Moisture Determination on Seeds of Honeylocust and Mimosa

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Moisture determinations with two hardseeded legumes-honeylocust, Gleditsia triacanthos *L., and mimosa,* Albizia julibrissin *Durazzini--clearly showed that seed coats must be ruptured in some fashion to allow all moisture to escape during ovendrying. With these species, cutting the seeds in half was equivalent to grinding for this purpose. In a comparison of grinders, a small coffee mill performed just as well as a Wily laboratory mill, but its durability with large hard seeds may be questioned. According to the Karl Fischer technique run on an automatic analyzer, ovendrying methods provided accurate measurements of moisture for honeylocust but underestimated moisture in the smaller mimosa seeds.*
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There are two primary reasons for measuring the moisture content of seeds. One is the requirement that all seeds bought and sold in international commerce be tested for germination, purity, and moisture content. The second is seed workers' need to know the condition of the seeds that they are extracting, cleaning, and storing. In commercial transactions, moisture determinations must be accurate and precise, and most countries use the procedures of the International Seed Testing Association (ISTA) for official tests. In routine seed handling, accuracy is also desirable but the precision required is much lower.

Seed moisture content is expressed as a percentage of wet weight (International Seed Testing Association 1985). In any test for moisture content in which loss of weight is equated with loss of moisture, anything that prevents complete loss of moisture from the sample during drying (such as an impermeable seed coat) will produce inaccurate results. Incomplete moisture loss will produce a weight loss that underestimates the moisture content of the sample when moisture is expressed as a percentage of sample weight.

In determinations of seed moisture in official tests (ISTA 1985), grinding is required for "large seeds" and all seeds of species listed in the ISTA rules (table 9A, section 9.5.4.). Large seeds are ground to ensure complete moisture removal and shorten the drying period (Grabe 1989). Although no leguminous tree species are listed in the ISTA rules (table 9A), past experience suggests that intact seeds (even small ones) with very hard coats may require grinding for complete moisture removal during ovendrying. Coarse grinding (50% of the material passing through a 4.0-mm mesh sieve) is required for "leguminous and tree seeds" (ISTA 1985), but it has been demonstrated that for oaks, simply cutting the acorns into two to four pieces provides complete drying and accurate determinations by the oven method (Bonner 1974). Many seed laboratories in developing countries of the tropics do not have the type of grinders required by the ISTA rules. The objective of this study was to determine if alternatives to grinding by the official test procedures can provide accurate measurements of moisture in selected leguminous tree seeds.

Materials and Methods

Seed material. Two leguminous species were used in the study: one with a relatively large seed, honeylocust (*Gleditsia triacanthos* L.), with 5,000 seeds/kg) and one with a small seed, mimosa (*Albizia julibrissin* Durazzini), with 24,000 seeds/kg). Four seed lots of each species were tested. All except one lot were collected in the locality of the Forestry Sciences Laboratory, Starkville, Mississippi, in 1987-89. One lot of honeylocust seeds from South Dakota was purchased from a commercial dealer. The seeds were extracted from their pods with a mechanical macerator and stored at 4 °C until the tests were run.

Preliminary tests were carried out on single lots at low moisture contents (near 10%) to establish procedures. The final test used four lots of each species and two moisture levels of each lot. Two levels were used to increase the repeatability of the results. A higher moisture level (approximately

15%) was achieved by placing samples in a darkened, moist chamber at 40 °C for 1 and 2 days for mimosa and honeylocust respectively. Following this treatment the seeds were returned to storage for 6 weeks before tests were performed.

Treatments. The following measurement techniques were compared on both species:

1. Oven method, intact seeds
2. Oven method, seeds ground in a Wiley mill
3. Oven method, seeds ground in an electric coffee grinder
4. Oven method, seeds cut in half
5. Karl Fischer technique with automatic moisture analyzer

A 1-mm mesh screen was used on the Wiley laboratory mill. The coffee grinder was an inexpensive model available in many retail outlets. Tests with the coffee grinder determined that the percentage of ground material that passed through a 3-mm mesh was 99% for mimosa and 83% for honeylocust. Mimosa seeds were cut in half with nail clippers, and honeylocust seeds were cut with small pruning shears.

Determinations of seed moisture according to Karl Fischer's analysis technique (Grabe 1989) served as the reference method. This technique is widely accepted as the most reliable method for determining seed moisture (Grabe 1989). Measurements were carried out on duplicate samples with an EM Science Aquastar V1B Karl Fischer automatic moisture analyzer. Seeds were first ground in the Wiley mill, and 1-g samples were placed in 50 ml of anhydrous methanol for 48 hours. Determinations of moisture were made on 1.0-ml aliquots.

Drying procedure. Ovendrying was carried out in a mechanical convection oven at 103 ± 1 °C for 17 hours (ISTA 1985). Samples were dried in round aluminum cans (47 by 22 mm) and cooled in glass desiccators over indicating silica gel (6 to 16-mesh) for 45 minutes before reweighing. Samples typically weighed 3 to 5 g, although a limited supply of mimosa seeds at the low moisture level required the use of some samples weighing only 1 g. Each measurement technique was replicated five times at both moisture levels in each seed lot. All samples were weighed to three decimal places on an electric pan balance, and moisture contents were expressed as a percentage of wet weight (ISTA 1985). Moisture percentages were transformed to their square roots (Steel and Torrie 1960) before analyses of variance (ANOVA) were run separately for each of the four species-moisture level combinations. Following ANOVA, treatment means were compared by Duncan's new multiple range test.

Results

Honeylocust. With this larger of the two leguminous seeds, rupturing the seed coat was necessary to completely dry seeds at low moisture contents. Measurements with intact seeds yielded significantly lower values than the other techniques (tables 1 and 2), demonstrating that not all moisture was removed during drying.* Cutting and grinding means (both mills) were not significantly different from results from the Karl Fischer technique, but the Wiley mill mean was significantly higher than the cutting mean (12.0 versus 11.4). There were also significant differences among the seed lots, as expected, probably due to differences in initial moisture contents and seed coat hardness.

Table 1—*Treatment means for both species and moisture levels*

Treatment	Seed moisture content (%)	
	Low	High
Honeylocust		
Intact	9.6 a	15.5 a
Cutting	11.4 b	17.8 b
Wiley mill	12.0 c	16.7 ab
Coffee mill	11.6 bc	17.6 b
Karl Fischer	11.6 bc	15.7 a
Mimosa		
Intact	5.3 a	10.0 a
Cutting	9.4 b	13.4 b
Wiley mill	9.6 bc	12.9 b
Coffee mill	9.8 c	13.4 b
Karl Fischer	11.7 d	15.6 c

All samples were dried for 17 hours in aluminum cans. Means not followed by the same letter differ at $P \leq 0.05$.

*Assuming the Karl Fischer technique is the correct determination, measurement techniques that yield significantly lower moisture contents do so as a result of incomplete drying due to moisture retention in the seed. For example, assume the correct wet and overdry weights of a seed lot are 100 g and 90 g (% moisture content of 10%). However, if due to incomplete drying the wet and overdry weights are 100 g and 95 g, respectively, the false moisture content is now 5%.

Table 2—*Analyses of variance for all species–moisture level tests*

Source	df	Sums of squares	Mean square error	F ratio
Honeylocust—low moisture				
Seed lot	3	0.1185	0.0395	3.58*
Treatment	4	1.7216	.4304	39.02*
Interaction	12	0.8945	.0745	6.76*
Error	80	.8825	.0110	—
Honeylocust—high moisture				
Seed lot	3	1.4640	0.4880	8.28*
Treatment	4	1.3839	.3460	5.87*
Interaction	12	2.4102	.2008	3.41*
Error	80	4.7147	.0589	—
Mimosa—low moisture				
Seed lot	3	1.0534	0.3511	86.63*
Treatment	4	13.8957	3.4739	857.06*
Interaction	12	0.3256	0.0271	6.69*
Error	80	.3243	.0041	—
Mimosa—high moisture				
Seed lot	3	2.0581	0.6860	27.90*
Treatment	4	6.4036	1.6009	65.10*
Interaction	12	0.3925	0.0327	1.33
Error	80	1.9672	.0246	—

*Significant at $P \leq 0.05$.

At the higher moisture level, there was not much difference among technique means (tables 1 and 2). There was no significant difference in moisture between intact seeds and seeds tested by the Karl Fischer technique (15.5 versus 15.7%). The other three treatments (cutting and the grinding mills) had higher means (16.7 to 17.8%), but no significant differences among them. Imbibition had apparently softened the seed coats so much that even intact seeds lost their moisture readily in the oven. There was also wide variation among the measurements at the high moisture level. Lot means were significantly different, as the treatment did not produce equal moisture levels in all lots.

Mimosa. In the smaller seeds of mimosa, the results were similar to those of honeylocust, except that the seed coat seemed to have a much greater effect on moisture loss. At the low moisture level, much more moisture was lost during drying from ground or cut seeds as compared to intact seeds (tables 1 and 2). There was little difference among means for cutting and grinding (both mills), but values from the Karl Fischer technique were significantly higher than means for all other techniques. As with honeylocust, there were significant differences among seed lots.

At the high moisture level of mimosa seeds, intact seeds still gave significantly lower moisture values than any other technique, but the differences were not as much as in the low moisture level (tables 1 and 2). There were no differences among results from cutting and grinding, but moisture determination by the Karl Fischer technique showed that none of the oven methods removed all of the moisture from these seeds. Differences among seed lots were significant at this level also.

Discussion

The results of this study clearly show that the hard seed coats of honeylocust and mimosa must be ruptured in some fashion to allow all moisture to escape during drying, although rupture appears to be more important for seeds with low moisture contents. It is also apparent that in most cases cutting the seeds in half can give results equal to those obtained with ground seeds. This finding could be very helpful to workers in remote field stations or in developing countries where equipment may be limited. The goal in official testing for this type of tree seed is to be accurate to within 0.1 % of true moisture content and to have no greater difference between replicates than 0.3% (ISTA 1985). Cutting should not be adopted for official testing without additional research, but it certainly would be suitable for routine moisture tests for internal use. Most workers agree that accuracy to within 1.0% of true moisture is adequate in those cases.

Although a comparison of grinding mills was not a major objective of this study, the results did show that there was no difference between Wiley and coffee mills for both species. It is possible that for bigger and harder seeds, the coffee mill would not perform as well. In fact, one blade on the coffee mill was broken while grinding honeylocust seeds. Some leguminous tree seeds are extremely hard, and even a Wiley mill would have trouble grinding them when they are dry. The coffee mill should be used on small seeds only. For this reason cutting is even more attractive as an alternative to grinding, although very large seeds might have to be broken with hammers instead of being cut with hand shears. Cutting is not as fast as grinding, but neither method is time-consuming.

For honeylocust seeds, there was good agreement between results of the Karl Fischer technique and the ovendrying methods with ground samples, especially at the low moisture level. The same procedures used with mimosa showed that none of the oven methods were apparently driving all of the moisture out; there was approximately 2% difference between the best oven methods and the Karl Fischer technique. One explanation could be that the silica gel was giving up moisture to the seed material as suggested by Hunt and Neustadt (1966). This is not likely, however, because it did not occur with honeylocust. Another possible reason is stronger chemical bonding of moisture in mimosa.

A weakness of this study was the excessive variation in sample moisture content, which resulted in part from differential uptake of moisture by individual seeds. The presence of insect larvae was another source of error that was difficult to avoid, especially when grinding seeds. High-moisture seeds do not grind as well as seeds with lower moisture levels either, and the ground material tends to form small clumps.

Four seed lots per species were used in this study to ensure a variety of seed conditions and characteristics. Significant differences in moisture content among lots demonstrated that this objective was met, although these differences do not detract from the main goal of the study. Significant interactions between seed lot and treatment in three of the four tests, however, is a different matter (table 2). This could mean that some techniques are not as effective with seed lots with certain characteristics (seed coat thickness, insect infestation, etc.). Additional tests with a greater number of seed lots would be useful in this regard.

Although more research with these and other leguminous tree seeds is recommended, several conclusions can be drawn from the present work:

1. Rupture of seed coats is required for total escape of moisture during drying, and cutting seeds in half seems equal to grinding them for this purpose.
2. The coffee mill proved equal to the Wiley mill in grinding these species, but the coffee mill may not be durable enough for repeated grinding of large seeds such as *G. triacanthos.*
3. Compared to moisture determinations made using the Karl Fischer technique, the oven methods used in this study gave accurate measurements of moisture in honeylocust but did not completely remove the moisture in mimosa seeds. This species deserves more study.

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