Use of LS 213 During Rooting of Vegetative Ornamental Cuttings: Experiment 2

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Summary: LS 213 had positive effects on plant growth, but results varied among species tested (angelonia, mimulus, vinca vine, and rosemary). The most significant results occurred when the substrate was treated with LS 213 at 1:20 (LS 213 : potting mix) during propagation. The increased plant growth may help propagators grow larger sized plants in a shorter amount of time. Due to variation, propagators will need to determine which plants have the greatest response.

Differences in growth were not evident when the rooted cuttings were transplanted and grown in 4” pots, except for rosemary. Gustafson reported differences between LS 213 and untreated plants being evident 30 to 60 days after transplant, with the LS 213 plants having more growth. For the plants grown in 4” pots, data was often taken 75 days after inoculation, which is beyond the timing when Gustafson reported differences.

The time of 30 to 60 days of when differences are noticed is supported by the fact that a second application of LS 213 did result in growth differences with angelonia and rosemary. Angelonia growth was more compact, while the growth of rosemary increased – being greater than the control, but not the single LS 213 application. Except for the potential of height control, the second application of LS 213 appears to have limited commercial application to angelonia or rosemary production. However an opportunity for LS 213 may be with tall growing herbs in which plant growth regulators are not registered.

Introduction: Since 1989, Gustafson LLC Chemical Co. and a number of universities have been conducting trials on vegetable transplants with naturally occurring biological inoculants. These bacteria, Paenobacillus macerans GB122 and Bacillus amyloliquefaciens GB99, are marketed as LS 213 or BioYield™. This class of bacteria are referred to as plant growth-promoting rhizobacteria (PGPR). Published research reports state that PGPRs promote plant growth (especially young transplants), early flowering, and improve early yield and grade quality of vegetables. These advantageous results have been consistent, with only a few exceptions where the results were equal to the untreated control, and suggest that the use of these PGPRs would be beneficial to the vegetable production.

PGPRs are weak colonizers of the root system and do not compete with other naturally occurring soil bacteria. Consequently for LS 213 to be effective, Gustafson noted that the roots must be inoculated with the LS 213 when the radicle is
emerging from the seed.

All previous studies with LS 213 have been conducted with vegetable transplants, but it may have beneficial qualities that can be utilized by floriculture plug producers. Preliminary trials indicated that LS 213 at 1:40 (LS 213 : potting mix) promoted axillary shoot development during rooting, but results varied by species.

The objective of this study was to determine the effects of LS 213 on plant growth of Rosemary, Mimulus cultivar 'Jelly Bean White', vinca vine, and Angelonia cultivar 'Deep Plum'.

**Experiment:** The four substrate treatments (Berger BM2) included:

1. Untreated control (only Berger BM2),
2. LS 213 incorporated at 1:20 (volume/volume) into Berger BM2,
3. LS 213 incorporated at 1:40 (volume/volume) into Berger BM2 [half the rate of treatment 2],
4. LS 213 incorporated at 1:60 (volume/volume) into Berger BM2, and
5. LS 213 incorporated at 1:80 (volume/volume) into Berger BM2.

**Propagation:** Tip cuttings of the four plant types were propagated in each of the substrate treatments on October 16th. The container was a 72-cell pack flat, which is a common size used by the industry for vegetatively propagated material. The 72-cell packs were cut into eight – 9 cell trays for the study, with six trays (blocks) being used for each treatment, and 9 replicated plants per block (Figure 1). Plants were placed under mist until November 21st for rooting. Three randomly selected plants from each of the 6 blocks were evaluated on November 21st for plant height (measured from the container rim to the highest growing point), plant diameter (measured at the widest point and then turned 90°), and fresh weight. Plants were then placed in a drying oven for 72 hours and dry weights were determined.

**4-Inch Pot Production.** Six additional plants of each plant type and treatment were transplanted into 4” pots using non-LS 213 treated Fafard 4P mix. Evaluation dates and data collected varied by species. For all plants except vinca vine, plant height, plant diameter, the number of shoots ≥1 cm long were counted (excluding the primary shoot), length of the 3 longest shoots measured (excluding the primary shoot), total dry weight of the shoots ≥1 cm long, and total plant dry weight were recorded. Flowering date was recorded when each individual angelonia plants bloomed. Data was collected for angelonia, rosemary, and vinca vine on January 10th. Data for mimulus was collected on February 9th. Data collected for vinca vine included length on the longest shoot and the number of shoots ≥1 cm long.

**Second LS 213 Inoculation.** Some previous research reports note that the effects of LS 213 were enhanced with a second application. To determine those effects, six randomly selected plants of angelonia and rosemary were transplanted into Fafard 4P which had been treated with LS 213 at 1:20. The plants were grown and evaluated similarly as the other plants.

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![Fig. 1. Propagation procedures used with the LS 213 experiment.](image-url)
Results:

Propagation Evaluation

Angelonia 'Deep Plum'. Plant height was the greatest with a LS 213 rate of 1:20, with the plants being 0.7 cm taller than the other LS 213 treatments and 1.5 cm taller than the untreated control (Figures 2 and 3). Rates of 1:40 to 1:80 were similar and all were greater than the untreated control.

Shoot fresh weigh (0.9 g) was greatest with the 1:20 LS 213 rate, which was similar to the 1:40 and 1:80 rates (Figure 4). The fresh weight of the untreated control was 0.6 g which was significantly less than for all other treatments.

Plant diameters (7.4 cm) and dry weights (0.11 g) were similar among all the treatments.

Due to the increase in plant height and fresh weight, the use of LS 213 appears to be advantageous with angelonia cutting production.

These results contrast Experiment 1 in which LS 213 rates of 1:20 resulted in smaller plant height and plant diameter when compared to the 1:40 rate or the untreated control. It should be noted that the cuttings in Experiment 1 were grown in a larger sized container (36-cell pack) and evaluated later in production.
Mimulus 'Jelly Bean White'. LS 213 treatments produced mimulus plants that were taller than the untreated control (Figures 5 and 6) and had a greater shoot fresh weight (Figure 7). All LS 213 rates, except 1:40 resulted in taller plants and greater fresh weights than the untreated control.

Plant diameters (6.5 cm) and dry weights (0.15 g) were similar among all the treatments.

With the increase in plant height and fresh weight, the use of LS 213 appears to be advantageous with mimulus cuttings.

Rosemary. Plant height was greatest with LS 213 rates of 1:20, with the plants being 0.9 cm taller than the untreated control (Figures 8 and 9). All other LS 213 treatments were similar to the untreated control.

Plant diameter was greatest with LS 213 rates of 1:40 (Figure 10). The 1:20 rate was similar to the untreated control.

Fresh weights were similar between the untreated control and 1:20 rate (Figure 11). All other LS 213 rates produced plants smaller than the control.

Dry weights were similar for all treatments (0.25 g).

These results indicate that the advantages of LS 213 during propagation vary among species. Based on this study, the use of LS 213 does not appear to be beneficial with rosemary production.
**Fig. 9.** The effects of LS 213 on cutting growth of rosemary.

**Fig. 8.** The effects of LS 213 on plant height of rosemary. [LSD = 0.6 cm, $P < 0.05$]

**Fig. 10.** The effects of LS 213 on plant diameter of rosemary. [LSD = 0.5 cm, $P < 0.01$]

**Fig. 11.** The effects of LS 213 on fresh weight of rosemary. [LSD = 0.11 g, $P < 0.001$]
**Vinca Vine.** Plant height was greatest with LS 213 rates of 1:20, with the plants being 1.9 cm taller than the untreated control (Figures 12 and 13). All other LS 213 treatments were similar to the untreated control. Plant diameters were similar for all treatments (4.8 cm).

**Conclusions:** The use of LS 213 during propagation appears to have advantages in increasing plant height and fresh weight, but results varied by species. The optimal rate of 1:20 in Experiment 2 was greater than the 1:40 rate that provided better results with angelonia in Experiment 1.

With the high value of vegetative cuttings, any production practice that promotes quick growth would be advantageous and would result in cost savings. Therefore, further exploration of LS 213 should be conducted by specialty propagators to determine which plants respond positively.

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**4-Inch Pot Production Evaluation**

**Angelonia 'Deep Plum'.** The growth of angelonia was not affected by LS 213 when the plants were transplanted into 4-inch pots. For all treatments, the number of days from propagating the cuttings until flowering was 75, plant height was 36.9 cm, plant diameter 17.1 cm, the number of shoots ≥1 cm long was 14.6, average length of the three longest shoots was 11.2 cm, total shoot dry weight was 1.3 g, and total plant dry weight was 2.86 g.

While visually there appeared to be some trends among the LS 213 treatments, this was negated due to considerable plant to plant variation (Figure 14).

**Mimulus 'Jelly Bean White'.** Mimulus growth also was not affected by LS 213 when the plants were transplanted into 4-inch pots. For all treatments, plant height was 47.5 cm, plant diameter 17.7 cm, the number of shoots ≥1 cm long was 29, average length of the three longest shoots was 10.8 cm, total shoot dry weight was 4.45 g, total shoot dry weight was 8.32 g, and the number of flower buds on the plant when the data was collected was 5.1 (Figure 15).
**Vinca Vine.** There were no effects from the use of LS 213 during propagation of vinca vine on subsequent plant growth when transplanted into 4-inch pots. The average length of the longest new shoots was 69.1 cm and the average number of new shoots produced was 2.7. A proliferation of shoots from the crown of the plant would be an advantageous trait, but this did not occur.

**Rosemary.** There were differences in rosemary growth during 4-inch production with the use of LS 213. Plant height was greater with LS 213 rates of 1:20 (1.2 cm taller) and 1:60 (2.2 cm taller), when compared to the untreated control (Figure 17). LS 213 rates of 1:40 and 1:80 were similar to the untreated control. Reasons for why efficacy varied was unclear.

Plant diameters were similar for all LS 312 treatments (13.2 cm).

The number of shoots \( \geq 1 \text{ cm} \) were statistically similar with LS 213 rates of 1:20 (14) and 1:60 (18.2), when compared to the untreated control (Figure 18). All other rates were similar.

Average length of the three longest shoots was greatest with a LS 213 rate of 1:20 at 7.4 cm, which was 1.8 cm longer than the untreated control (Figure 19).

Both total shoot dry weight (Figure 20) and total plant dry weight (Figure 21) were greatest with a LS 213 rate of 1:20.

**Conclusions:** Rosemary was the only plant to have any differences in plant growth among the treatments when transplanted into 4-inch pots after inoculation during propagation. Some growth differences during 4-inch production did not occur during propagation. It is unclear why these results varied.
Fig. 17. The effects of LS 213 on plant height of rosemary when grown in 4-inch pots. [LSD = 1.5 cm, $P \leq 0.05$]

Fig. 18. The effects of LS 213 on shoot number of rosemary when grown in 4-inch pots. [LSD = 4.3 cm, $P \leq 0.05$]

Fig. 19. The effects of LS 213 on average shoot length of rosemary when grown in 4-inch pots. [LSD = 1.2 cm, $P \leq 0.05$]

Fig. 20. The effects of LS 213 on total shoot dry weight of rosemary when grown in 4-inch pots. [LSD = 0.24 g, $P \leq 0.01$]
Second LS 213 Treatment During Pot Production Evaluation

Angelonia 'Deep Plum'. The incorporation of LS 213 into the Fafard 4P substrate at 1:20 when the rooted cuttings were transplanted into 4-inch pots had significant effects on plant growth, when compared to inoculating plants with LS 213 at 1:20 only during propagation or the untreated control. Flowering date was delayed by 6 days with the use of LS 213, when compared to the untreated control (Figures 22 and 23).

Plant height was 17% shorter when LS 213 was used a second time, when compared to the control (Figure 24).

The number of side shoots produced which were ≥1 cm long was less when LS 213 was used (Figure 25). But the average length of the 3 longest shoots was greatest when LS 213 was...
Fig. 24. Comparing the effects on plant height of angelonia with a second LS 213 application. [LSD = 3.7 cm, $P<0.001$]

Fig. 25. Comparing the effects on number of shoots of angelonia with a second LS 213 application. [LSD = 5.3, $P<0.01$]

Fig. 26. Comparing the effects on average shoot length of angelonia with a second LS 213 application. [LSD = 3.4 cm, $P<0.05$]

Fig. 27. Comparing the effects on total shoot dry weight of angelonia with a second LS 213 application. [LSD = 0.43 g, $P<0.01$]
only used during propagation (Figure 26).

The incorporation of LS 213 at 1:20 resulted in a lower dry weight of both the side shoots (Figure 27) and total plant dry weight (Figure 28).

Rosemary. The second application of LS 213 resulted in the tallest plants, which were similar in height with those treated only once, but 2.4 cm taller than the untreated control (Figures 29 and 30).

Average shoot length (Figure 31), total shoot dry weight (Figure 32), and total plant dry weight (Figure 33) followed the same trend, with the single LS 213 resulting in the largest increase.

**Conclusions:** The second application of LS 213 when the rooted cutting is being transplanted into the final growing container does not appear to offer any commercial production advantages. At this stage of production, maximizing plant growth to hasten the time to market is the desired outcome. The second LS 213 application at 1:20 resulted in less plant growth with angelonia. This height control effect may be beneficial for some crops, but in commercial greenhouse production, the use of a plant growth regulator spray would be more practical. The growth of rosemary was greatest when LS 213 was used, but in most cases plant growth was greatest when LS 213 was incorporated only prior to propagation. The time and cost of a second application has no clear advantages.

**Overall Study Conclusions:** LS 213 had some positive effects on plant growth, but results varied among species. The most significant results occurred when the substrate was treated with LS 213 at 1:20 during propagation. The increase in plant growth may help propagators grow larger sized plants in a shorter amount of time. Results varied by plant type and propagators will need to determine which plants have the greatest response.

Differences in growth were not evident when cuttings were transplanted and grown in 4” pots.
Fig. 30. The effects on plant growth of rosemary with a second inoculation of LS 213 at 1:20.

Fig. 31. Comparing the effects on average shoot length of rosemary with a second LS 213 application. [LSD = 1.4 cm, $P \leq 0.05$]

Fig. 32. Comparing the effects on total shoot dry weight of rosemary with a second LS 213 application. [LSD = 0.25 g, $P \leq 0.01$]

Fig. 33. Comparing the effects on total plant dry weight of rosemary with a second LS 213 application. [LSD = 0.26 g, $P \leq 0.01$]
except for rosemary. Gustafson reported differences between LS 213 and untreated plants being evident 30 to 60 days after transplant, with the LS 213 plants having more growth. For the plants grown in 4” pots, data was often taken 75 days after inoculation, which is beyond the timing when Gustafson reported differences.

The time of 30 to 60 days of when differences are noticed is supported by the fact that a second application of LS 213 did result in growth differences with angelonia and rosemary. The growth response of a second LS 213 application resulted in opposite effects with angelonia and rosemary. Angelonia growth was more compact, while the growth of rosemary increased – being greater than the control, but not the single LS 213 application. Except for the potential of height control, the second application of LS 213 does not appear to have any commercial applications to the vegetatively propagated cutting production. However, plant growth regulators are registered and used regularly. An opportunity for LS 213 may be with tall growing herbs in which plant growth regulators are not registered.

Acknowledgments: Uniroyal Chemical Co. for grant support, Berger Co. and Fafard Co. for the root substrate, and Scott’s Co. for the fertilizer. Thanks is also expressed to Lloyd Traven, Peace Tree Farm, Kintersville, PA, Al Newsom of Ball Seed Co., and Jack Williams of the Paul Ecke Ranch for supplying the cuttings.

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