# Effects of stratification, germination temperature, and pretreatment with gibberellic acid and hydrogen peroxide on germination of 'Fry' muscadine (*Vitis rotundifolia*) seed.

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*Additional index words*. propagation, $H_2O_2$

Abstract: Germination of muscadine seed has frequently been low and irregular in the University of Georgia breeding program. A systematic study was undertaken to determine the best seed treatments and germination conditions for muscadine seed. Open pollinated seeds of 'Fry' muscadine were used for all treatments. Stratification of seeds was performed by placing dry seed in damp vermiculite at 4C for periods of 0, 30, 60, and 90 d. The 90 d stratification period gave the highest germination percentage, with successively lower germination in the shorter stratification treatments. Pretreatment of seeds prior to stratification with three rates (0.5, 1.0, and 2.0 M) of hydrogen peroxide ($H_2O_2$) and four rates (1, 2, 4, and 8 $g \cdot L^{-1}$) of gibberellic acid ($GA_3$) were used in an attempt to promote germination. Low rates of $H_2O_2$ (0.5 M) and $GA_3$ (1 $g \cdot L^{-1}$) were beneficial in some instances, while high rates of $GA_3$ were detrimental. Nicking the seed coats prior to stratification and soaking seeds in running water after stratification were ineffective in promoting germination. Germination temperatures of 32/22C (8h/16h) were superior to 22/22C, 27/22C, and 37/22C.

The genus *Vitis* contains two subgenera, *Euvitis* (bunch grapes) and *Muscadinia* (muscadine grapes). The muscadine grape, *Vitis rotundifolia* Michx., is the only commonly

cultivated member of the *Muscadinia* subgenus. Muscadine grapes are native to the southern United States and have been cultivated for over 400 years. The muscadine grape differs from the familiar bunch grape (*Vitis labrusca, V. vinifera,* and their various hybrids) in several morphological characteristics including that they have smaller clusters, the berries abscise from the cluster (shatter) at maturity, the tendrils are unbranched, and the berries have thick skins and a unique fruity aroma.

The University of Georgia has been breeding muscadine grapes for nearly 100 years, and routinely germinates large seedling populations. In the only published study on muscadine seed germination to our knowledge, Nesbitt et al. (1976) found that germination rates in excess of 90% could be obtained by cold-stratifying seed in moist sand for 80 to 100 d before planting. Unfortunately we are unable to replicate those results, and the breeding program has been hampered by erratic germination timings and low germination rates of muscadine seed.

Barriers to germination have been better studied in *Euvitis* grapes, and these studies provide a starting point to investigations into muscadine seed germination. *Euvitis* seed generally has very low germination rates until endodormancy has been removed (Ellis et al., 1983). Dormancy removal is generally achieved by cold-stratification of the seeds for a period of three to four months (Einset and Pratt, 1975), but in many varieties this results in only modest germination rates (Ellis et al., 1983; Scott and Ink, 1950; Selim et al., 1981). Grape seeds have a thick, tough seed coat that can be a mechanical barrier to germination. Attempts at scarifying seeds with sulfuric acid provided minimal benefits and was harmful to seed viability if not handled carefully (ChaiWei and ShyiKuan, 2003; Scott and Ink, 1950), however, Ramirez (1968) found that nicking seeds during extraction with a blender blade was beneficial to germination. Treatment of grape seeds with gibberellic acid ($GA_3$) prior to stratification has generally been

found to promote germination (Kachru et al., 1972; Pal et al., 1976; Selim, et al., 1981; Yeou-Der et al., 1967). Ellis et al. (1981) found that the effect of the $GA_3$ could be improved by an additional pretreatment in 0.5 M $H_2O_2$ prior to the $GA_3$ pretreatment. In addition, an alternating germination temperature of 30/20C, with the higher temperature being applied for 8 h every 24 h was superior to a single germination temperature. Kachru et al., 1972 attribute grape seed dormancy to a water soluble inhibitor, most likely abscisic acid, which can be removed with leaching by running water.

The goal of this study was to test various seed treatments in an attempt to improve overall germination rates of muscadine seed and lower the lengthy stratification periods now employed.

## Materials and Methods

*Plant material.* Open pollinated seeds of 'Fry' muscadine were used for all treatments. Fully mature 'Fry' berries were collected from vines located at the University of Georgia – Tifton Campus. Seed was collected on 13 Sept. 2005 for Expt. 1 and Expt. 2, and on 20 Sept. 2006 for Expt. 3. Seed was extracted, washed, float checked, and air dried for several days to approximately 23% moisture. Dry seed was then packed in polyethylene bags and stored at 4C prior to treatment initiation.

*Expt. 1: Effects of 0.5M $H_2O_2$ and* 1 g•$L^{-1}$ *$GA_3$ pretreatment and cold stratification period on germination rate*. Seed received one of the following pretreatments prior to cold stratification 1) 48 h water soak; 2) 24 h soak in 0.5 M $H_2O_2$ followed by a 24 h soak in water; 3) 24 h soak in water followed by a 24 h soak in 1 g•$L^{-1}$ gibberellic acid ($GA_3$); 4) 24 h soak in 0.5 M $H_2O_2$

88 followed by a 24 h soak in 1 g•$L^{-1}$ $GA_3$. Pretreatments were carried out by placing 25 seeds in
89 10 mL of solution at 22C. After the final soak seeds were washed 3× in sterile distilled water
90 and packed in 50 mL of damp sterile vermiculite and sealed in polyethylene bags. Seed was
91 stratified at 4C $\pm$ 2C in a dark refrigerator for periods of 0, 30, 60 or 90 d. Pretreatments were
92 applied so that all stratification treatments concluded on 12 Jan. 2006. Each pretreatment ×
93 stratification period treatment was applied to four replications of 25 seeds. For germination tests
94 seeds were planted in 8 × 8 × 13 cm pot in moist vermiculite. Pots were placed in an incubator
95 set at an alternating cycle of 30/24C with the higher temperature being applied for 8 h of each 24
96 h cycle. Treatments were monitored weekly for 6 weeks for the presence of germinated seed.
97 Seeds were considered to have germinated when the cotyledons extended above the surface of
98 the vermiculite. At the end of the germination period ungerminated seed was removed and tested
99 for viability by a tetrazolium test. This was done by bisecting the seed with a knife and
100 incubating one half the bisected seed in a 0.5% tetrazolium chloride solution for 2 h at 40C and
101 observing the color change.
102
103 *Expt. 2: Effects of $GA_3$, $H_2O_2$, running water, and seed coat nick pretreatments on germination*
104 *rate*. Seed received one of 4 rates of $H_2O_2$ (0, 0.5, 1.0, or 2.0 M) or one of 5 rates of $GA_3$ (0, 1,
105 2, 4 or 8 g•$L^{-1}$) prior to stratification. Pretreatments were applied by soaking 25 seeds in 10 mL
106 of solution for 24 h at 22C. A seed coat nick pretreatment was applied by clipping the basal end
107 of the seed coat with a pair of fingernail clippers and then soaking the seed for 24 h in water
108 prior to stratification. After pretreatment, seeds were stratified as described in Expt. 1 for 60 d.
109 After stratification, a running water treatment was applied by placing seeds that had received 0
110 M $H_2O_2$ and 0 g•$L^{-1}$ $GA_3$ pretreatments in cotton bags under a running faucet of 22C water for 1,

2, 4, or 8 d prior to planting. Each treatment was applied to four replications of 25 seeds. Seed was planted on 15 May 2006. For germination tests, seeds were treated as described in Expt. 1 except that each replication of 25 seeds was placed in a 3.8 L pot in a greenhouse set for a minimum temperature of 21C and maximum temperatures were 30 to 35C. Seed germination was recorded weekly for a period of 5 weeks.

*Expt. 3: Effect of germination temperature, seed stratification period, and seed type on germination rate.* Seed was pretreated by soaking seeds for 24 h in 0.5 M $H_2O_2$ followed by a 24 h soak in 1 g•$L^{-1}$ $GA_3$. Seed was then cold stratified as described in Expt. 1 for periods of 0, 30, 60, or 90 d. The effect of seed type was investigated by taking a batch of seed at the beginning of the experiment and keeping it moist. This seed was then handled the same way as the dry seed in regards to pretreatments and subjected to a 90 d stratification period. Pretreatments were applied so that all stratification periods ended on 11 Jan. 2007. Each pretreatment × stratification period treatment was applied to four replications of 25 seeds. Each replication was planted in 8 × 8 × 13 cm pots of moist vermiculite in growth chambers. Chambers were set for 8 h light at 22, 27, 32, or 37C, followed by 16 h darkness at 22C. Pots were monitored weekly for 8 weeks for the presence of germinated seed. The rate of seedling emergence was evaluated by calculating the average week of seedling emergence for all germinated seeds of a replication.

*Data analysis.* Final germination percentage and percentage viable seed of Expt. 1 was analyzed using a three-way analysis of variance (SigmStat) with interactions tested using Fisher's least significant difference test. Percent viable seed was calculated as the number of germinated seed and the number of seed tested as viable via the tetrazolium test divided by the total number of

seed in the replication. Percentage data was arcsine-square root transformed for statistical analysis, but raw data is reported. Regression analysis (Minitab) was used to determine the best-fit relationship between $H_2O_2$ and $GA_3$ concentration pretreatment and germination percentage in Expt. 2. Arcsine-square root transformation did not improve these relationships, so untransformed data is presented. One-way ANOVA was used to test for differences in germination percentage between seed coat nick and running water treatments versus the control. Percentage data was arcsine-square root transformed for statistical analysis. The effect of germination temperature, stratification period, and seed type on final germination percentage and rate of seedling emergence in Expt. 3 was tested using general linear model analysis (Minitab). Germination percentage was arcsine-square root transformed for statistical analysis, but raw data is reported. The data for the 0 d of stratification was not included in the statistical analysis of rate of seedling emergence because no seed germinated in some treatments.

Results

Stratification period had a strong effect on final germination percentage, with germination rate increasing up to the maximum 90 d period (Table 1). Both the 0.5 M $H_2O_2$ and 1.0 g•$L^{-1}$ $GA_3$ pretreatments increased final germination percentage. The positive effect of the pretreatments was most prominent when the seed had received less than the maximum 90 d stratification period. A combination of both pretreatments together gave superior results to either pretreatment alone. Neither pretreatment had an effect on seed viability (Table 2), but the shorter stratification periods had significantly lower seed viability. This was likely due to the poor germination of these treatments, giving a greater chance for the seeds to rot in the germination

media. Even in the highest germination treatments, final germination rates were still much lower than seed viability, indicating that dormancy had not been fully broken by any of the treatments.

In the second experiment, higher concentrations of $GA_3$ and $H_2O_2$ were tested in the pretreatments to determine if further benefits could be realized. A stratification period of 60 d was chosen for this experiment because that period had given the largest effect with pretreatments in Expt. 1. In contrast to the first experiment, $H_2O_2$ pretreatment was not found to be associated with germination percentage in this experiment, although the 0.5 M $H_2O_2$ treatment had a numerically higher germination rate (data not shown). The $GA_3$ pretreatment was found to reduce germination (Fig. 1), although this was mostly due to a reduction in germination at the 4 and 8 $g \cdot L^{-1}$ rates. Mechanically scarifiying the seed coat by nicking the basal end of the seed did not significantly affect seed germination rate. Placing seeds in running water for 1 to 8 d after stratification was also ineffective in promoting germination (data not shown).

Germination temperature had a significant effect on final germination rates, with the 32/22C regime giving the highest germination rate overall (Table 3). This temperature profile was resulted in an average of nearly double the control of 22/22C, and was especially advantageous after the shorter stratification periods. Average time to emergence decreased with increasing stratification period and increasing germination temperature (Table 4). Seedlings emerged approximately 0.7 weeks sooner at the 37/22C temperature than at the 22/22C temperature. Interestingly, emergence at the 32/22C temperature was significantly slower than at 27/22C. This may be due the larger percentage of seed that germinated at 32/22C, whereas at the cooler temperature only the most vigorous seed germinated. Drying of the seed had no effect on final seed germination percentage (Table 3), but did slow the rate of germination (Table 4).

## Discussion

Muscadine grapes are native to the southern U.S. where winter temperatures commonly fluctuate from mild to cold. It is not surprising, therefore, that muscadine seeds have a relatively pernicious endodormancy that prevents them from germinating during brief warm periods in the winter. In addition, muscadine seed has a very thick seed coat to protect the seed as it travels through the digestive tract of animal dispersers. As a result, muscadine seed germination can be slow and erratic. Once the seed germinates, however, growth is usually quite vigorous. In our breeding program, seed is typically planted in January so that seedlings can be transplanted in the field in March. This allows vines a maximum amount of time to grow in the first year, and a large percentage will flower the second year. This process is hampered in some crosses by delayed or poor germination, necessitating a better protocol to remove seed dormancy. Ideally such a protocol would be relatively easy to apply to progenies of several thousand seed and would require a relatively short stratification period.

As a starting point, this experiment investigated the effects of the $GA_3$ and $H_2O_2$ pretreatments recommended by Ellis et al. (1983) for *Euvitis* grape seed. $GA_3$ is an exogenous growth regulator which promotes germination by stimulating the activation of food mobilizing enzymes (Hartmann and Kester, 1983). The mode of action of $H_2O_2$ in the promotion of germination is unclear, but may involve the scarification of the seed coat (Chien and Lin, 1994; Keeley and Fotteringham, 1998) or oxidation of germination inhibitors (Ogawa and Iwabuchi, 2001). While both pretreatments promoted muscadine germination in Expt. 1, the benefits were marginal and did not substitute for longer stratification periods. In addition, even the best combination of seed treatments still left over 30% of the seed with unbroken dormancy as judged

by the viability of ungerminated seed. This is in contrast to Ellis et al. (1983) where these pretreatments resulted in the germination of virtually all viable seed.

Given the marginal efficacy of the previous pretreatments, Expt. 2 investigated a wider range of concentrations of $GA_3$ and $H_2O_2$. Increasing the $H_2O_2$ concentration was ineffective in promoting germination, and increasing the $GA_3$ concentration resulted in a decrease in germination. Concentrations of $GA_3$ of 2 $g \cdot L^{-1}$ were also found to by Ellis et al. (1983) to cause the death of a percentage of seed in some seed lots. The $H_2O_2$ pretreatment appears to be marginally valuable since it gave a significant benefit in the first experiment and while not significant was numerically better in the second experiment. The 1 $g \cdot L^{-1}$ $GA_3$ treatment may also be beneficial since it was favorable in the first experiment and only appeared to be inhibitory at the higher rates in the second experiment. Mechanically scarifying seeds by nicking the seed coat did not improve germination, suggesting that dormancy is not controlled by the impermeability of the seed coat. Leaching the seeds after stratification was also ineffective in reducing seed dormancy, also in contrast to what was found in *Euvitis* (ChiaWei and ShyiKuan, 2003; Ellis et al., 1983).

Germination temperatures have a strong influence on the germination of *Euvitis* seed. Ellis et al. (1983) found that an alternating temperature of 30/20C resulted in increased germination over a single temperature of 20 or 30C. Results from Expt. 3 indicate that muscadine seed germination is improved by providing at least a brief period of warmer temperatures. However, a single warm temperature was not tested, so it is not clear if a single warm temperature would provide the same benefit. We have previously noticed that muscadine seedling progenies planted later in the spring emerge sooner than those planted earlier in the

year, and the results of Expt. 3 confirm the observation that warmer temperatures increase the speed of germination.

As a practical matter, the results of these experiments suggest that a 0.5 M $H_2O_2$ and a 1 $g \cdot L^{-1}$ $GA_3$ pretreatment are not likely to be harmful and will benefit muscadine seed germination in at least some instances. Seed should be stratified at least 90 d and germinated in an environment where daytime temperatures are allowed to reach 30C.

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282

283 Table 1. Effect of $H_2O_2$ and $GA_3$ pretreatments and stratification period on muscadine seed final

284 germination percentage.

| Pretreatments | | Seed stratification period (d) | | | | |
|---|---|---|---|---|---|---|
| $H_2O_2$ (M) | $GA_3$ ($g \cdot L^{-1}$) | 0 | 30 | 60 | 90 | Avg. |
| 0 | 0 | 0%[z] | 11% | 31% | 55% | 24% |
| 0.5 | 0 | 0% | 17% | 41% | 51% | 27% |
| 0 | 1 | 3% | 12% | 47% | 58% | 30% |
| 0.5 | 1 | 4% | 24% | 52% | 63% | 36% |
| | Avg.[y] | 2% | 16% | 43% | 57% | |

| Significance | *df* | *P* value |
|---|---|---|
| Stratification period (SP) | 3 | *** |
| $H_2O_2$ | 1 | * |
| $GA_3$ | 1 | *** |
| SP × $H_2O_2$ | 3 | NS |
| SP x $GA_3$ | 3 | NS |
| $H_2O_2$ x $GA_3$ | 1 | NS |
| SP x $GA_3$ x $H_2O_2$ | 3 | NS |

285 [z]Percent germinated seed after 6 weeks.

286 [y]Means followed by same letter are not significantly different according to Duncan's multiple

287 range test, $P \leq 0.001$.

288 NS, *,***Nonsignificant or significant at $P \leq 0.05$, or 0.001, respectively.

289

Table 2. Effect of $H_2O_2$ and $GA_3$ pretreatments and stratification period on muscadine seed viability.

| Seed stratification period[z] (d) | Viable seed[y] (%) |
|---|---|
| 0 | 88 c |
| 30 | 89 bc |
| 60 | 97 a |
| 90 | 95 ab |

| Significance | |
|---|---|
| Stratification period (SP) | *** |
| $H_2O_2$ | NS |
| $GA_3$ | NS |
| SP × $H_2O_2$ | NS |
| SP x $GA_3$ | NS |
| $H_2O_2$ x $GA_3$ | NS |
| SP x $GA_3$ x $H_2O_2$ | NS |

[z]Combined data across all $H_2O_2$ and $GA_3$ pretreatments.

[y]Viable seed percentage is the number of germinated seed plus ungerminated seed which stained as viable divided by the total number of seed tested. Means followed by same letter are not significantly different according to Fisher's least significant difference, $P \leq 0.001$.

NS,.***Nonsignificant or significant at $P \leq 0.001$, respectively.

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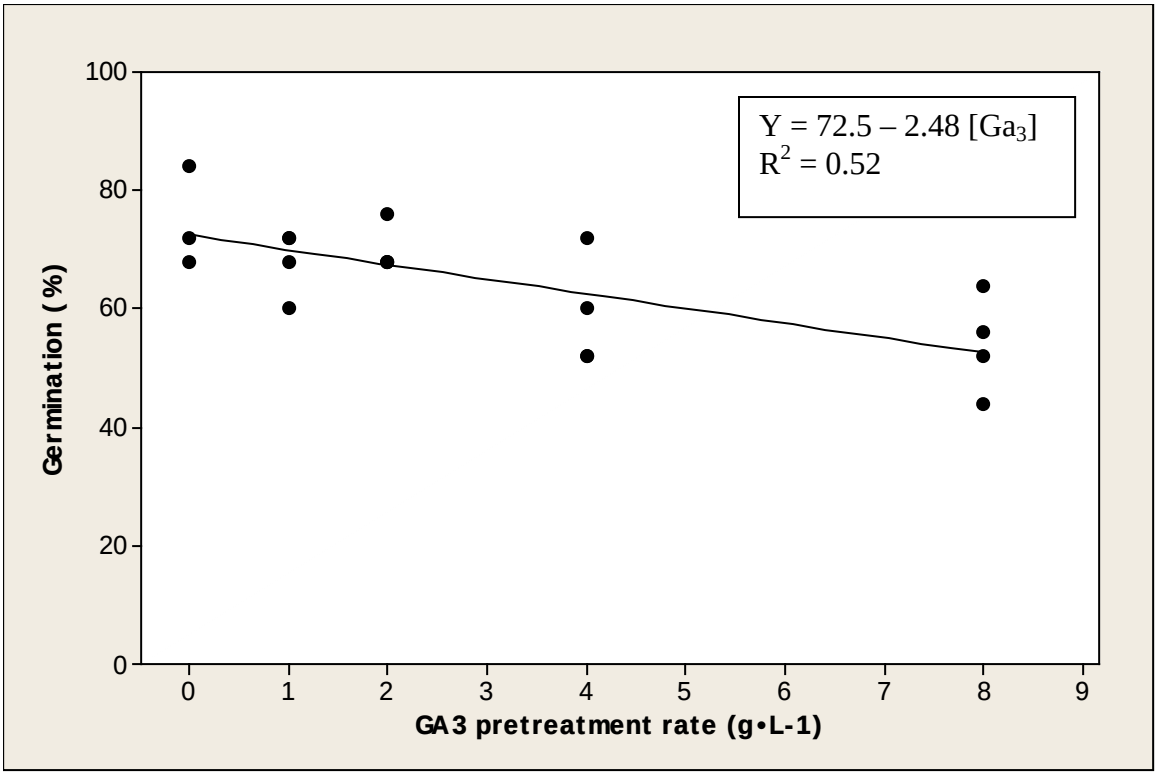


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303 Fig. 1. Regression of percent germination of ‘Fry’ muscadine seed on rate
304 of $GA_3$ pretreatment. For each replication, 25 seeds were soaked in 10 mL
305 of the $GA_3$ solution for 24 h and then stratified for 60 days. Four
306 replications of 25 seeds were conducted for each $GA_3$ pretreatment rate.
307 Final germination percentage was calculated after 5 weeks.
308
309
310

Table 3. Effects of germination temperature, stratification period, seed type on 'Fry' muscadine seed germination percentage.

| Stratification period (d) | | 0 | 30 | 60 | 90 | 90 | |
|---|---|---|---|---|---|---|---|
| Seed type[z] | | | | Dry | | Fresh | Avg. |
| Germination temp | 22/22C[y] | 0%[x] | 11% | 22% | 49% | 38% | 21% |
| | 27/22C | 2% | 7% | 28% | 53% | 50% | 23% |
| | 32/22C | 16% | 36% | 44% | 68% | 73% | 41% |
| | 37/22C | 7% | 23% | 47% | 36% | 46% | 28% |
| | Avg. | 6% | 19% | 35% | 52% | 52% | |

| Significance | *df* | |
|---|---|---|
| Stratification period (SP) | 3 | *** |
| Germination temp (GT) | 3 | *** |
| Seed type | 1 | NS |
| SP × GT | 9 | ** |

[z]Dry seed was dried for several days before processing, fresh seed was never allowed to dry.

[y]Temp 1 / temp 2. Seeds were placed in light at temperature 1 for 8 h and in darkness at temperature 2 for 16 h.

[x]Final germination percentage after 8 weeks.

NS, **.***Nonsignificant or significant at $P \leq 0.01$, or 0.001, respectively.

322 Table 4. Effect of germination temperature, stratification period, and seed handling on

323 rate of 'Fry' muscadine seedling emergence.

| Stratification period (d) | | 30 | 60 | 90 | 90 | |
|---|---|---|---|---|---|---|
| Seed type[z] | | Dry | Dry | Dry | Fresh | Avg. |
| Germination temp | 22/22C[y] | 4.5[x] | 4.2 | 3.7 | 4.1 | 4.1 |
| | 27/22C | 4.3 | 3.3 | 3.2 | 3.6 | 3.6 |
| | 32/22C | 4.5 | 3.7 | 3.4 | 3.8 | 3.9 |
| | 37/22C | 3.6 | 3.2 | 3.1 | 3.7 | 3.4 |
| | Avg. | 4.2 | 3.6 | 3.3 | 3.8 | |

| Significance | *df* | |
|---|---|---|
| Stratification period (SP) | 3 | *** |
| Germination temperature (GT) | 3 | *** |
| Seed type | 1 | *** |
| SP × GT | 9 | NS |

324 [z]Dry seed was dried for several days before processing, fresh seed was never allowed

325 to dry.

326 [y] Temp 1 / temp 2. Seeds were placed in light at temperature 1 for 8 h and in darkness at

327 temperature 2 for 16 h.

328 [x]Average week of seedling emergence.

329 NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

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