Evaluation of Ginseng (*Panax quinquefolius*) Germination and Aeroponic Growth in Response to Stratification Duration, GA_3, De-coating, and Media

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Abstract: Abstract: Ginseng (*Panax quinquefolius*) is a well-known and commonly used Chinese herb for its physical strength development properties (Choi, 2008). Other effects which have been discovered be modern science includes improved brain functions, pain relieving effects, enhanced immune system and liver function, as well as anti-stress and anti-fatigue effects (Choi, 2008). In its natural habitat, ginseng grows in heavily shaded forest type locations. However, a great deal of failure has occurred in the process of trying to grow this plant in a production setting (Hankins, 2000). Wood cultivation and wild simulated grown ginseng can take anywhere from six to twelve years for it to fully mature (Beyfuss, 1998). Our objective was to evaluate methods to enhance germination rates and uniformity while producing seedlings for aeroponic production. Three hundred warm stratified Ginseng seeds obtained from Hardings Ginseng Farm were cold moist stratified for six weeks. Half of the seeds were soaked in a 0.1% gibberellic acid (GA_3) solution prior to stratification (Pre-strat), while the other half received the same GA_3 soak at the end of the stratification (Post-strat). After 6 weeks, 64% of the Pre-strat seeds, and 76% of the Post-strat seeds exhibited endocarp splitting (smiling). 150 smiling seeds of each group were randomly selected and the non-smiling seeds were returned to the stratification environment for an additional 8 weeks. Half of each group (6 week strat) were then de-coated, resulting in 75 seeds of each treatment; Pre-strat with coat, Pre-strat de-coated, Post-strat with coat, and Post-strat de-coated. All 300 seeds were then planted in coarse sand and placed onto an automated mist bench. Seedling emergence was observed over an eight week period. The seeds stratified for an additional 8 weeks were observed for germination after the full 14 week stratification. Eighteen seedlings at the one true leaf stage were randomly assigned to three media treatments and placed in an automated aeroponic system where growth was evaluated over 12 weeks. Seeds treated with GA3 prior to stratification emerged at significantly higher rates than seeds treated after the 6 week stratification. De-coating yielded no significant difference. Overall, germination was lower in all 6 week stratification treatments compared to seeds stratified for 14 weeks. Seedling growth parameters differed in response to media in the aeroponic unit.

Figure 2. Photo of a three prong American Ginseng growing in its native habitat (Cannon Co., TN).

INTRODUCTION:

Ginseng is an herb which has been demonstrated to have beneficial medical applications. According to the University of Maryland Medical Center "…ginseng is effective in boosting the immune system, and as an antioxidant" (Ehrlich, 2014). Additionally, they stated that "Other studies show that American ginseng might have therapeutic potential for inflammatory diseases" (Ehrlich, 2014). These are just examples of the numerous reputed benefits for which this plant can be cultivated. This is an important crop which could potentially enhance or even save lives. Because this particular crop has so much worth, both medically and economically, the natural ginseng population is slowly, but steadily being driven towards extinction due to over harvesting (Burkhart, 2016). In order to avoid such a fate from happening, we need to find out how to more efficiently cultivate it. Growing ginseng in a controlled wild environment has its own downsides. Since this plant takes anywhere between 5-7 years to mature, there is ample opportunity for stands to succumb to detrimental biotic and abiotic factors. A solution to these challenges would be to grow these crops in a hydroponics unit so that one may control the environment better, thereby reducing losses while increasing the growth rate.

There has been little to no published research regarding production practices for hydroponically grown ginseng. To help fill this information gap, our objective was to evaluate alternative methods for growing ginseng in a more efficient manner. First, we hypothesized that the required cold moist stratification duration could be shortened by using GA_3 and removing the seed coat. Secondly, we evaluated three different soilless substrates as media in an aeroponic unit.

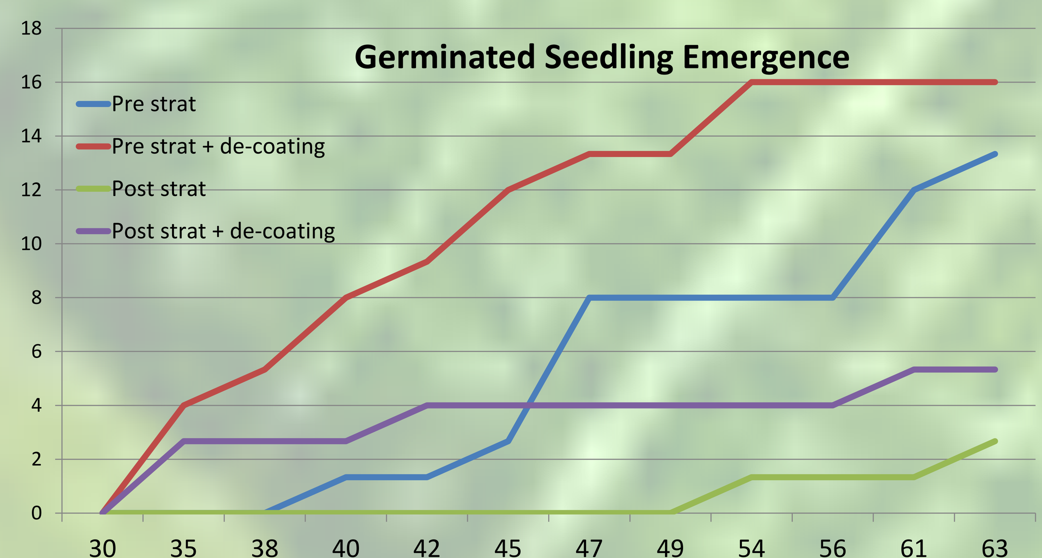

Figure 1. A sequential depiction of mean cumulative germination percentages over the course of the experiment. Germination percentages are represented by the y-axis, and the x-axis represents time, in the number of days, since removing the seeds from their 6 wk cold moist stratification.

Table 1. A numerical depiction of mean germination rates and percentages among the treatments. T_1 represents the number of days (after 6 wk stratification) to first germination. T_{50} is the amount of time, in days (after 6 wk stratification), for half of the seeds to sprout. Mean germination percentage is shown for seeds stratified for 6 or 14 weeks. Values denoted by a similar letter were not significantly different.

			Germination %	
	T_1	T_{50}	6 wks	14 wks
Pre-strat GA_3	44a	50.5a	13.3a	45.3*
Pre-strat GA_3 + de-coating	37a	40.7a	16.0a	-
Post strat GA_3	59a	58.5a	2.7b	8.9*
Post strat GA_3 + de-coating	46a	46.0a	5.3b	-

* Actual non-replicated germination percentage occurring during stratification.

Acknowledgements: This project was funded with a Middle Tennessee State University Undergraduate Research Experience and Creative Activity Grant. Additional material support was provided by the Tennessee Center for Botanical Medicine Research and the MTSU Ginseng Initiative.

MATERIAL AND METHODS:

Germination experiment

- Warm stratified seeds were cold moist stratified for six weeks.
  - Seeds obtained from Hardings Ginseng Farm; Friendsville Maryland
- Half of the seeds were soaked in a 0.1% gibberellic acid (GA_3) solution prior to stratification, while the other half received the same GA_3 soak at the end of the stratification.
- After 6 wks of stratification, 150 smiling seeds of each group (pre- or post-strat GA_3) were randomly selected.
- Half of each group were then de-coated, resulting in 75 seeds of each treatment:
  - Pre-strat with seed coat intact
  - Pre-strat de-coated
  - Post-strat with seed coat intact
  - Post-strat de-coated.
- All 300 seed were then planted in coarse sand and placed onto an automated mist bench.
- Seedling emergence was observed over an eight week period.

Growth experiment

- 18 seedlings were transplanted at the first true leaf stage into an aeroponics unit under 50% shade cloth.
- 3 media treatments were used:
  - Perlite
  - Sphagnum peat moss
  - 50:50 mixture of perlite and sphagnum peat moss
- Relative leaf chlorophyll content was assessed weekly using a Spad-502 Chlorophyll Meter
- The seedlings were then observed for seven weeks before destructive sampling.
  - Fresh and dry weights of foliage and roots

Data Analysis:

- Data was analyzed using Proc GLM in SAS version 9.3. Data were subjected to analysis of variance and means separation testing was conducted using Tukey-Kramer at $\alpha=0.05$
- T1 = Time to first germination; T_{50} = germ. rate, calculated as follows: $T_{50} = t_i + \frac{(0.5N - ni)\,(ti - tj)}{n_i - nj}$

RESULTS

Germination Study

- GA_3 applied to de-coated seeds prior to 6wk stratification had the highest percentage of emergence at 16.0% (Table 1)
- GA_3 applied Pre-stratification was significantly higher than GA_3 applied post-strat (Table 1)
- De-coating increased rate of germination (Table 1)
- De-coating did not significantly improve germination percentage (Table 1)
- Even with GA_3 and de-coating, stratification for 6 wks was lower than seeds stratified for 14 wks (Table 1)

Aeroponics Study

- Chlorophyll content similar among media trials (Table 3)
- Seedlings grown in peat performed poorly, experiencing complete mortality (Table 3; Fig. 4 and 5)
- Mixed media produced significantly higher foliage and root fresh and dry weight (Fig. 4 and 5)

Table 2. A comprehensive list of the amounts of nutrients used in the areoponic nutrient solution.

Nutrient Source	%
A - Calcium Nitrate (Tetrahydrate)	38.718
A - Potassium Nitrate	32.018
A - Chelated Liquid Iron	3.749
B - Copper Sulfate (Pentahydrate)	0.005
B - Manganese Sulfate (Monohydrate)	0.076
B - Sodium Molybdate (Dihydrate)	0.008
B - Boric Acid	0.174
B - Magnesium Sulfate (Heptahydrate)	18.535
B - Potassium Monobasic Phosphate	6.717
B - Zinc Sulfate (Dihydrate)	0.000

Figure 3. Ginseng seedlings in the Areoponic unit. Note the variability in survival between the different soil media treatments.

Table 3. A depiction of the mean Spad values (relative chlorophyll content of the leaves) among the ginseng plants in the areoponic unit. Measurements were obtained with a Spad 502 Chlorophyll Meter (Spectrum Technologies).

	31-Mar	5-Apr	14-Apr	21-Apr
Perlite	29.3	26.9	27.5	29.5
Mix	29.8	28.5	30.3	33.4
Peat	31.1	28.5	25.6	

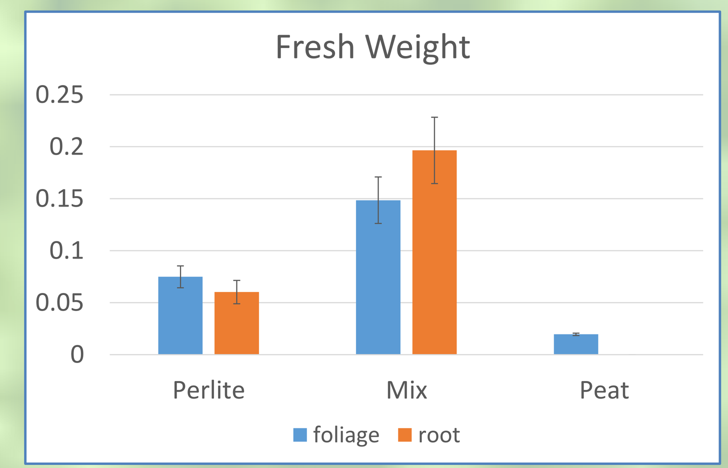

Figure 4. A depiction of mean fresh weights of ginseng foliage and roots at the end of the experiment. The weight in grams is represented by the y-axis, and the x-axis indicates the media treatments. Standard error bars are shown.

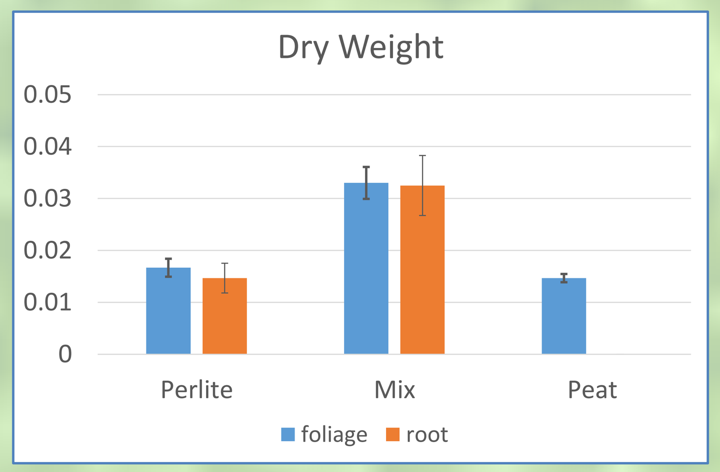

Figure 5. A depiction of mean dry weights of ginseng foliage and roots at the end of the experiment. The weight in grams is represented by the y-axis, and the x-axis indicates the media treatments. Standard error bars are shown.

CONCLUSION: *Panax quinquefolius* could have a high degree of importance to the horticultural industry. Because of its growing popularity and potential medical impact, it serves as an economically valuable product. Our work was done to determine if germination percentage and growth rate were affected through physical and/or chemical manipulation of the seed coat along with differing media environments in the aeropoinics unit. Our results showed that de-coating the seeds prior to planting , while increasing the rate of germination, did not increase germination percentages. Where as pre-stratifying in gibberellic acid does by 16.0%. This could prove useful to the grower in knowing that removing the seed coat does not justify the time and labor required. In regards to the aeroponic stage of the experiment there was little to no difference between all of the trials in regards to chlorophyll content, however, there was a major variance in the mortality rate amongst the trials. The perlite and the peat/perlite mixture performed the best due in part to lower mortality, while seedlings in the peat experienced complete mortality. Increased foliage and root weights from seedlings in the mixed media indicate that a combination of peat and perlite may be a suitable combination for a hydroponic ginseng substrate.

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