The effect of gibberellins (GA_3 and GA_{4+7}) and ethanol on seed germination of *Rosa eglanteria* and *R. glauca*

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Introduction

Germination of rose achenes (seeds) can be challenging due to both low germination percentages and germination occurring erratically over time (Buckley, 1985; Davis, 2010). Germination inhibitors are present in rose seeds that prevent them from germinating at inopportune times. Since inhibitors are primarily found in tissue surrounding the embryo, freeing the embryo of surrounding tissue allows for germination to proceed. However, excising embryos can be a slow and challenging process and it is easy to damage embryos in the process. Don Holeman (2010) has made great progress optimizing the process. For germination of a large number of seeds other methods to overcome germination barriers are primarily sought.

Factors such as temperature during both seed maturation and stratification, scarification through mechanical and chemical means, drying of seeds, red light, and plant growth regulators can affect germination frequency and uniformity (Buckley, 1985; Gudin *et al.*, 1990; Nyholm, 1955; VonAbrams and Hand, 1956; Yambe *et al.*, 1995; Yambe and Takeno, 1992; Zlesak, 2005). Even with the identification of methods which can enhance germination under some circumstances, there has not been any easily applied method that has removed dormancy and universally solved germination challenges. This has led researchers to continue to explore ways to enhance rose seed germination.

One frequently explored method to enhance seed germination in many species, including roses, has been gibberellic acid (GA), also called gibberellins. GA is one of the 17+ plant hormones and is commonly associated in plants with stem elongation and enhanced seed germination. Stem elongation is the plant response that has allowed it to first be identified and characterized and "Foolish seedling" disease in rice was the model that led to its discovery. Rice plants with this disease grow tall and readily fall over. Yield of such rice seedlings is reduced. The disease was discovered to be associated with the fungus *Gibberella fujikuroi*. This fungus produces GA and the additional GA available to the plant because of this fungus led to excessive stem elongation. GA can be produced by fungus in leaf mold and often seeds on the forest floor in such situations have better germination.

GA is naturally produced in plants and tends to be found in greater concentration in growing points (shoot and root), fruits, seeds, and tubers. This hormone encourages cell division and also elongation, together allowing for stem elongation. GA is routinely used to encourage stem elongation. There are products on the market such as Promalin® and Fascination® that combine GA and the cytokinin (plant hormone class that encourages cell division) benzlaminopurine (BA). One use of such products is to encourage more buds growing into stems (due to BA) on stock plants and then shoot elongation (due to GA) of such stems for more efficient cutting production. GA is also proposed to be associated with the control of recurrent / non-recurrent flowering of roses (Roberts *et al*., 1999) and is also used to control timing of flowering in potted florist azaleas. In addition, application of GA to the base of stigmas after pollination has been associated with better hip retention and seed set in roses (Dubois and de Vries, 1986; Ogilvie *et al*., 1991).

GA is intimately associated with release of dormancy of seeds of many species. It commonly has an inverse relationship with the dormancy instilling hormone abscissic acid (ABA). During the course of cold stratification of seeds with physiological (hormonal) dormancy, ABA concentrations generally decline and GA concentrations generally increase until dormancy is released. When dormancy has been released, seeds will germinate when the environmental conditions are in place to support germination (right temperature, oxygen, moisture, etc.).

For many species, soaking seeds in a solution of GA can overcome dormancy and jump start germination. Higher germination percentages and faster and more uniform germination of treated seeds are possible. If ABA is still at relatively high concentrations, germination can proceed but the seedlings can be abnormal. Soaks of GA are commonly used to overcome the dormancy for instance in fresh seed of potatoes in potato breeding programs. When I was earning my Masters degree with the potato breeding project at the University of Minnesota, an overnight soak of 500-2000ppm GA was what we would commonly use to get our potato seeds germinating right away in spring from our crosses made in the greenhouse during the winter months. In addition, GA soaks of tubers have also helped in some instances to release dormancy and get plants growing again soon after field harvest so we could grow plants for crosses during the winter in the greenhouse.

One of the largest commercial applications of GA is for beer production to uniformly start germination of barley seeds for the malting process. Naturally, after grain seeds are imbibed with water, GA from within the embryo moves in the seed and triggers the outer layer of cells of the seed (aleurone layer) to begin to produce an enzyme that breaks down starch (alpha amylase) into simple sugars. These sugars are what fuel the germination process. For beer making, using some GA to jump start the process allows for high and uniform levels of alpha amylase to be produced before the seeds are killed. The enzyme is still present to help break

down the starch molecules after the embryo is killed to fuel microbe growth for the fermentation process.

There are well over 120 GA's that have been identified. They share a common chemical structure, but differ based primarily on positioning and structure of side chains. Different plants have greater or reduced sensitivity to some GA's versus others. Although there are many different GA's that have been identified, GA_3 and GA_{4+7} are the two main forms that are commercially available. Many of the others can be purchased as well, but demand tends to be low and they are expensive. GA_3 and GA_{4+7} are commonly sold because many plants tend to be sensitive to them and they are relatively efficient and economical to produce and purify from fungi. GA_3 is the most commonly available form and GA_{4+7} is a mixture of two GA's because the fungi used to synthesize these tend to produce both of them together and there is added cost to separate them.

GA_3 is the primary GA that has been used for seed germination studies, including roses. The effect of GA in rose seed germination has been variable. Many have found it did not improve germination and others have found that it has improved germination typically to a minimal extent. Unfortunately, it has been traditionally difficult to publish a scientific manuscript with negative results, although it would be very valuable to others to know something did not have a significant effect. Manuscripts that have reported GA did not improve germination typically have explored multiple factors in regards to germination and it was possible to report the non-significant effects of GA in light of the greater context of factors explored. The reviews of Davis (2010) and Buckley (1985) highlight some of these studies. One notable exception where GA enhanced germination is the manuscript by Hosafcı *et al.* (2005) where they looked at germination of difficult to germinate species within the section Caninae and obtained better germination with GA_3 treatments after the second cold stratification treatment.

In a conversation with Dr. Jerry Cohen about rose seed germination and the variable and primarily non-beneficial effect of GA_3, he asked if people have tried GA_{4+7}. He pointed out that GA_{4+7} has been shown to be much more effective on apples than GA_3 for fruit set management and promoting fruit quality. Apples are another member of the Rosaceae family. I searched and couldn't find any references on GA_{4+7} and its role in rose seed germination. It was, however, the form of GA used by Dubois and de Vries (1986) to enhance fruit/seed set. The following experiment was conducted to explore the effect of GA_{4+7} on rose seed germination.

Materials and Methods

In order to develop an experiment that would help to answer this question I wanted to work with roses that can be challenging to germinate rather than easier to germinate roses that could germinate well with or without GA. The two rose species I chose to work with were

Rosa eglanteria (=*R. rubiginosa*) and *R. glauca* (=*R. rubrifolia*). *Rosa glauca* has been the most challenging species for seed germination I have ever experienced and *R. elganteria* has been somewhat challenging, but warm stratification before cold stratification has proven to be very effective at improving germination (Zlesak, 2008). These species are within the Caninae section of the genus *Rosa* and species within this section tend to have low and erratic germination and typically better germination in year two than one using traditional stratification and sowing techniques.

I collected open pollinated hips off of an especially beautiful pentaploid *R. eglanteria* clone (hips collected 10/19/2008) that Joan Moneith kindly shared with me years ago and open pollinated hips from the *R. glauca* (hips collected 10/5/2010) plant growing at the University of Minnesota Landscape Arboretum Nelson Shrub Rose Garden. Within a couple days of collection, I extracted the seeds from the hips and stored them in moist paper towels in baggies at room temperature until the GA treatments were administered.

There were four different treatment solutions and two warm stratification time periods before a common 12 weeks of cold stratification (4C). The four separate treatment solutions seeds were soaked in were:

- 500ppm GA_3 (also containing 2.85% ethanol)
- 500ppm GA_{4+7} (also containing 2.85% ethanol)
- Water
- 2.85% ethanol

In order to gain a strong understanding of the effect of GA_{4+7}, the three other treatments were used as controls for comparison. 500ppm GA was chosen because it is a common GA concentration used by others studying rose seed germination with GA_3 and a GA_3 treatment was included since it has been the standard GA tried. A water treatment was used to know what to expect without exogenously applied GA. The ethanol treatment was necessary because a little bit of ethanol is needed to dissolve and get GA into solution (3ml of 95% ethanol was needed to fully dissolve the GA before bringing it up to 100ml of final solution) and it is important to try to separate out and understand the effect of ethanol versus GA.

On November 3, 2008 about 20g of seeds of each species were placed into each of the treatments. A separate cup of 100ml of solution was used for each treatment / species combination. Seeds were allowed to soak in their respective solution for 24 hours. Eight groups of 100 seeds each were counted out for each species x solution combination and placed into separate zip-sealed sandwich baggies with 80cc of moistened peat moss.

Baggies were stored at room temperature for additional warm stratification. On December 14th four baggies of each species x treatment combination were placed into cold stratification (~10 and 8 weeks of warm stratification for *R. glauca* and *R. elganteria*, respectively) for time one. On December 30th the remaining baggies were put into cold stratification (~12 and 10 weeks of warm stratification for *R. glauca* and *R. elganteria*, respectively) for time two. There-

fore, for each species, solution, time combination there were four replications of 100 seeds. Previous experiments suggested that as warm stratification duration increased, germination after cold stratification generally improved for these species (Zlesak, 2008). If GA was able to compensate for time in warm stratification, having multiple warm stratification durations would hopefully help detect that. After a common 12 week cold stratification (4C), baggies were placed on the basement floor at home (~55-60F) and germination was monitored weekly for 14 weeks. Each week germinating seedlings were removed from the baggies and counted and the baggies were resealed. Additional moisture was added to the baggies as needed.

Results

The Analysis of Variance (a statistical test that explores the effects of factors) on the final percent germination after 14 weeks indicates that there was a significant difference in germination between the two species (*R. eglanteria* had significantly better germination than *R. glauca*), treatments (the water treatment was significantly better than the other three treatments and the other three treatments were not significantly different from each other), and duration of warm stratification (the longer duration of warm stratification resulted in better germination) (Table 1). In addition, the species x treatment interaction was significant which can be explained in part due to low germination for *R. glauca* in general across all treatments and greater germination percents and differentiation between treatments for *R. eglanteria*.

Table 1. Mean squares from analysis of variance for effects due to rose species, GA treatment, time, and their interactions on final germination percent.

Factors	Mean Squares
Species	32942.3**
Treatment	258.2**
Time	121.0*
Species x treatment	141.8**
Species x time	7.6
Treatment x time	10.5
Species x treatment x time	2.7

** Significant at P=0.01, * significant at P=0.05.

Looking at the final germination percents for each species/treatment/time combination it is interesting to learn that there were no significant differences across treatments for *R. glauca*, although the means for the ethanol treatments were numerically lower than the other treatments (Table 2). For *R. eglanteria* the water treatment contributed to significantly greater germination than ethanol and the GA treatments for time one grouped with the ethanol

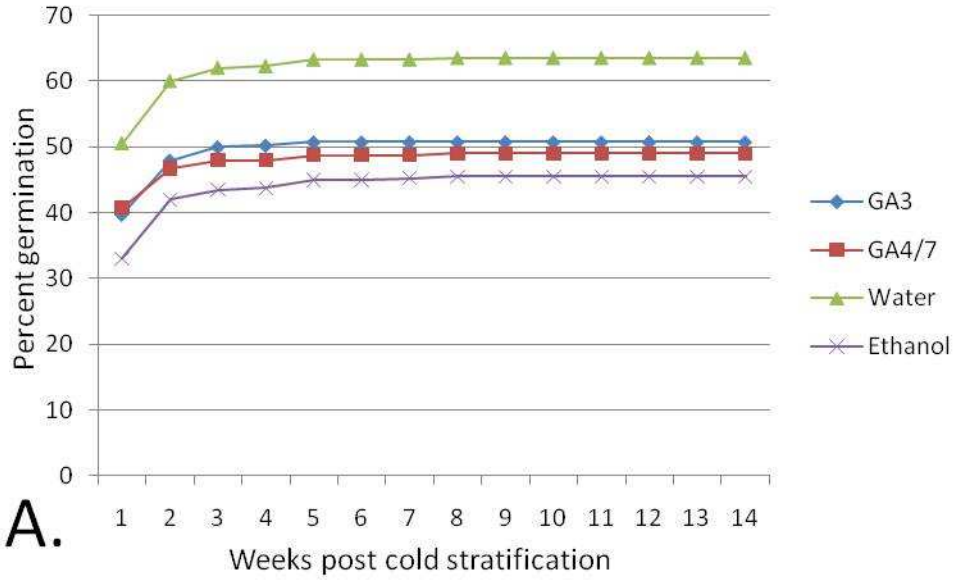
treatment and for time two they were indistinguishable from either the ethanol or water treatments.

Table 2. Final germination percentage after 14 weeks with standard deviation for the sixteen rose species / treatment / time combinations.

Species	Treatment	Time	% Germ.*	S.D.
R. glauca	GA_3	1	8.3c	2.9
		2	9.5c	5.4
	GA_{4+7}	1	8.0c	0.0
		2	10.5c	3.9
	Water	1	9.0c	1.2
		2	10.3c	1.3
	Ethanol	1	4.8c	4.3
		2	8.0c	6.3
R. eglanteria	GA_3	1	50.8b	5.9
		2	53.0ab	4.5
	GA_{4+7}	1	49.0b	1.4
		2	55.0ab	3.7
	Water	1	63.5a	4.5
		2	64.3a	8.3
	Ethanol	1	45.5b	7.5
		2	50.3b	5.1

* Percent germination values followed by the same letter do not significantly differ using Tukey's HSD at P=0.05.

Cumulative germination over time indicates that maximum germination was reached within just a few weeks and that maximum germination was reached sooner when the warm stratification duration was longer (Figure 1).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Percent germination
A.
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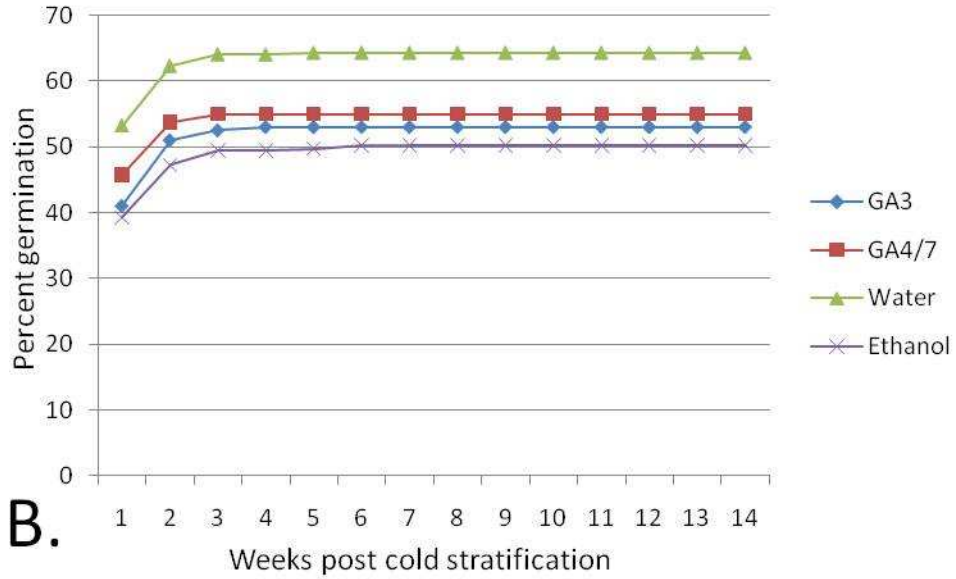
Weeks post cold stratification
GA3
GA4/7
Water
Ethanol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Weeks post cold stratification
GA3
GA4/7
Water
Ethanol

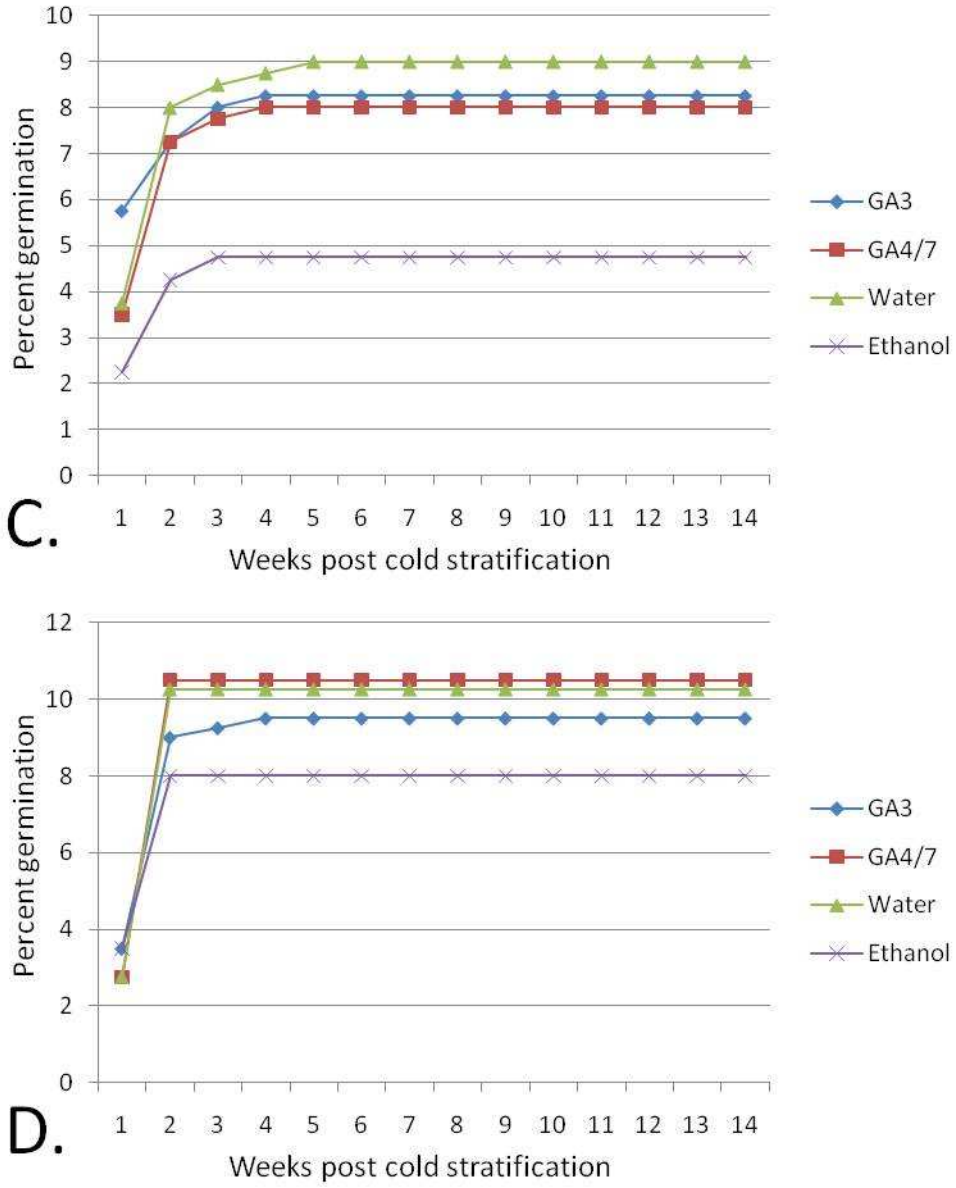

Figure 1. Cumulative germination curves for *R. eglanteria* for warm stratification durations one (A.) and two (B.) and *R. glauca* warm stratification durations one (C.) and two (D.).

Discussion

Unfortunately, GA_{4+7} did not result in dramatically enhanced germination in this experiment. The effect of GA_{4+7} was not significantly different than GA_3. It appears that the small amount of ethanol needed to get GA into solution is the culprit that in some cases has led to a slight reduction in germination relative to the water control. Perhaps with different species or under different circumstances different results would be possible. However, this data is in agreement with the majority of reports that external applications of GA (either GA_3 or GA_{4+7}) does not lead to enhanced germination in roses.

It was interesting to find (using ANOVA) that the post hoc test (Tukey's HSD) grouped the GA treatments with water and the ethanol treatment was distinctly different. Perhaps the GA treatments (which contain ethanol) to a small degree improved germination slightly relative to the ethanol only treatment. Since GA is known to encourage cell division and elongation, it may have slightly helped to counter/heal the effect of cell injury from ethanol. The slight increase in final germination in warm stratification time two versus time one for the GA treatments probably contributed to the GA treatments statistically grouping with water when all data across treatments and species were taken into account versus the ethanol only soak.

In order to circumvent the dormancy issues of rose seeds, one can carefully remove the embryo from the mature seed and free it from ABA so it can germinate right away under suitable germination conditions as Don Holeman (2009) reports. Besides physically freeing each rose embryo from its outer coatings, it appears that the most promising tool that we have to enhance germination continues to be proper management of stratification temperatures and durations. Additionally, in some instances, the following have also been shown to be helpful:

- enzyme treatments to soften the hard outer pericarp
- removing seeds from ripe hips as soon as possible so ABA does not continue to transfer from hip tissue and accumulate in seeds
- preventing seeds from drying out between harvest and stratification
- providing red light.

References

BUCKLEY, F.C. 1985. *Germination of rose achenes: a review and analysis of practices and some suggestions for future investigations*. 1st ed. The Amateur Rose Breeders Association, England.

DUBOIS, L.A.M. and D.P. DE VRIES. 1986. The effect of gibberellins A_{4+7} on fruit set and seed set in unpollinated and pollinated 'Sonia' roses. *J of Plant Growth Regulation* 4:75-80.

DAVIS, L. 2010. A century of rose germination studies. www.rosehybridizers.org/A%20century%20of%20rose%20germination.pdf

GUDIN, S., ARÉNE, L., CHAVAGNAT, A. and C. BULARD. 1990. Influence of endocarp thickness on rose achene germination: genetic and environmental factors. *HortScience* 25:786-788.

HOLEMAN, DON J. 2009. A manual of technique for the extraction and in-vitro germination of mature plant embryos with emphasis on the rose. www.rosehybridizers.org/embryoculture.pdf

HOSAFCI, H., ARSLAN, N., and E.O. SARIHAN. 2005. Propagation of dog roses (*Rosa canina* L.) by seed. *Acta Hort*. 690: 159-164.

NYHOLM, I. 1955. Germination trials of *Rosa rugosa* (In Danish). *Dansk Skovforenings Tidsskrift*. 40:143-150.

OGILVIE, I., CLOUTIER, D., ARNOLD, N., and P.Y. JUI. 1991. The effect of gibberellic acid on fruit and seed set in crosses of garden and winter hardy *Rosa* accessions. *Euphytica* 52: 119-123.

ROBERTS, A.V., BLAKE, P.S., LEWIS, R., TAYLOR, J.M., and D.I. DUNSTAN. 1999. The effect of gibberellins on flowering in roses. *J of Plant Growth Regulation* 18:113-119.

VONABRAMS, G.J. and M.E. HAND,.1956. Seed dormancy in *Rosa* as a function of climate. *American J of Botany* 43:7-12.

YAMBE, Y. and K. TAKENO. 1992. Improvement of rose achene germination by treatment with macerating enzymes. *HortScience* 27:1018-1020.

YAMBE, Y., TAKENO, K., and T. SAITO. 1995. Light and phytochrome involvement in *Rosa multiflora* seed Germination. *Journal of the American Society for Horticultural Science* 120:953-955.

ZLESAK, D.C. 2005. The effects of short-term drying on seed germination in *Rosa*. *HortScience* 40:1931-1932.

ZLESAK, D.C., 2008. Warm stratification enhances germination of *Rosa* section Caninae species. *HortScience* 43:1268.