HAITI AGROFORESTRY RESEARCH PROJECT

SOUTH EAST CONSORTIUM FOR INTERNATIONAL DEVELOPMENT/

AUBURN UNIVERSITY

February, 1991

This work was performed under USAID Contract No. 521-0217-C-00-0004-00.

EFFECTS OF SEED TREATMENT METHODS ON GERMINATION OF *SIMAROUBA GLAUCA* var. *LATIFOLIA* Cronq.

by

Fritz Vaval and Joel Timyan

SECID/AUBURN AGROFORESTRY REPORT NO. 24

ACKNOWLEDGMENTS

The authors would like to extend their appreciation to the following people who aided in the implementation of this study: Raynold Orilien and Georges Marcellus for helping set up the laboratory research at the PADF seed center; St. John Dorsanville for supervising the propagation of seedlings at the ODH nursery; Duverger Vernus for collecting laboratory and nursery data; and Sigrid d'Aquin for data analyses.

EFFECTS OF SEED TREATMENT METHODS ON GERMINATION OF *SIMAROUBA GLAUCA* var. *LATIFOLIA* Cronq.

CONTENTS

EFFECTS OF SEED TREATMENT METHODS ON GERMINATION OF

SIMAROUBA GLAUCA var. *LATIFOLIA* Cronq.

EXECUTIVE SUMMARY

The seed of *Simarouba glauca* var. *latifolia* exhibits dormancy problems during storage and subsequent germination efforts. Five pre-germination treatments were selected to investigate methods to break post-harvest dormancy and increase both rate and total germination of seed. Complete removal of the kernel from the seed coat and seed coat cracking improved germination in both the nursery and the laboratory for SECID 2290, a seedlot that was handled by conventional procedures. PADF 945, a seedlot that differed by an additional step of tumbling the seed in an abrasive drum, exhibited no differences between the manual scarification methods and the control. Water soaks did not improve germination for either seedlot. Correlation between laboratory and nursery germination totals was poor, mainly due to differences in germination substrate. Germination in the laboratory was lower than germination under nursery conditions, the former being adversely affected by fungal contamination. Seedling heights measured two months after sowing in the nursery showed poorest growth when the kernel was planted without its seedcoat. Further studies should be directed toward proper handling and testing of simarouba seed from seed harvest to country-wide distribution in Haiti. These include optimal tumbling procedures to condition bulk seed and germination tests that correlate better with performance in the nursery.

EFE PLIZYE TRETMAN SEMANS SOU JEMINASYON BWA FRENN

REZIME

Gen pwoblem nan *Simarouba glauca* var. *latifolia* (bwa blan, frenn) pou stoke e jèmen semans yo. Nou te chwazi senk (5) trètman pou bay semans yo avan nou seme yo nan pepinyè-a. Trètman sa yo te chwazi pou etidye efè yo gen sou vitès jèminasyon ak pousantaj jèminasyon. Ankò, nou te aplike tout 5 trètman yo avek 2 lo semans: PADF 945 e SECID 2290. Lè nou te retire jèm nan nan po semans la oubyen le nou te kraze po-a, nou te amelioye jèminasyon nan laboratwa e pepinyè pou SECID 2290, yon lo semans ki te netwaye jan nou kon fe nomal. Nou pa te jwen mem rezilta pou PADF 945, yon lo semans ki te resevwa yon pli bon netwayaj nan yon machin ki te fabrike pa Proje Pyebwa. Nan PADF 945, nou pa te jwen diferans ant semans ki pa te resevwa trètman (sa nou rele "kontwol") ak semans ki te reserwa trètman yo. Trampe semans nan dlo, ni fret, ni cho, pa-t bay pli bon jèminasyon. Jèminasyon nan laboratwa pa te koresponn ak jèminasyon nan pepinyè. Jèminasyon nan laboratwa te mwens ke jèminasyon nan pepinyè, akoz mous champinyon ki te atake semans nan laboratwa. Kondisyon nan pepinyè evite pwoblem champinyon atake semans. Ote plantil apre de (2) mwa te mezire pou efè trètman sa yo gen sou jan frenn ap develope nan pepinyè. Ote pou tout trètman te parey sof pou jem ki te retire kompletman nan po-a. Ote plantil sa yo te mwens. Si nou gen lot etid kap fet nan semans bwa frenn, fok nou adrese pwoblem kolèksyon e netwayaj semans la avan nou distribye nan pepinyè yo tout kote an Ayiti. Metod Proje Pyebwa te sevi pou netwaye semans yo dwe etidye plis, paske metod sa gen posibilite pou nou pare semans pli byen e mwen chè pase lot metod yo. Nou bezwen rezoud pwoblem jèminasyon nan laboratwa pou nou kapab gen yon teknik pou evalye kalite semans avan nou distribye yo nan pepinyè.

I. INTRODUCTION

Simarouba glauca var. *latifolia* Cronq., known in Haiti as frenn or bois blanc, and in English as simarouba or princess tree, is a common tree species of the subtropical moist forest of Hispaniola. Simarouba is native to the Greater Antilles, Mexico and Central America (Liogier,1985).It grows well on both basalt and limestone derived soils from sea level to 800 meters.

In Haiti, *S. glauca* var. *latifolia* is much more common than the endemic *S. berteroana*, though the latter is appreciated for its higher quality of wood. The two species may hybridize, though the extent of hybridization is unknown. Due to its relatively fast growth, *S. glauca* is an important source of utility wood in house construction and medium grade furniture; other products include fuelwood, oil extracted from the seed and a source of ingredients in folk remedies (Rath, 1987; Jenkins, 1989).

The fruiting of this species peaks during the summer period between May-July. Several problems are associated with the simarouba seed: 1) the seed stores poorly which limits the sowing of fresh seed in the nursery to a single season and 2) germination and emergence rates of the seed are not uniform due to dormancy factors. Stored seed that has tested 70% viable by the tetrazolium test has failed to germinate (Colin Hughes, personal communication).

II. OBJECTIVES

Simarouba seed is generally sown without any pre-germination treatment. However, methods have evolved in several containerized nurseries in Haiti that seem to increase germination rate and uniformity. Several methods are currently used to break dormancy: 1) slightly cracking the endocarp to allow water imbibition, but not so much as to allow the kernel to rot; 2) extracting the kernel from the endocarp; 3) immersing the seed first in 80°C water and letting the seed soak for 24 hours and 4) soaking the seed in cold water for 24 hours. This study investigates whether these methods are effective, particularly since the mechanical scarification methods are labor intensive and costly. The major objectives of the study include:

1. Determine the pre-treatment methods that maximize total germination and germination rate of simarouba in the nursery.

2. Correlate laboratory germination with nursery emergence.

3. Study the effect that seedlot quality has on the general conclusions of the study.

III. METHODS AND MATERIALS

Study Sites

The study was conducted at two sites. Laboratory analyses were conducted at the tree seed center of Proje Pyebwa (Pan American Development Foundation (PADF)) located at Delmas # 31 in Port-au-Prince. All nursery trials were completed at the Operation Double Harvest (ODH) nursery in Roche Blanche near Croix des Bouquets.

Seed Collection and Handling

The seeds were harvested during May 1990, corresponding to the peak harvest period of simarouba in most areas of Haiti. Two bulked seed lots were harvested and are referred in this study as SECID 2290 and PADF 945. SECID 2290 was harvested during the period of May 12-18 from elite tree candidates located in two separate regions of Haiti (**Appendix A**). PADF 945 was harvested from a single location near Grand Goâve on May 30. Though both seedlots were extracted from the pulp and air dried, PADF 945 was rotated in a drum lined with sandpaper for 1.5 hours.

Seed Moisture Determinations

Seed moisture determinations were performed for each of the seed lots prior conducting the pre-germination treatments. Methods followed ISTA (1985) rules. A comparison of seed moisture contents was made between ground seed, conforming to ISTA standards, and unground seed. Furthermore, moisture contents of the seed was compared to kernel moisture contents.

Pre-germination Treatments

Each seedlot was divided into five parts and received the following pre-germination treatments:

TREATMENT	DESCRIPTION
HOT SOAK	Complete seed immersed in hot water at 80 °C and soaked for 24 hours
KERNEL	Kernel extracted completely from the endocarp
COLD SOAK	Complete seed soaked in cold water for 24 hours
CRACK	Endocarp cracked longitudinally
CONTROL	No treatment applied

Germination

The germination tests were initiated June 20,1990. The pre-germination treatments were tested in the laboratory and the nursery. Germination in the laboratory was conducted under ambient temperature (daily minimum 22 °C and maximum 28 °C) and light

conditions utilizing plastic Lamarre trays. Four replicates of 100 seeds per treatment were placed on top of Kimpak germination paper saturated with distilled water.

The nursery trials utilized the large Winstrip container, consisting of 15 cm high zig-zag sheets of plastic fitting together to form cells approximately 5 cm x 5 cm (375 cc volume). Fafard Growmix was selected as the potting medium to minimize the effect of the potting medium on germination and growth of the seed treatments. Four replicates of 100 seeds per treatment were sown in 8 Winstrip cases (50 seeds/case). Daily counts of germinated seed in both laboratory and nursery were conducted at the beginning of the 7th day and continued until the 30th day.

Seedling Heights

Two months after the seed treatments were sown in the nursery, seedling height was measured to determine the effect of pre-germination treatment on early seedling growth. Forty seedlings were randomly selected from the inner portion of the Winstrip case (i.e., edge seedlings were eliminated from the analysis) for each combination of seed lot and pre-germination treatment. These were measured to the nearest 0.1 cm. Sample sizes for treatments exhibiting poor germination in the nursery were less than forty seedlings for the cold soak treatments of both seedlots; 10 seedlings for PADF 945 and 23 seedlings for SECID 2290.

Statistical Analyses

All statistical analyses were performed using SAS Version 6.04 software (SAS Institute, Inc., Box 8000, Cary, NC 27512-8000) installed on a NEC Powermate 286 Plus. ANOVAs were computed for a 2 X 2 X 5 factorial experiment (Snedecor and Cochran, 1980). The factors were seed lot (2 levels), germination method (2 levels) and pre-germination treatment (5 levels). Comparison of means were conducted by the Duncan-Waller and Studentized t-test procedures.

IV. RESULTS

Seed Moisture Content

The results of the seed moisture determinations no difference in moisture content of the unground seed and the ground seed. However, moisture content of the kernel was half that of the whole seed which included the endocarp. Moisture content, wet weight basis, of seed for PADF 945 and SECID 2290 was 10% and 9.5%, respectively. Kernel moisture contents were 6% and 5% for PADF 945 and SECID 2290. It is not likely that these differences are significant, though insufficient replications were conducted to analyze for differences. It is not known the interaction effect of seed moisture content on the seed treatments.

Analysis of Variances

The ANOVA (**Appendix B**) supports rejecting the null hypothesis that germination totals are equal 1) between seed lots, 2) between laboratory and nursery, and 3) between pre-germination treatments. All differences were significant at the 0.0001 probability level. All interaction effects were significant. The general linear model could explain 87% of the variation observed in the germination experiments.

Comparison of Pre-germination Treatments

The main objective of this study was to show the effect of five seed treatments on the germination dynamics of simarouba seed. Any seed treatments prior to sowing in the nursery attempts to increase the uniformity, rate and germination totals of a given seed lot. These three variables were analyzed for both seed lots, since it would be natural that the interaction between seed quality and seed treatment would effect germination differently.

Table 1 shows the comparison of total germination means using the Duncan's Multiple Range Test. PADF 945 showed significant differences between treatments in the nursery; no differences were exhibited in the laboratory. SECID 2290 showed differences both in the laboratory and the nursery. The best treatments for PADF 945 were not the best for SECID 2290. Overall, the cold soak treatment exhibited poor germination for the two seed lots in both the laboratory and the nursery.

Table 1. Comparison of germination means for *Simarouba glauca* var. **latifolia** seed lots by pre-treatment method in the laboratory and the nursery. Means followed by the same letter are not significantly different at the 95% probability level.

Seed Lot	Treatment	Nursery Germination (%)	Laboratory Germination (%)
PADF 945	Kernel	76.75 a	36.50 a
	Control	70.50 a	34.25 a
	Hot soak	67.50 ab	25.25 a
	Cracking	59.50 b	51.75 a
	Cold soak	10.00 c	27.00 a
SECID 2290	Cracking	57.75 a	11.25 a
	Kernel	54.00 a	12.75 ab
	Control	31.50 b	3.75 b
	Cold soak	30.00 b	3.50 b
	Hot soak	28.50 b	3.25 b

It is logical that pre-germination treatments would effect not only total germination percentages, but also the rate of germination. Rate of germination is important to insure that the seedlings develop uniformly in the nursery and that seedling quality be maintained. In general, the same trends in germination rate that were shown in the laboratory were exhibited in the nursery.

Figure 1 compares germination rates for PADF 945. Changes in germination velocities occurred in both the laboratory and the nursery. In general, the treatments that exhibited the highest total germination were also the treatments that most rapidly germinated. As mentioned previously, these treatments showed interaction effects (i.e., laboratory results were not comparable to nursery results). **Figure 2** compares germination rates of SECID 2290. Emergent rates in the nursery fall into two groups: the rapid and high germination of the kernel and cracking treatments versus the slower rate and lower germination totals of the control and water soaks.

The final result of any difference in pre-germination treatment would be the effect that changes in germination rate may have on seedling growth in the nursery. A comparison of the mean heights for the five seed treatments is shown in **Table 2.**

Table 2. Average height of *Simarouba glauca* after two months in nursery. Means followed by the same letter are not significantly different.

TREATMENTS	N	PADF 945 HEIGHT (cm)	N	SECID 2290 HEIGHT (cm)
Control	40	11.915 a	40	11.091 a
Hot soak	40	11.820 a	34	11.160 ab
Cracking	40	11.103 ab	40	10.463 ab
Cold soak	10	10.350 b	23	11.587 ab
Kernel	40	10.012 b	40	10.163 b

It is interesting to note that the poorest growth for both seedlots was exhibited by seedlings germinated from the kernel without the seedcoat. However, from a nursery management perspective, these differences are not as important as the differences in germination vigor (i.e., germination rate + germination total) due to pre-germination treatment.

Comparison of Laboratory and Nursery Germination

Germination in the laboratory is tested at the PADF seed center prior to distribution to nurseries throughout Haiti. In

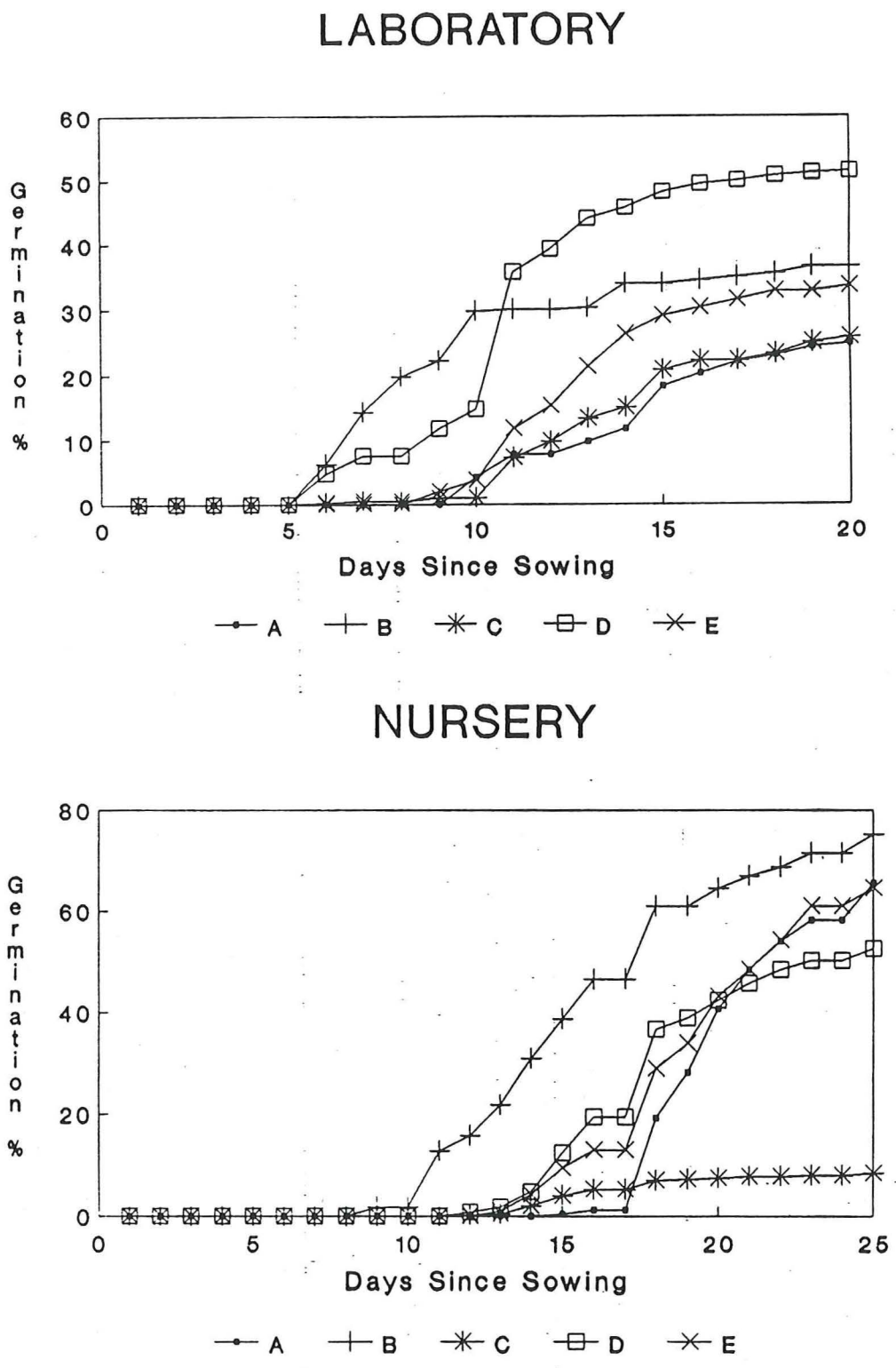
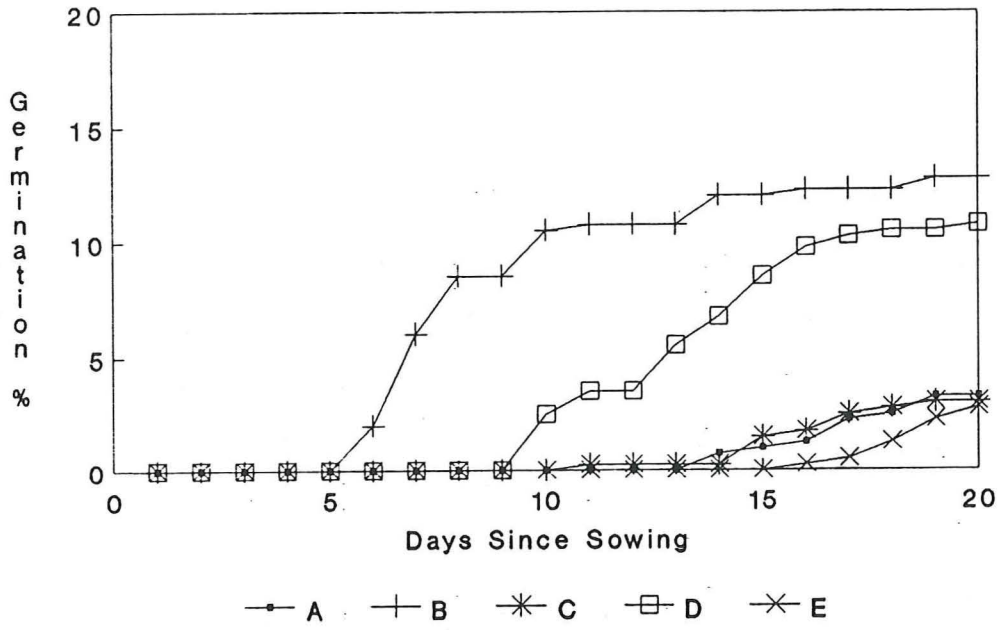


Figure 1. Cumulative germination of pre-sowing seed treatments for *Simarouba glauca*, PADF lot No. 945. A = Hot water at 80 degrees C. B = Kernel only. C = Cold water for 24 hours. D = Split endocarp. E = Control - no treatment.

LABORATORY



NURSERY

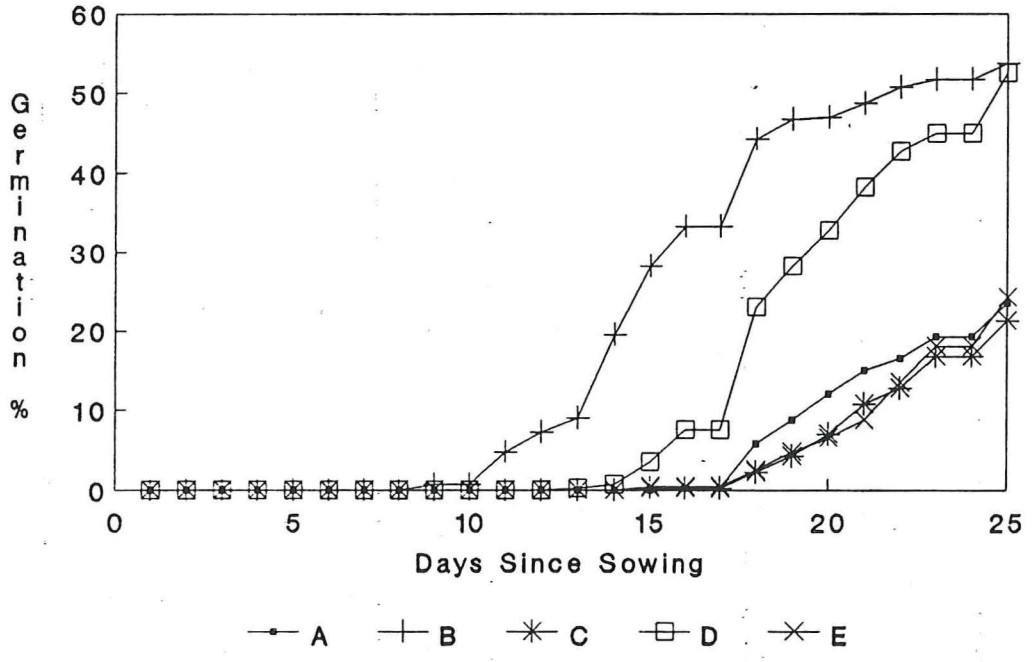


Figure 2. Cumulative germination of pre-sowing seed treatments for *Simarouba glauca*, SECID lot No. 2290. A = Hot water at 80 degrees C. B = Kernel only. C = Cold water for 24 hours. D = Split endocarp. E = Control - no treatment.

general, it is assumed that test results in the laboratory are indicative of what is to be expected in the nursery. This may be true for many species. However, for the simarouba seed lots in this study, the data show that correlations between laboratory and nursery results are poor. One possible reason for the difference is the typical mold infestations that attack simarouba seed under the humid conditions of the germinator. Mold is very difficult to control with simarouba seed. In previous experiments, mold problems still existed after the trays had been washed with Chlorox and the distilled water used for germination experiments was mixed with either dilute solutions of Captan or Chlorox. No means to control fungal contamination during germination in the laboratory has been discovered.

The two seed lots were analyzed for differences in germination between the laboratory and the nursery. **Figure** 3 compares the germination totals of PADF 945 for each of the seed treatments. With the exception of the cold soak treatment which resulted in the poorest germination of simarouba seed, germination totals were higher in the nursery than in the laboratory. The treatments that exhibited the highest germination in the nursery were significantly different from the laboratory results. These were the hot soak and the extracted kernel treatments.

Figure 4 shows the lab/nursery comparison for SECID 2290. The difference in germination totals between the laboratory and the nursery were greater for SECID 2290 than PADF 945. This may be a natural variation, in which case this observation is of no consequence. However, if the additional handling procedure of PADF 945 had an effect on germination aside from the fact that no two seedlots are the same, than the data suggests that some pre-germination treatment is necessary to optimize germination performance. In all cases, the nursery totals were significantly greater than the laboratory results, indicating that poor seed germination in the laboratory is not necessarily indicative of performance in the nursery.

Appendix C provides the Studentized t-Test results comparing the difference between laboratory and nursery data for both seed lots. A significant test rejects the hypothesis that the two germination means of a given seed treatment are equal in the laboratory and the nursery.

Seed Lot Comparisons

The two seed lots, PADF 945 and SECID 2290, differed considerably in their performance, both in the laboratory and the nursery. PADF 945 performed better overall than SECID 2290, particularly in the laboratory. Many factors not tested in this study could have contributed to the difference. The PADF seed lot was collected at one location and time, minimizing the spatial and temporal factors that effect seed viability. PADF 945 also

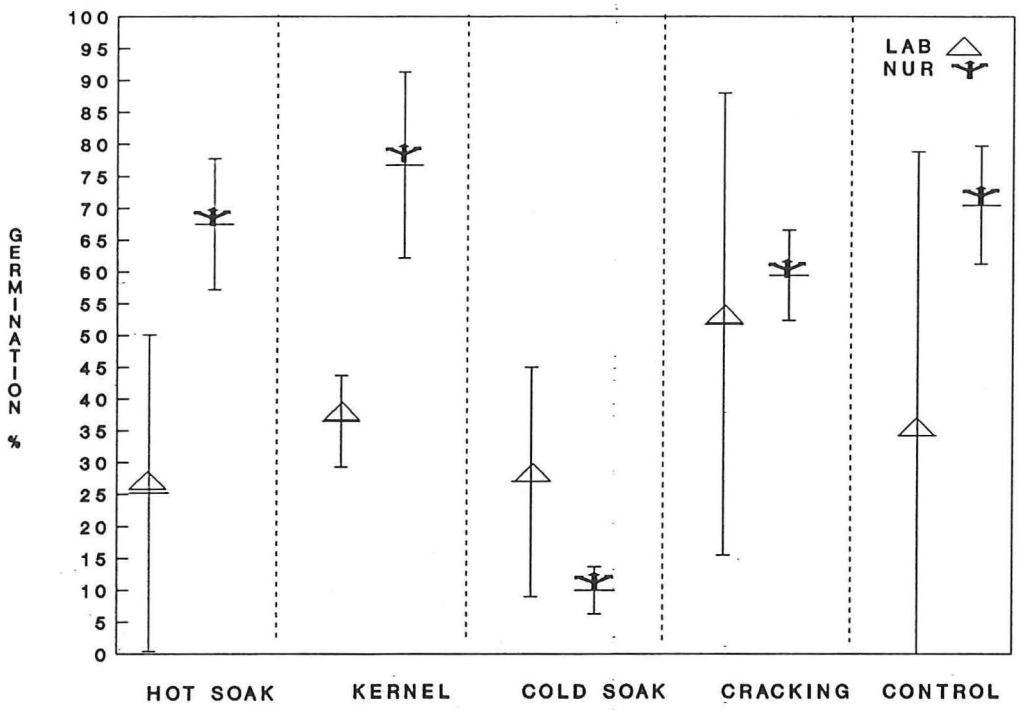


Figure 3. Comparison of laboratory and nursery germination means for PADF 945 by pre-germination treatment. Error bars estimate the 95% confidence interval of the mean.

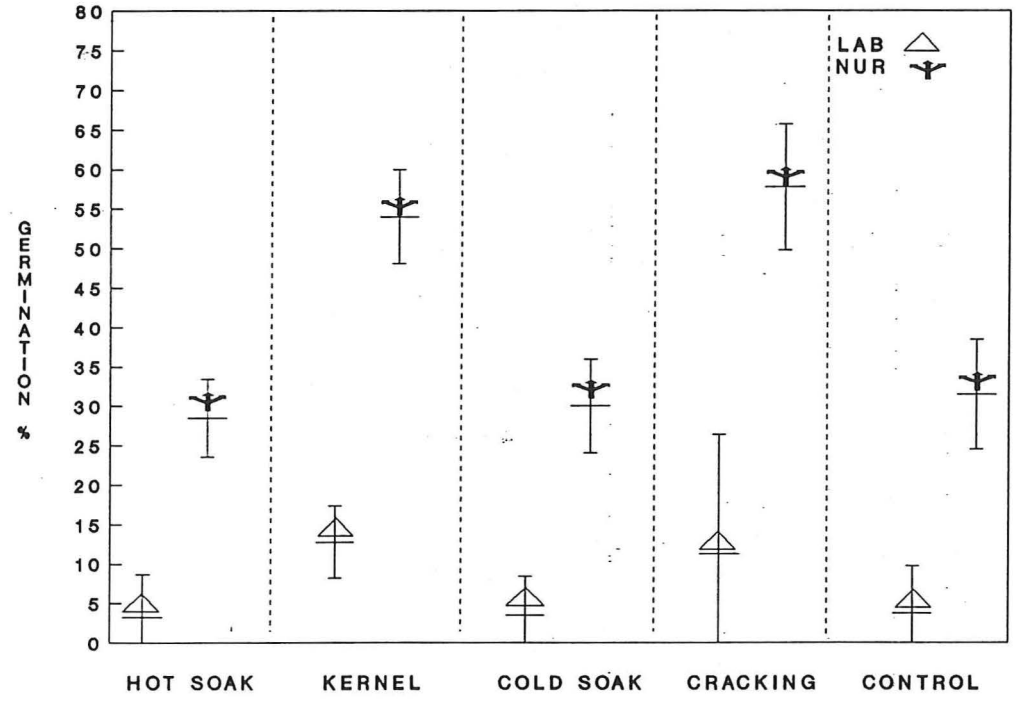


Figure 4. Comparison of laboratory and nursery germination means for SECID 2290 by pre-germination treatment. Error bars estimate the 95% confidence interval of the mean.

underwent an additional handling treatment in the rotating abrasive drum. The SECID seed lot was collected from individual trees located in two regions of Haiti during an 18 day period. No doubt air drying times and handling conditions were different as well.

The effect that seed lot quality had on the pre-germination treatments both in the lab and the nursery was analyzed by a Studentized t-Test procedure. The results are summarized in Appendix D. Significant differences existed between the seed lots for every pre-germination treatment in the laboratory. PADF 940 exhibited higher germination than SECID 2290 for all treatments. This was not the case in the nursery where germination differences between the two seed lots were not as great. In fact, SECID 2290 germinated significantly higher than PADF 940 for the cold soak treatment; no significant difference was shown for the cracking treatment.

CONCLUSION AND RECOMMENDATIONS

The results obtained from this study on seed treatments and the various germination methods for *Simarouba glauca* led us to draw the following conclusions:

1. Simarouba seeds exhibit significant differences in germination rates when pretreated. These differences were affected by the quality of the seed lot, but whether this difference in quality was natural or due to differences in seed handling procedures was not tested. Less differences occurred with PADF 945 which was also the seed lot that had been cleaned and perhaps partially scarified by tumbling in an abrasive drum prior to treatment. Cracking or removal of the hard seed coat favored germination of SECID 2290 which had not been previously tumbled. Simarouba seeds seem to react negatively to cold water soaks, though no reason can be given at this time.

2. There is a poor correlation between germination rates in the laboratory and nursery emergence rates. The germination tests revealed that simarouba seeds germinated better in the nursery. This is mostly due to the fungal contamination in the germination trays, though the combination of light, moisture and biological factors in the nursery must be considered as an important effect. The problem of how best to test seed prior to distribution to nurseries countrywide is still not solved with simarouba. Germination with sand as a germination substrate maybe a viable alternative to blotting paper, in order to control fungal growth. Another alternative would be to sterilize the seed with hydrogen peroxide or mercuric chloride, though concentrations would have to be worked out as to avoid excessive interaction with germination. The objective is for laboratory results to be a closely correlated with nursery results as possible. There should be no significant differences between germination in the laboratory and the PADF and CARE nurseries, unless these differences are due to handling

procedures after the seed has left the seed center.

3. The differences observed between PADF 945 and SECID 2290 could not be determined in this study. However, the additional seed handling treatment conducted by PADF by tumbling the seed in an abrasive drum for 1-2 hours merits further testing as a pre-germination treatment. This type of scarification, if effective, is also the cheapest method to scarify bulk quantities of seed. Manual procedures of cracking the endocarp or extracting the kernel from the endocarp are labor intensive and did not significantly improve the germination of PADF 945 in the nursery. However, these treatments did significantly improve germination for SECID 2290 that was not tumbled.

4. Dormancy factors that negatively effect germination vigor can be partially overcome by cracking or extracting the kernel from the endocarp. This study indicates that some type of scarification is required to optimize germination performance. The soaking treatments did not improve germination in the nursery any better than the control. The differences in seedling height growth due to the removal of the seedcoat is not as important as the effect on rate and total germination of simarouba seed.

LITERATURE CITED

International Seed Testing Association. 1985. **International Rules for Seed Testing**, Seed Sci. and Technol. 13, (2), 299-355.

Jenkins, M. 1989 *The Useful Trees of Haiti: A Selected Review*, 263 pp.

Liogier, A.H. 1985. La Flora de la Española. III Cent. San Pedro de Macoris vol. 56, San Pedro de Macoris, Rep. Dominicana, 431 pp.

Rath, T. 1987. *Simarouba glauca* - an exotic edible oil seed, *Indian Forester* 113 (1): 73-76.

APPENDIX A

Summary of seed harvested for this study.

HARVEST

SEED LOT NO.	FAMILY NO.	HARVEST	LOCALITY	COMMUNE
SECID 2290	201	28/05/90	MOUSSIGNAC	MIRAGOANE
	203	28/05/90	MOUSSIGNAC	MIRAGOANE
	228	12/05/90	FELICIAN	LASCAHOBAS
	257	18/05/90	CHABANNE	PETIT GOAVE
	281	28/05/90	SAVANNE LONGUE	LASCAHOBAS
	284	17/05/90	SAVANNE LARAINE	LASCAHOBAS
PADF 945		30/05/90	CARREFOUR FAUCHE	GRAND GOAVE

CLEANING/HANDLING

SECID 2290: Seeds were cleaned by removing pulp with water. Dried in the shade for several days prior to bulking. Seed sown in the laboratory 20 June, 1990 and the nursery 21 June, 1990.

PADF 945: Seeds were cleaned by removing pulp with water. Dried in the shade for 3 days then tumbled in a sandpaper lined drum for 1.5 hours. Seed sown in the laboratory 20 June, 1990 and the nursery 21 June, 1990.

APPENDIX B

ANOVA OF SIMAROUBA GLAUCA PRE-GERMINATION TREATMENT STUDY

VAR: GERMINATION PERCENTAGE AFTER 30 DAYS.

SOURCE	DF	SS	MSE	F	Pr > F
Model	19	42880.7375	2256.8809	21.51	0.0001
Error	60	6295.7500	104.9292		
Corrected Total	79	49176.4875			

R-Square	C.V.	Root MSE	GERM Mean
0.871976	29.46708	10.24349	34.762500

SOURCE	DF	TYPE I SS	MSE	F	Pr > F
LOT	1	9923.5125	9923.5125	94.57	0.0001
METHOD	1	15318.1125	15318.1125	145.99	0.0001
TREATMENT(TRT)	4	8286.0500	2071.5125	19.74	0.0001
LOT*METHOD	1	667.0125	667.0125	6.36	0.0144
LOT*TRT	4	2587.3000	646.8250	6.16	0.0003
METHOD*TRT	4	3009.7000	752.4250	7.17	0.0001
LOT*METHOD*TRT	4	3089.0500	772.2625	7.36	0.0001

APPENDIX C

Results of Paired Comparisons t-Test between Laboratory and Nursery Germination Experiments.

PADF 945

Seed Treatment	N	Laboratory	Nursery	T	Pr>|T|
HOT WATER	4	25.25	67.50	5.00	0.0024
KERNEL	4	36.50	76.75	7.87	0.0002
COLD WATER	4	27.00	10.00	2.94	0.0260
CRACK	4	51.75	59.50	0.67	0.5295
CONTROL	4	34.25	70.50	2.53	0.0445

SECID 2290

HOT WATER	4	3.25	28.50	10.96	0.0001
KERNEL	4	12.75	54.00	17.49	0.0001
COLD WATER	4	3.50	30.00	10.89	0.0001
CRACK	4	11.25	57.75	8.67	0.0001
CONTROL	4	3.75	31.50	9.62	0.0001

APPENDIX D

Results of a Student's t-Test procedure comparing the germination totals of PADF 945 and SECID 2290 for the various treatment levels analyzed in this study.

TREATMENT LEVELS I	II	PADF	SECID	T	Pr > T
LAB	HOT SOAK	25.25	3.25	2.75	0.0331
LAB	KERNEL	36.50	12.75	8.88	0.0001
LAB	COLD SOAK	27.00	3.50	4.00	0.0072
LAB	CRACKING	51.75	11.25	3.28	0.0169
LAB	CONTROL	34.25	3.75	2.16	0.0744
NURS	HOT SOAK	67.50	28.50	10.89	0.0001
NURS	KERNEL	76.75	54.00	4.59	0.0037
NURS	COLD SOAK	10.00	30.00	9.10	0.0001
NURS	CRACKING	59.50	57.75	0.52	0.6189
NURS	CONTROL	70.50	31.50	10.75	0.0001