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The Effects of Seed Treatments on Germination of Dormant *Fraxinus americana* L. and *Fraxinus pennsylvanica* Marsh. Seeds

University Honors Program Senior Thesis

Jennifer A. Ashley Plant and Soil Science

Introduction

White ash (*Fraxinus americana* L.) and green ash (*Fraxinus pennsylvanica* Marsh.), members of the Oleaceae family, are two tree species native to North America. They are the two most common species of the commercial ashes. They are economically important because of their high quality wood, which is strong yet flexible. It is these characteristics that make them desirable for tool handles, sporting equipment such as boat oars and baseball bats, paneling, and furniture. They are also utilized as landscape trees, where they are prized for their habit and fall color.

White ash and green ash are propagated primarily by seed, although some propagation has been done via vegetative cuttings, bud grafting, and micropropagation. However, there are problems associated with seed propagation of ash such as low or sporadic germination rates and variable dormancy requirements (Preece et al. 1995). It has long been assumed that both white ash and green ash needed either a cold, moist stratification treatment, or a combined treatment of heat followed by stratification, in order to overcome seed dormancy (Bonner 1974: Young and Young 1992).

There appear to be at least three basic factors involved in ash seed dormancy. These factors are immature embryos, internal chemical and hormonal agents (Bonner 1974; Dirr and Heuser 1987; Stinemetz and Roberts 1984; Young and Young 1964), and oxygen-impermeable pericarps (Villiers and Wareing 1964).

The standard technique used to mature white ash and green ash embryos is a warm treatment designed to afterripen the embryos. For white ash, the seeds are kept between 20 and 30°C (68 and 86°F) for 30 days; green ash seeds are thought to require 20°C (68°F) for 60 days (Dirr 1998). The stratification period is believed to overcome

dormancy related to the internal factors (Bonner 1974; Young and Young 1992). White ash seeds are typically stored at 5°C (41°F) for 60 days; green ash seeds are kept at 0-5°C (32-41°F) for 120 days (Dirr 1998).

It is well documented that gibberellin (GA) in seeds enhances germination, whereas abscisic acid (ABA) induces dormancy in seeds. It is also known that the actual concentrations of these two hormones are not as important as the relative proportion to one another. It has been shown that dormant ash seeds contain higher levels of ABA than GA (Villiers and Wareing 1965). Sondheimer et al. (1968) found that the pericarp of dormant *F. americana* actually contained much higher concentrations of ABA than did the actual seed, and that the concentrations in both dropped greatly after dormancy was broken and the seeds germinated. It was also found that GA₃ and GA₁ enhanced germination of excised embryos in *F. ornus, F. americana*, and *F. excelsior* (European ash), while applications of ABA inhibited germination of the embryos (Sondheimer and Galson 1966). It has also been shown that exogenous applications of GA₃ could overcome seed dormancy in white ash (McBride and Dickson 1972; Stinemetz and Roberts 1984) and in green ash (Tinus 1982).

There has also been evidence reported that applications of GA have no effect on ash seed germination—leading to the conclusion that there are other factors affecting ash dormancy. Villiers and Wareing (1964) found that seeds of *Fraxinus excelsior* that were soaked in GA experienced no increased germination, and Arrillaga et al. (1992) found that applications of GA₃ had no effect on germination of *Fraxinus ornus* (flowering ash). Preece et al. (1989) also discovered low germination rates (20%) when white ash seeds were placed in an in vitro environment containing GA₃. Recently, Preece et al. (1989 and 1995) have shown that nonstratified ash seeds can attain high germination rates (>80%) in an in vitro environment by simply excising approximately one-third of the seed opposite the radicle end. They maintain that this indicates the need to reexamine the dormancy requirements of ash. Because extremely immature seeds were shown to germinate at high rates, it seems unlikely that immature embryos would be the sole cause of dormancy. It was also shown that uncut white ash seeds without a pericarp did not germinate well, despite being exposed to GA. This would indicate that oxygen-impermeable pericarps would also be an unlikely source of dormancy.

Based on this evidence, it seems likely that the cause of white ash and green ash dormancy lies in the seed coat. It is possible that the testa is impermeable to oxygen, rather than the pericarp. However, Preece et al. (1995) holds that the testa may be a source of germination inhibitors. When the seed coat is cut, the seed can quickly imbibe water and expand beyond the coat and its inhibitors.

The goal of this experiment was to facilitate seed propagation of *F. americana* and *F. pennsylvanica*. The experimental objectives were to determine if cut, non-stratified, "dormant" ash seeds will germinate in soil or petri dishes and to determine if cut ash seeds require any special treatments (e.g. fungicides) to germinate and emerge in greenhouse conditions.

Materials and Methods

Samaras were collected from white and green ash trees in Carbondale, Illinois, during October 1999. They were stored dry in a dark cooler at 4°C until they were used in spring 2000. Each treatment listed hereafter was performed on both species. Seeds that received the cutting treatment had approximately one-third of their length removed opposite the radicle end by cutting cross-wise. Seeds that were germinated in petri dishes were placed on paper towels that were moistened with deionized water. The dishes were sealed with parafilm and then, unless otherwise noted, placed under cool white fluorescent light at a photosynthetic photon flux of approximately 35μ mol·m⁻²·s⁻¹ and a 16 hour photoperiod. Seeds that were sown to a depth of 1.0 cm in the greenhouse were placed in 7.5 x 12.5 x 20.0 cm packs in a peat-lite medium (2 sphagnum peat:1 perlite:1 vermiculite, by volume).

Seeds that were disinfested were prepared as follows. The apical tip was cut to "mark" the end for future reference. If the pericarp was to be removed, it was done at this time. The seeds were surface disinfested for 30 minutes in a 1% NaClO solution containing two drops of Tween 20 per liter of solution. They were then rinsed in sterile, deionized water for five minutes. Approximately one-third of the seed opposite the radicle was then removed and discarded.

In petri dish experiments, germination was measured. A seed was considered to have germinated when the cotyledons began to emerge from the seed and turn green. In the greenhouse experiments, seedling emergence was measured. Seedlings were considered to have emerged when any part of the plant appeared through the surface of the growing medium.

Effects of Light and Darkness Petri Dish Experiment

The seeds were divided into four treatments: cut with pericarp remaining, cut with pericarp removed, uncut with pericarp remaining, and uncut with pericarp removed. The seeds were placed in moist 100 x 15 mm petri dishes and sealed. For each treatment, ten seeds were placed in a dish; this represented one replication. There were five replications of each petri dish kept in darkness, and five replications that were placed under cool white fluorescent light.

Surface Disinfestation Petri Dish Experiment

The seeds were divided into two treatments: cut with pericarp and cut without pericarp. The seeds were surface disinfested in the 1% NaClO-Tween 20 solution, placed in moist 100.0 x 15.0 mm petri dishes, and sealed. All treatments were placed under cool white fluorescent light. Five seeds of a treatment were placed in a dish, and each experimental treatment was performed twice.

Effects of Chemical Treatments Petri Dish Experiment

All seeds used in this experiment were cut with the pericarp removed. There were four different treatments. One group of seeds was a placed in a petri dish containing a 0.13% solution of Physan disinfestant (10% n-alkyl dimethyl benzyl ammonium chloride and 10% n-alkyl dimethyl ethylbenzyl ammonium chloride). One group was placed in a dish containing a 0.065% solution of Ban-Rot fungicide (15% 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole and 25% thiophanate-methyl). The third group was placed in a dish with a 1.17% solution of Zero-Tol disinfestant (27% hydrogen dioxide). The final group received the aforementioned 1% NaClO-Tween 20 disinfestation treatment. Five seeds were placed in each dish (representing one replication), and each treatment was repeated five times.

Cut Seeds Greenhouse Experiment

The seeds were divided into the four treatments of cut with pericarp, cut without pericarp, uncut with pericarp and uncut without pericarp. The seeds were sown to a

depth of 1.0 cm in peat-lite medium and placed in the greenhouse under the natural photoperiod. The experiment was conducted in late winter and early spring, from February 18 to April 26. During the earlier parts of the experiment, the greenhouse was kept at a day temperature of 24°C (75°F) and a night temperature of 18°C (65°F). However, later in the season, the day temperature would sometimes vary five to ten degrees higher or lower. Each pack contained two seeds of each treatment, for each species. This was performed five times.

Surface Disinfestation Greenhouse Experiment

In this experiment, only cut seeds without the pericarp were used. There were three treatments: NaClO-Tween 20 surface disinfestation, deionized water soak, and cut only. The soaked seeds were placed in deionized water for 30 minutes. The seeds were sown in the greenhouse as before. Five seeds of each treatment, for each species, were placed in a pack. This experiment was conducted in two runs. There were eight replications done, and all were performed in early spring. The first four replications ran from March 10 to April 26, and the second four went form March 24 to April 26.

Results

Effects of Light and Darkness Petri Dish Experiment

None of the seeds in either the light treatment or the dark treatment germinated, even after two months of observation. This was true for both the white ash and the green ash. Most of them became infected with bacteria and fungi.

Surface Disinfestation Petri Dish Experiment

None of the seeds with pericarps germinated. The green ash seeds germinated first, and at a higher rate, than the white ash seeds (Table 1). After 20 days, 50% of green

Table 1. Effects of pericarp removal on percent germination of cut F. *americana* and F. *pennsylvanica* seeds that have been surface disinfested in a 1% NaClO-Tween 20 solution

				Nu	mber of	Days Fr	om Sov	ving		
		10	14	17	20	24	28	31	45	49
Species	Seed Treatment				Percen	<u>t Germi</u>	nation ²			
F. americana	With Pericarp	0	0	0	0	0	0	0	0	0
	No Pericarp	10	10	10	20	20	20	20	20	20
F. pennsylvanica	With Pericarp	0	0	0	0	0	0	0	0	0
•	No Pericarp	30	30	40	50	50	50	50	50	50

^zThe percent germination is based on 10 seeds.

ash seeds germinated, compared to only 20% germination for white ash. No additional seeds of either species germinated after 20 days following sowing.

Effects of Chemical Treatments Petri Dish Experiment

The NaClO treatment produced the best results. Germination occurred after only seven days. The white ash germinated at 84%, whereas 36% of the green ash seeds germinated (Table 2). There was again a small amount of microbial infection, but it was not nearly pronounced as with the following three treatments.

The seeds in the Physan treatment did not germinate at all. The Ban-Rot fungicide did almost nothing to promote germination. Although the white ash seeds did not germinate, 4% of the green ash seeds germinated. Similar results were seen with the Zero-Tol disinfestant. However, the green ash did not germinate at all, whereas 8% of the white ash seeds germinated (Table 2). With both treatments, germination was slow, and seedlings did not emerge until 24 days after sowing. For all three of the aforementioned treatments, many of the seeds were infected with fungi and bacteria. Cut Seeds Greenhouse Experiment

As was the case in the petri dishes, almost none of the seeds that were still inside the pericarp emerged. The one exception was the green ash seeds that were uncut and intact. However, only one seedling emerged. Otherwise, all of the other seeds that were uncut also did not emerge (Table 3).

The seedlings that emerged were those that were cut and removed from their pericarp. The green ash emerged at 70%, and the white ash emerged at 20%. However, they did not emerge until 31 days after sowing.

			N	<u>lumber o</u>	f Days Fr	om Sowi	ing	
		7	11	13	23	27	31	37
Species	Seed Treatment			Perce	nt Germi	n <u>ation^Z</u>		
F. americana	Ban-Rot fungicide	0	0	0	0	0	0	0
	Zero-Tol disinfestant	0	0	0	0	4	4	8
	Phyzan disinfestant	0	0	0	0	0	0	0
	NaClO disinfestant	60	72	76	84	84	84	84
F. pennsylvanica	Ban-Rot fungicide	0	0	0	4	4	4	4
1 1	Zero-Tol disinfestant	0	0	0	0	0	0	0
	Phyzan disinfestant	0	0	0	0	0	0	0
	NaClO disinfestant	8	20	32	32	36	36	36

Table 2. Effects of fungicides and surface disinfestation on germination rates of F. americana and F. pennsylvanica seeds that have been cut and removed from their pericarps

^zThe percent germination is based on 25 seeds.

Surface Disinfestation Greenhouse Experiment

Emergence times for both species remained fairly consistent between the two experimental runs. In Run 1, rapid emergence occurred at approximately Day 18 and in Run 2, it began at approximately Day 19.

The green ash once again emerged at a much higher rate than the white ash (Tables 4A and 4B). The green ash emerged up to 80% in the first run of the experiment and up to 65% in the second run, while the white ash only emerged up to 20% in the first run and 25% in the second run.

The first run of the experiment clearly showed that the green ash also emerged sooner than the white ash. The green ash began emerging ten days after sowing, while the white ash did not emerge until after eighteen days. Similar results were seen in the second run. Over 40% of the green ash seedlings emerged by Day 19, but only 5% of the ash seedlings emerged at this time.

There seemed to be little consistency between the emergence rates of the two different experimental runs. In the first run, the white ash emerged almost evenly across all treatments, attaining 15% with the NaClO-Tween 20 disinfestation treatment and the unsoaked treatment, and 20% with the soaking treatment. However, in the second run, it emerged at 25% with the NaClO-Tween 20 treatment, 15% with the soaking treatment, and 0% with the unsoaked treatment. In Run 1, the green ash had the best rates of emergence with the unsoaked treatment, reaching 80% compared to 45% with both the NaClO-Tween 20 and soaking treatments. Then, in Run 2, emergence was nearly even across treatments. Green ash emerged at 65% with the NaClO-Tween 20 treatment, 60% with the soaking treatment, and 50% with the unsoaked treatment.

Table 3. Effects of pericarp removal and seed tip excision on emergence rates of F. americana Image: A second se
and F. pennsylvanica seeds sown in a greenhouse growing medium

			nber of Da		
		31	35	39	45
Species 1	Seed Treatment		Percent E	mergence	2
F. americana	Cut Without Pericarp	10	10	20	20
	Cut With Pericarp	0	0	0	0
	Intact Without Pericarp	0	0	0	0
	Intact With Pericarp	0	0	0	0
F. pennsylvanica	Cut Without Pericarp	60	70	70	70
	Cut With Pericarp	0	0	0	0
	Intact Without Pericarp	0	0	0	0
	Intact With Pericarp	0	0	10	10

^zThe percent emergence is based on 10 seeds.

Table 4A. Effects of surface disinfestation on emergence rates of *Fraxinus americana* and *Fraxinus pennsylvanica* seeds sown in a greenhouse growing medium that have been cut and removed from their pericarps—Run 1

			<u>1</u>	<u>lumber o</u>	<u>f Days Fi</u>	rom Sowi	ng	
		10	14	18	24	32	39	47
Species	Seed Treatment			Perce	ent Emer	gence ^z		
F. americana	NaClO Disinfestation	0	0	5	15	15	15	15
	Water Soak	0	0	15	15	20	20	20
	Unsoaked	0	0	0	5	10	15	15
F. pennsylvanica	NaCIO Disinfestation	5	15	30	40	45	45	45
	Water Soak	0	5	15	45	45	45	45
	Unsoaked	0	0	20	75	80	80	80

^zThe percent emergence is based on 20 seeds.

Table 4B. Effects of surface disinfestation on emergence rates of *Fraxinus americana* and *Fraxinus pennsylvanica* seeds sown in a greenhouse growing medium that have been cut and removed from their pericarps—Run 2

		Number of Days From Sowing				
		19	26	33		
Species 5	Seed Treatment	Perc	cent Emerge	ence ²		
F. americana	NaClO Disinfestation	5	10	25		
	Water Soak	5	10	15		
	Unsoaked	0	0	0		
F. pennsylvanica	NaClO Disinfestation	45	65	65		
• •	Water Soak	40	55	60		
	Unsoaked	10	40	50		

^zThe percent emergence is based on 20 seeds.

Discussion

The first conclusion that can be reached is that the seeds of both white ash and green ash can germinate in either light or darkness. This is because the seeds germinated when placed under the cool white fluorescent light, as well as when they were buried 1.0 cm deep in the greenhouse growing medium. Therefore, the other experimental variables must be examined.

It appears that the greatest hindrance to seed germination in petri dishes is the invasion of microorganisms. When the seeds were placed in the petri dishes, they were exposed to an environment of humid, stagnant air. This atmosphere is conducive to the growth of bacteria and fungi, such as the *Botrytis* fungus. These pathogens infect the seed, rotting it before it has the opportunity to germinate. This was clearly seen in many of the experiments.

This was likely the cause of the lack of germination in the Effects of Light and Darkness Petri Dish Experiment. Neither the seeds nor the equipment was disinfested in the experiment, meaning that numerous microorganisms and their spores, etc. were present.

Rot was also seen in the Surface Disinfestation Petri Dish Experiment. This is because, although the seeds were surface disinfested, there were still some remaining sources of contamination. The paper towels that were put into the petri dishes were not disinfested. The tools used (beakers, forceps, scalpel, etc.) were also not sterilized. Finally, the experiment was performed in a laboratory, not in a sterile environment.

Had a sterile environment been created, the likelihood of pathogen infection would have been greatly reduced. This could have been done by sterilizing all equipment used. The experiment could also have been performed in a sterile environment, such as in a laminar-flow hood. These conditions probably account for the high rates of germination achieved by Preece et al. (1989 and 1995).

The results of this experiment also seem to indicate that the presence of the pericarp inhibits germination. This appears to be true for both cut and uncut seeds. In the Surface Disinfestation Petri Dish Experiment, no ash seeds of either species that were kept inside their pericarps germinated. In the Cut Seeds Greenhouse Experiment, only one seed out of forty seeds germinated when the pericarp was not removed.

One explanation is that the pericarp contains chemical inhibitors to germination. One such inhibitor is abscisic acid (ABA), which the pericarp has been shown to contain in large quantities. (Sondheimer et al. 1968), Removing the pericarp eliminates its chemical inhibitory properties, allowing the seed to germinate.

Another possible factor relates to the anatomy of the seed and its pericarp. While the seed is growing on the ash tree, the pericarp serves as its protection against the elements; the pericarp is exposed to inclement weather, insects, and pathogens. It can therefore be assumed that it is completely contaminated with microorganisms. When the seed is then placed in a warm, moist environment, such as in a petri dish or a greenhouse, the microbes are provided with conditions optimum for their growth. This explains the abundance of microbial growth on ash seeds that were still in the pericarp.

However, the microbial growth was also seen on seeds that had been surface disinfested. One possible explanation is that the grooved, porous nature of the pericarp makes it impossible to completely disinfest. There is simply too much surface area, and too many crevices. However, the source of contamination may have also come from the aforementioned use of unsterilized equipment.

The presence of an intact testa also appeared to be detrimental to seed germination. In the Cut Seeds Greenhouse Experiment, only one uncut seed out of forty germinated. This was true for seeds both inside and out of the pericarp. All experimental treatments that displayed high rates of germination or emergence were performed using cut seeds. This seems to rebuke the theory that the pericarp works to prevent germination, instead of the seed coat (Villiers and Wareing 1964). Rather, it provides further evidence to the theory that the seed coat may possess chemical germination inhibitors (Preece et al. 1995) or may be impermeable to oxygen.

The Effects of Chemical Treatments Petri Dish Experiment strongly indicates that the NaClO-Tween 20 surface disinfestation treatment greatly enhances germination of dormant white ash and green ash seeds. As an acid, the NaClO is a strong oxidizer. It may have had the ability to oxidize part of the seed coat, making it somewhat more permeable. This would have aided the seed in imbibing water and obtaining oxygen. However, it is also possible that it was simply a stronger oxidizing agent than the Zero-Tol or Physan disinfestants, and therefore killed more microorganisms, providing better surface disinfestation.

However, another possibility is that the NaClO-Tween 20 solution contained some chemical or compound that induced germination in the seeds. It could have enhanced the gibberellin or antagonized the abscisic acid contained in the seed. If there are, in fact, chemical germination inhibitors inside the seed coat, it could have somehow overcome them. This is clearly an area that merits further investigation. The experiments that were performed in the greenhouse portray a different picture of the requirements needed for ash seed germination. In the Cut Seeds Greenhouse Experiment, good emergence rates were attained for seeds of both white ash and green ash that had been cut and removed from their pericarps. While the white ash only emerged at 20%, the green ash emerged at 70%. This is almost as high as the 80% germination rates attained by Preece et al. (1989 and 1995) in a sterile, in vitro environment.

The Surface Disinfestation Greenhouse Experiment seems to indicate that cut seeds that are removed from their pericarps will germinate under almost any conditions. Emergence was seen for seeds with the NaClO-Tween 20 disinfestation treatment, the soaking treatment, and the unsoaked treatment. This suggests that once the seed embryo is given the opportunity to escape whatever barriers exist in the seed coat, it will germinate so long as it has the proper requirements of water, oxygen, optimum temperature, etc.

Throughout the experiment, the green ash and white ash seeds continually germinated at vastly different rates. In the Surface Disinfestation Petri Dish Experiment, the green ash seeds germinated at 50%, whereas the white ash seeds only germinated at 20%. However, in the other petri dish experiment, Effects of Fungicides Petri Dish Experiment, the white ash germinated at 86% in the NaClO-Tween 20 treatment, and the green ash germinated at 34%. In both of these experiments, the seeds all received the same NaClO-Tween 20 disinfestation. The only difference between the two that can be noted is the temperature of the room in which the petri dishes were placed. The former experiment was performed in mid-winter, and the room was kept at approximately 21°C

(70°F). On the other hand, the latter experiment was conducted from late winter through mid-spring. On some days, the room would reach over 27.7°C (80°F).

This seems to suggest that white ash needs higher temperatures for germination that green ash. This may be because white ash seeds are short and thick, having a small surface area to volume ratio. Higher ambient air temperatures would be required to warm the entire seed to the optimum temperature for germination. Cool temperatures would slow this process. On the other hand, green ash seeds are long and thin with a high surface area to volume ratio. Less energy would be needed to warm the seed, so germination could occur at lower temperatures. Likewise, high temperatures could overheat the seat, hindering germination.

This theory is further supported by the results of the Cut Seeds Greenhouse Experiment and the Surface Disinfestation Greenhouse Experiment. In both experiments, the green ash emerged sooner than the white ash. This coincides with the fact that the seeds were sown in late winter, when it was cooler, but continued to grow through the spring, as the weather warmed up.

The results of this experiment have profound implications regarding the commercial production of *Fraxinus americana* and *Fraxinus pennsylvanica*. If the seeds do not require intricate afterripening or stratification treatments, commercial propagators and nurseries could save a great deal of time, labor, and money. The methods employed in this experiment are simple and extremely inexpensive to instate. Non-afterripened, seeds could be used. Fewer seeds would be lost during the storage process. Finally, ash seeds could be sown any time of the year so long as they were provided with the

optimum requirements for germination. This would allow growers to gain a time advantage in the production process.

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