvest and rooting percentage, mean root number, and mean primary root length were calculated.

The experimental design was a randomized complete block design with five substrate treatments and six cutting subsamples of each species per substrate. The treatments were replicated 6 times. Data were analyzed with linear models using the GLM procedure of SAS (Version 9.2; SAS Institute Inc., Cary, NC). Each species made up a separate experiment.

To determine substrate air space, container capacity, total porosity, and bulk density, substrate samples were adjusted to 35% volumetric water content and four replications of each were measured using the procedure described by Fonteno and Harden (2).

Results and Discussion: Overall percent rooting was high for all species (≥95%), except forsythia (25.6%), and unaffected by substrate ERC content (Table 1). Many forsythia cuttings desiccated when mist was reduced (to 6 s every 16 min) 40 days after the experiment began. Warm greenhouse conditions likely forced forsythia cuttings to initiate shoot growth before adventitious root initiation.

Mean root number per rooted cutting and mean root length were not significantly affected by substrate ERC content with any species, except spreading euonymus for which the response to ERC was quadratic in nature (Table 1). Euonymus root number peaked at 0% and 100% ERC with means of 36.3 and 25.6 roots, respectively. Root length of spreading euonymus declined linearly [from 2.8 to 0.9 in (7.1 to 2.3 cm)] with

increasing ERC content and a corresponding increase in bulk density [from 5.6 to 10.6 $lb \cdot ft^{-3}$ (0.09 to 0.17 $g \cdot cm^{-3}$)]. Chong (1) observed a similar inverse relationship between root length and substrate bulk density after growing burning bush [*Euonymus alatus* (Thunb.) Siebold] in substrates containing composted municipal solid waste. Kirkham (3) demonstrated that increasing bulk density increases the work roots must do to elongate. This may explain why cuttings grown in substrates with large ERC contents typically had shorter roots.

Herbaceous cuttings rooted well among treatments (Table 1). Average rooting of all coleus treatments combined was 94.7% with a mean of 12.3 roots per rooted cutting and a mean root length of 2.2 in (5.5 cm). Cuttings of English ivy rooted at 98.9% with a mean root number of 11.8 and mean root length of 1.8 in (4.5 cm). Similarly, cuttings of lantana rooted at 97.2% with a mean root number of 7.8, and mean root length of 1.4 in (3.5 cm). These results demonstrate that cuttings of many species can root successfully in ERC substrates.

Maronek et al. (4) provide recommended ranges for physical properties of propagation substrates. In the current experiment, all substrates were within the recommended ranges for air space (15% to 40%) and container capacity (20% to 60%) (data not shown). Total porosity was above the recommended range (40% to 60%) in all cases, but was closest (79.2%) in the standard 3 perlite:1 peat (v/v) substrate. High porosity can lead to poor contact between substrate and cutting tissue (4), but in this experiment, high porosity did not seem to be a problem as most species rooted well. Bulk density was below the recommended range of 18.7 to 49.9 $lb \cdot ft^{-3}$ (0.3 to 0.8 $g \cdot cm^{-3}$) and only reached 10.6 $lb \cdot ft^{-3}$ (0.17 $g \cdot cm^{-3}$) at the highest ERC content. Similarly, Starr (5) determined the bulk density of his 100% ERC substrate to be 11.2 $lb \cdot ft^{-3}$ (0.18 $g \cdot cm^{-3}$). The range for bulk density recommended by Maronek et al. (4) is influenced by the ballast needed in substrates used for liner production. Though substrates from this experiment may not be dense enough for container production, they all appear suitable as propagation substrates.

ERC substrates have excellent potential for cutting propagation. Although roots of certain species such as spreading euonymus and possibly forsythia may develop poorly in ERC substrates, other species, including English ivy, lantana, and coleus, root well in substrates containing up to 100% ERC. Propagators seeking alternatives to perlite should consider ERC as a component of their propagation substrate.

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Table 1. Percent rooting, mean root number, and mean root length of stem cuttings inserted into substrates containing 0%, 25%, 50%, 75%, or 100% (by vol.) *Juniperus virginiana* chips (ERC). Individual treatment means are given for spreading euonymus.

Species	Rooting (%)[z]	Roots (no.)	Root Length (in)
Coleus	94.7[NS]	12.3[NS]	2.2[NS]
English Ivy	98.9[NS]	11.8[NS]	1.8[NS]
Forsythia	25.6[NS]	3.6[NS]	0.6[NS]
Lantana	97.2[NS]	7.8[NS]	1.4[NS]
Spreading Euonymus	96.1[NS]	24.6[y]	1.7[x]
0% ERC	97.2[w]	36.3	2.8
25% ERC	100.0	20.1	2.3
50% ERC	97.2	20.7	1.6
75% ERC	88.9	20.2	1.0
100% ERC	97.2	25.6	0.9

[z]Combined mean of all treatments; n=36 stem cuttings per treatment.
[NS]Treatment differences were not significant at $P \leq 0.05$.
[y]Treatment differences were significant at $P \leq 0.01$ and followed a quadratic trend.
[x]Treatment differences were significant at $P \leq 0.01$ and followed a linear trend.
[w]Treatment mean for spreading euonymus.

Hormone Impact on Rooting of *Syzygium buxifolium* Stem Cuttings

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Index Words: blight, boxwood, hormone, new plant, rooting, stem cutting, syzygium

Significance to Industry: Due to pressure from boxwood blight, alternatives to boxwood are desperately needed. *Syzygium buxifolium* is an evergreen shrub or small tree with boxwood-like leaves. It has a similar landscape appearance to common boxwood. To support breeding and selection of this new crop for the nursery industry, we investigated clonal propagation using stem cuttings. The results of this study should enhance the market potential and nursery production of this plant.

Nature of Work: *Syzygium buxifolium* Hook. et Arn. (boxwood syzygium; Myrtaceae) is an evergreen shrub or small tree. Although the majority of *Syzygium* species are distributed in tropical areas, boxwood syzygium is native to the forest area or scrub in the mountains of Central and South China (subtropical or temperate zones), as well as South Japan and Vietnam (1). The plant has a habit and growth features similar to boxwood and is an ideal plant to substitute for boxwood in the landscape, especially considering the pressure of boxwood blight (2). Boxwood syzygium has been widely grown in China for bonsai and landscape plants (3,4,5). The UGA woody plant research laboratory has initiated a breeding and selection project with *Syzygium buxifolium* and hopes to select some potential cultivars which could substitute for boxwood in our Georgia and southeastern U.S. landscapes.

To regenerate a new cultivar for commercial production, rooting of stem cuttings is the most common method used in the nursery industry (6). On 29 Jan. 2014, semi-hardwood stem cuttings (10-15 cm long) of *Syzygium buxifolium* were collected from one-year-old seedlings at the Horticulture Farm of the University of Georgia. Cuttings were placed into black plastic bags and sprayed with water immediately after being removed from the mother plants. Leaves along the bottom 3-5 cm were stripped and cuttings received the following treatments: 1: Control (CK); 2: K-IBA at 1,000 ppm; 3: K-IBA at 3,000ppm; 4: K-IBA at 8,000ppm; 5: Hormodin #1 (1,000 ppm IBA); 6: Hormodin #2 (3,000ppm); 7: Hormodin #3 (8,000ppm); and 8: K-IBA at 5,000ppm + Hormodin #2. For application of Hormodin powder, cuttings were first dipped into water and then dusted with Hormodin powder. Treated cuttings were randomly inserted into the rooting media, which contained Fafard 3L Mix (main ingredients: peat moss and bark) and perlite at 1:1(v:v). For K-IBA application, cuttings were dipped into the concentrations for 10-15 seconds, then air-dried for at least 10 minutes before placing them into the

rooting medium. For the double dips, cuttings were treated with liquid hormone first, and then the powder. All cuttings were rooted in 32-cell flat trays and thoroughly watered before placing them on the mist bench. The mist bench was covered with 70% shade cloth and the mist system was set for 20 seconds every 20 minutes for the first week, then 10 seconds every 20 minutes thereafter.

A randomized complete block design was used in this experiment with 4 replicates for each treatment and 8 subsamples (cuttings) per replicate per treatment. Rooting percentage, number of roots, and average length of roots were collected on the 14 May 2014 (after 15 weeks). All data were analyzed with SAS and mean separations were run with LSD (α=0.05).

Results and Discussion: Since the rooting percentage for the control was 68.8% and the root quality (as indicated by total root length (cm) per cutting) was acceptable, rooting of stem cuttings from one-year-old seedlings should not be too difficult and *Syzygium buxifolium* can be regenerated from stem cuttings. Application of rooting hormones did significantly affect the rooting percentage and root quality. The highest rooting percentage, 81.3%, was obtained using Hormodin #3 (8,000 ppm IBA). A negative effect was observed using Hormodin #2 (3,000 ppm IBA), which only yielded 21.9% rooting. Root quality followed a similar trend (Table 1).

Boxwood syzygium responded significantly to hormone concentration. Better results were obtained using either higher or lower hormone concentrations. Concentrations at 3,000 ppm reduced both rooting percentage and root quality greatly. Application methods resulted in significant differences in rooting. Use of 8,000 ppm powder yielded the highest rooting percentage (81.3%) and root quality (19.4cm). Double dips had 78.1% rooting, but no significant difference from than that of liquid hormone (62.5%). Double dip treatment yielded 10.2 cm roots per cutting, which was significantly lower than that of both liquid K-IBA at 8,000 ppm and Hormodin #3 powder (Table 1). Since double dips increased the amount of work and did not yield better results, it is not recommended.

Although Hormodin #2 significantly reduced rooting percentage and root quality, much greater callus formation was observed (Table 1). It is possible that lower powder hormone concentrations (1,000 ppm and 3,000 ppm) might help the callus formation. The callus could produce roots after the second application of hormones (data not presented). Further studies should address the relationship between callus formation and root initiation for boxwood syzygium.

Commercial propagation of *Syzygium buxifolium* can be carried out using stem cuttings and powder hormone at 8,000 ppm (Hormodin #3) recommended for better rooting percentage and higher quality of liners.

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Table 1. Impact of rooting hormones on the rooting percentage and root quality of *Syzygium buxifolium* stem cuttings.

Treatment	Rooting (%)	Total root length (cm)	Callus formation (%)
Control	68.8a*	8.8bcd	25.0c
K-IBA 1,000 ppm	75.0a	14.6ab	25.0c
K-IBA 3,000 ppm	59.4a	7.9bcd	34.4bc
K-IBA 8,000 ppm	62.5a	17.1a	31.2bc
Hormodin #1 (1,000ppm IBA)	31.3b	5.3cd	54.2ab
Hormodin #2 (3,000ppm IBA)	21.9b	2.9d	75.0a
Hormodin #3 (8,000ppm IBA)	81.3a	19.4a	15.6c
K-IBA 5,000 ppm + Hormodin	78.1a	10.2bc	21.9c

*Different letters in the same column indicate a significant difference at $\alpha = 0.05$.