

ACCELERATED AGING TEST

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The accelerated aging (AA) test utilizes the environmental factors commonly associated with seed deterioration, namely storage temperature and relative humidity. A single layer of seed is placed on a screen tray which is inserted into an inner chamber (plastic box) containing a small volume (40 ml) of water (Figure 1). The inner chamber is then placed into an accelerated aging (outer) chamber and aged at high temperatures (41 to 45°C) for a specific period of time (i.e., 72 h). During the aging period the seeds take up water from the humid environment within the inner chamber and are stressed at high temperatures and seed moisture. High vigor seed deteriorate slower than low vigor seed and seed lots can be separated into various vigor levels.

Most of the initial investigations of accelerated aging were conducted at Mississippi State University (Helmer et al., 1962; Delouche, 1965; Delouche and Helmer, 1967; Rushing, 1969) with the primary emphasis directed toward predicting the relative storability of seed for several crop species. Delouche and Baskin (1973) reported that the deterioration of a high and low vigor seed lot in AA and in warehouse storage followed similar trends (Figure 2). They reported significant correlations between

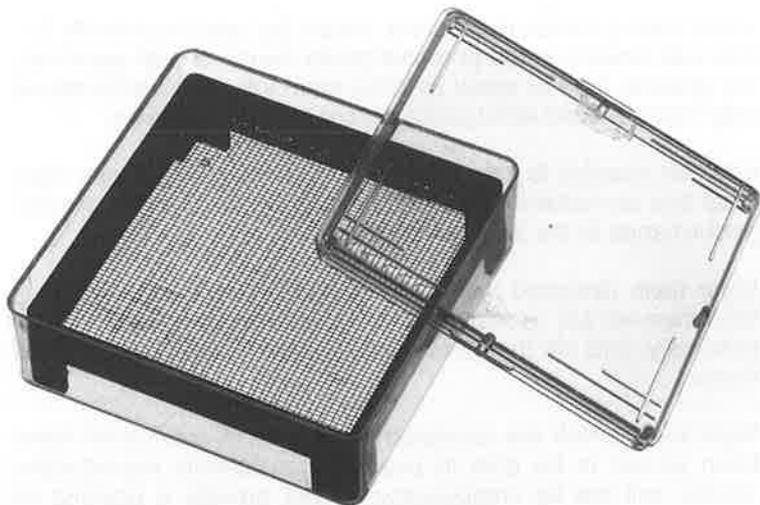


Figure 1. Inner chamber plastic box with screen frame to hold seed for aging.

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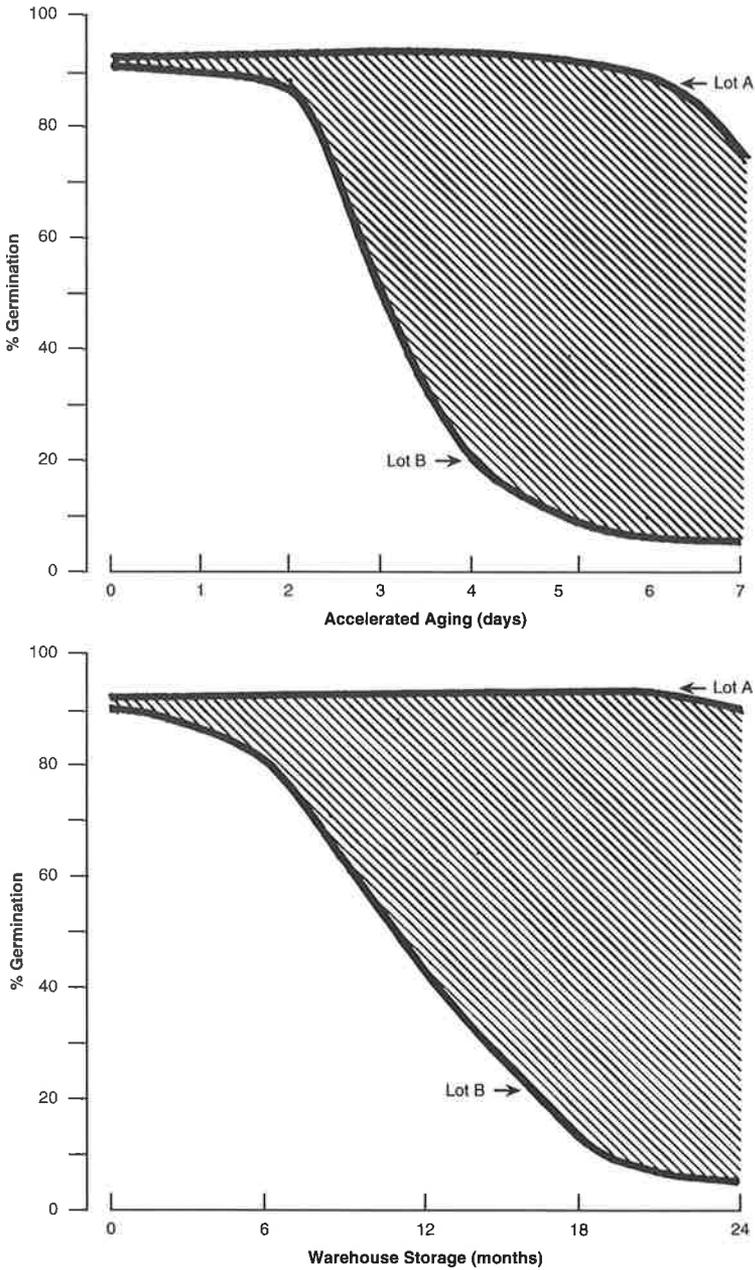


Figure 2. Germination response of two lots of crimson clover seed after AA at 40°C for periods up to 7 days and open storage for periods up to 24 months at State College, MS (Delouche and Baskin, 1973).

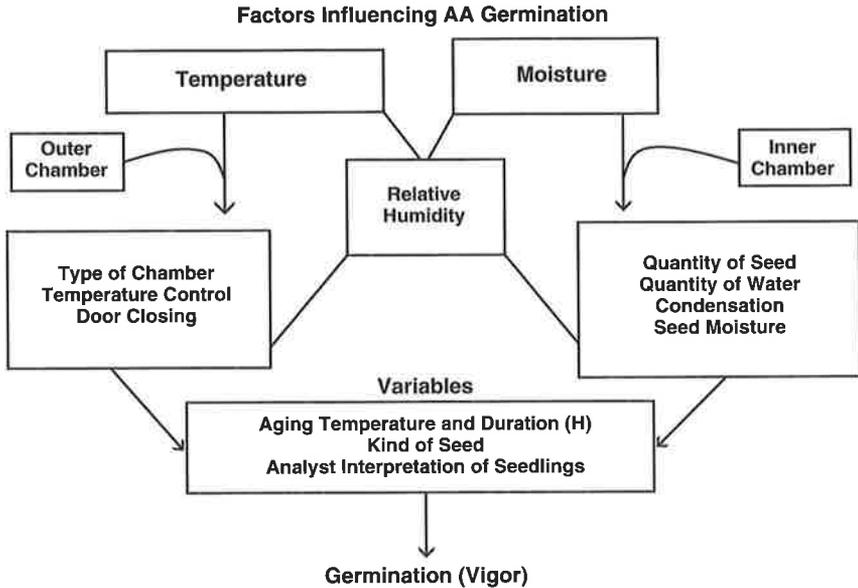


Figure 3. Seed moisture and aging temperature are the most important variables of AA test. Both are influenced by many factors which can be controlled.

the initial accelerated aging germination prior to storage and the standard germination following a specified time in storage.

In recent years the accelerated aging test has been evaluated as an indicator of seed vigor in a wide range of crop species (Baskin, 1970; Bishnoi and Delouche, 1975; Roos and Manalo, 1971; Byrd and Delouche, 1971; TeKrony and Egli, 1977; Tomes, 1985). This resulted in the test being included as one of seven suggested tests to determine seed vigor when the AOSA Seed Vigor Committee published the first Handbook of Vigor Testing in 1983 (AOSA, 1983). The test received additional support from this committee in 1988 when it was listed as the first test in the recommended section of the Vigor Testing Handbook for soybean. Recent surveys of seed testing laboratories have shown that the accelerated aging and cold tests are the most frequently used vigor tests in North America (TeKrony, 1983).

In this presentation I will discuss several factors which influence germination when using the accelerated aging test. The two most pertinent factors are temperature and seed moisture (Figure 3). The moisture which accumulates in the seed during the aging period is primarily influenced by the environment in the inner chamber (plastic box), while the temperature is controlled by the outer chamber. Several variables may act independently or in combination to alter temperature and seed-moisture during the aging period (Figure 3). All of these variables can be controlled but must be precisely monitored to achieve uniformity of test results.

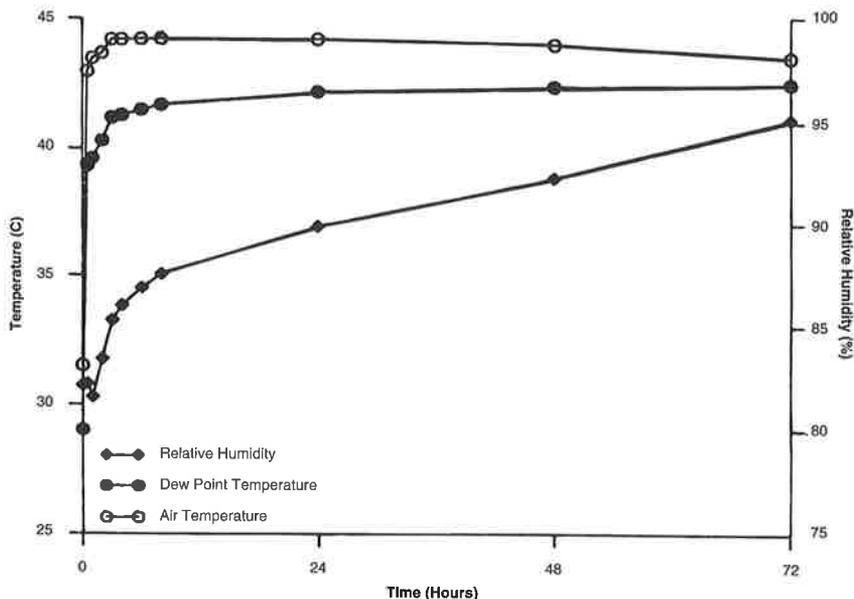


Figure 4. Changes in temperature and relative humidity inside an individual inner chamber (sample box) during 72 h aging period (Tomes et al., 1988).

SEED MOISTURE

In 1988 Tomes et al. reported the effect of relative humidity on seed moisture by measuring both air and dew point temperature inside the inner chamber (containing soybean seed) over 50 ml of water. They found that the relative humidity increased to 90% after 24 h and to 95% at the end of the 72 h aging period (Figure 4). Although this was lower than the 100% RH previously reported, it was adequate to raise the seed moisture to approximately 30% (FWB). This was consistent with earlier studies by McDonald and Phaneendranath (1978) which showed similar moistures for soybean seed aged for 72 h at 41°C (Table 1). They also reported that the initial water levels in the inner chamber (30 to 130 ml) did not affect seed moistures and germination at the end of the aging period. Thus, the initial

Table 1. Influence of three water levels in inner chamber (plastic box) on soybean seed moisture and germination after 72 h at 41°C (McDonald and Phaneendranath, 1978).

Water level (ml)	Seed moisture	Germination
	----- % -----	
30	30.8	78
80	30.9	74
130	32.1	74

Table 2. Influence of position in the wire mesh basket for glass jar (inner chamber) on seed moisture and germination following aging 41°C, 96 h (McDonald and Phaneendranath, 1978).

No. seeds tested	Seed position	Seed moisture	Germination
		----- % -----	
200	Top 1/3	24.8	28
	Middle 1/3	24.7	28
	Bottom 1/3	28.2*	8*

* Significant change at α 0.05.

levels of water in the inner aging chamber are not that critical, provided adequate water is available to provide a continuous surface of water under the seed during the entire aging period. We commonly use 40 ml, which is adequate and small enough to lower the risk of splashing water onto the seed when moving the inner chamber boxes on a tray during testing.

The quantity of seed and its placement in the inner chamber is extremely important when conducting the AA test. Studies by McDonald and Phaneendranath (1978) showed that the layering of soybean seed in a glass jar resulted in variable seed moisture at the end of a 96 h aging period (Table 2). They also reported that the seed in the lower position (closest to the water surface) had higher moisture and lower germination. This resulted in the recommendation that a single layer of seed (regardless of the species being tested) be placed on the screen of the inner chamber (plastic box) aging chamber.

Initial tests with soybean seed in our laboratory by Tomes et al. (1988) indicated that seed size influenced seed moisture at the end of the aging period and germination. They verified this by sizing two seed lots into four size fractions using round hole screens (Table 3) and aging 200 seed of

Table 3. Effect of sample and seed size on final seed moisture after aging at 41°C for 72 h (Tomes et al., 1988).

Screen ^a size	Seed size	Sample size			
		40 g		200 seed	
		Seed number	Final seed ^b moisture	Weight/ 200 seed	Final seed moisture
cm	mg seed ⁻¹		%	g	%
0.56	112	357	33.3	22	37.1
0.64	163	248	32.7	32	34.0
0.71	225	178	33.0	45	31.8
0.79	297	135	31.2	59	29.0
LSD ($\alpha=0.05$)			NS		2.4

^a Seeds were screened to uniform sizes by passing through sieves with 0.56, 0.64, 0.71 and 0.79 cm diameter openings.

^b Represents the seed moisture obtained after aging at 41°C for 72 h.

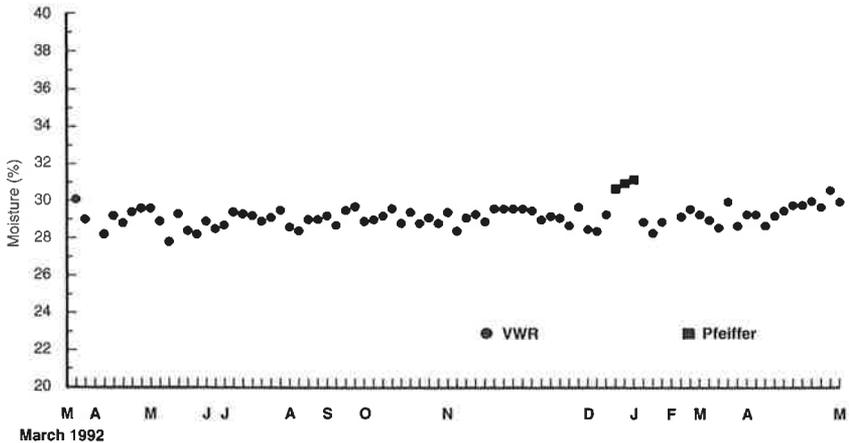


Figure 5. Seed moisture of check soybean sample following aging in VWR and Pfeiffer outer chambers from March, 1992 through April, 1993 (Conditions: 42 g of seed, 40 ml water, 41°C, 72 h).

each fraction for 72 h at 41°C. The final seed moistures ranged from 37% (FWB) for the smallest seed size to 29% for the largest size, which resulted in lower germination for the small seed fraction. If they weighed 40 g samples of each size fraction and aged the seed for the same time, the final seed moisture was much more uniform with only a two percentage point range from the small to the large seed. Thus, the final seed moisture provides an excellent quality control check for the amount of seed moisture imbibed during the aging period, which influences germination. For this reason we routinely remove a small sample from the inner chamber at the end of the aging period to determine the seed moisture. I recognize that this may be impractical when you are running many AA tests in a routine laboratory, but I strongly recommend that seed moisture is determined on your check sample. The seed moisture of the check soybean sample in our research laboratory for the past year is shown in Figure 5. The seed moisture ranged from 28 to 30% when seed were aged in the water-jacketed chamber and was slightly higher (31%) for the three tests conducted in the Pfeiffer chamber.

If the final seed moisture is an important quality check for the AA test, the logical question is what effect does initial seed moisture have on final seed moisture? Tomes et al. (1988) evaluated this by preconditioning two soybean seed lots to three moisture levels (8.0, 10.5, 13.5%) and aging each for 48 and 72 h (Table 4). Although the initial seed moisture range was 5.5 percentage points, the final seed moistures did not differ significantly and varied by only 2.1 percentage points. There were no significant differences in final germination in the high vigor seed lot. The low vigor seed lot showed lower germination at the high moisture level. Within the moisture

Table 4. Effect of initial seed moisture on final seed moisture content (SMC) and germination following aging at 41°C (Tomes et al., 1988).

Seed lot	Initial seed moisture	Aging time			
		48 h		72 h	
		SMC	Germ	SMC	Germ
		----- % -----			
High vigor	8.0	27.9	97	31.9	91
	10.5	29.3	97	32.6	90
	13.5	29.5	95	32.6	90
Low vigor	8.0	28.7	76	32.3	64
	10.5	29.5	71	32.4	60
	13.5	29.9	65	34.4	47

LSD (α 0.05): SMC=3.1; Germ=8

range of most seed lots received by the seed laboratory, the initial seed moisture would have little effect on final seed moisture or germination.

Another factor that can affect the final seed moisture in the inner chamber is the amount of condensation that accumulates in the outer aging chamber during the test. This condensation most frequently occurs in those aging chambers that have a heating element immersed in water in the bottom of the chamber. When condensation occurs at the top of the chamber, precautions must be made to prevent droplets of water from falling on the top of the inner chamber box. If water accumulates on the lid of the plastic box, the temperature differential between the air inside the box and the temperature of the water on the top of the box will cause condensation on the inside of the lid. When these water droplets fall onto the seed, the seed moisture will increase quickly to higher than normal levels and the germination will decline. We prevent this condensation from dropping onto the inner chamber boxes in our Pfeiffer aging chamber by placing absorbent cellulose material on the top shelf above the inner chamber aging boxes.

Several factors have been discussed which can influence the seed moisture levels which accumulate during the AA test. Fortunately, all of these factors can be controlled by the seed analyst if the precautions listed above are taken.

TEMPERATURE

The second major factor which influences AA results is temperature, which is primarily controlled by the outer aging chamber (Figure 3). Tomes et al. (1988) evaluated the effect of temperature by testing several soybean seed lots at 37, 39, 41, 43, and 45°C across several seed moisture levels. They concluded that aging temperatures of 37°C were too low, while 45°C was too high. They also reported that minor changes in aging temperature of 1 to 2°C would significantly reduce the germination of both low and high vigor seed lots (Figure 6). This clearly showed the importance of precisely controlling temperature during the AA test.

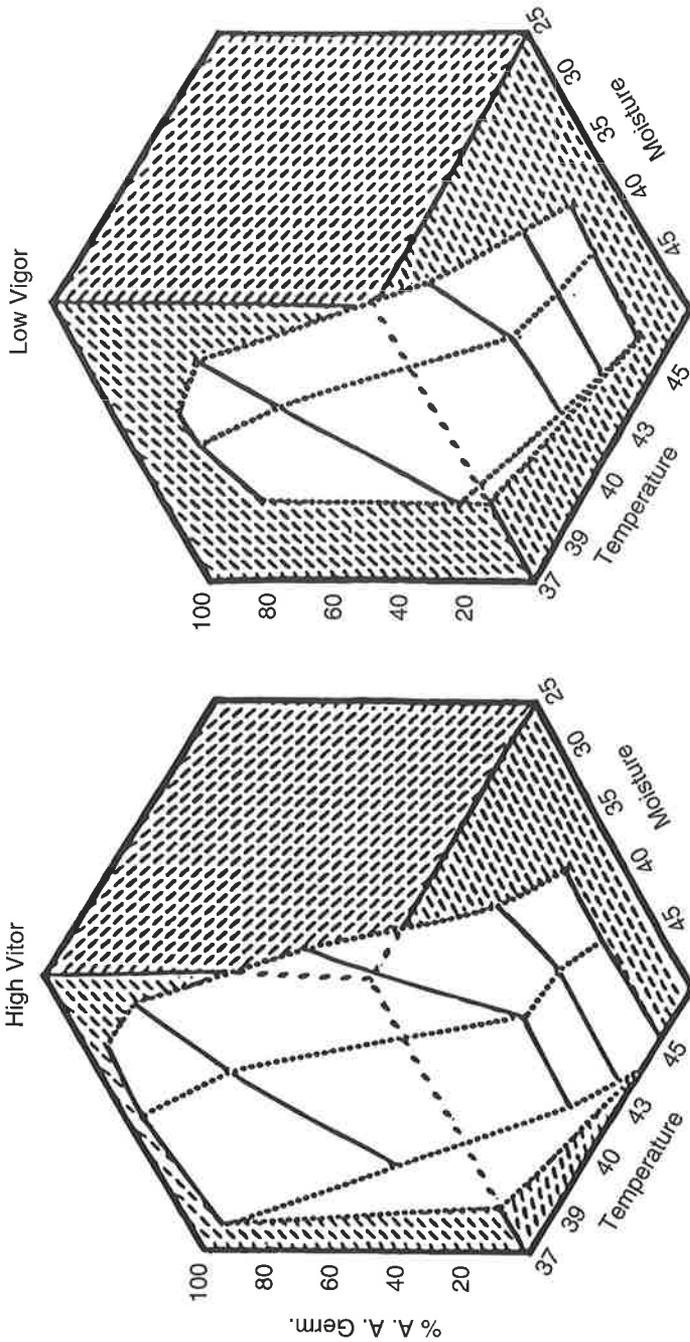


Figure 6. The effect of temperature and final seed moisture on germination of two soybean seed lots after aging for 72 hours (Tomes et al., 1988).

Table 5. Mean air temperature and recovery time to 41°C in the outer and inner chambers after 0.5 and 1.0 min of outer chamber door openings with nine inner chambers per shelf (Tomes et al., 1988).

Door opening time	Shelf position ^a	Outer chamber		Inner chamber	
		Temperature depression (below 41°C)	Recovery time	Temperature depression (below 41°C)	Recovery time
min		°C	min	°C	min
0.5	T	7	21	2	15
	M	6	24	1	20
	B	15	14		6
1.0	T	14	34	2	37
	M	7	38	3	18
	B	17	27	2	18
LSD ($\alpha=0.05$)		0.9	11.7	0.3	5.4

^a Shelf positions in outer chamber; T=top; M=middle; B=bottom.

Temperature uniformity during aging is primarily influenced by the type of AA outer chamber and the precision of temperature controls. The new water-jacketed aging chambers provide excellent temperature control, no condensation problems and are presently used by many laboratories that routinely conduct the AA test. It is necessary to provide a water source inside the chamber (a pan of distilled water on the bottom shelf). This allows the final seed moistures to reach the same level recommended in the AOSA Seed Vigor Testing Handbook. Other outer aging chambers which are frequently used for the AA test have a heating element immersed in water at the base of the chamber. These chambers frequently have condensation problems and usually do not have precise temperature controls. Thus, laboratories using these chambers will commonly attach a separate temperature control (with higher precision) as described in the AOSA Handbook (1983). Dry incubators or ovens have also been used for the AA test, however they are not recommended because of variable temperature and lower final seed moistures. Regardless of the outer chamber used, precise temperature control must be maintained within plus or minus 0.3°C of the desired aging temperature. This is best accomplished by using a water-jacketed type of AA chamber.

To maintain uniform and constant temperatures during aging, the door of the outer chamber must remain closed for the entire duration of the test. Tomes et al. (1988) evaluated the influence of opening the door for 0.5 or 1.0 minutes on the temperature in both the outer and inner chambers (Table 5) with nine inner chambers per shelf at a top, middle, and lower placement in the Pfeiffer outer chamber. They reported that opening the door for 1.0 minute lowered the temperature in the inner chamber 2 to 3°C and the outer chamber 7 to 17°C. The recovery time for both locations was approximately 30 minutes. Thus, if the outer door was opened two times for 1.0 minutes during a 72 h aging period a constant temperature could not be maintained

Table 6. Variables for accelerated-aging test for several crop species using 50 ml water in inner chamber (plastic box).

Crop	Weight seed/box	No. AA boxes	Aging Temperature	Time in chamber	Seed moisture following aging*
			°C	h	%
Soybean	42g	1	41	72	28–31
Corn	40g	2	45	96	26–29
Wheat	20g	1	41	72	28–30
Sorghum	15g	1	43	72	28–30
Tall fescue	1g	1	41	72	47–53
Red clover	1g	1	41	72	39–44
Tomato	1g	1	41	72	44–46
Pepper	2g	1	41	72	40–45
Lettuce	0.5g	1	41	72	38–41
Onion	1g	1	41	72	40–45
Canola	1g	1	41	72	39–44
Tobacco	0.2g	1	43	72	40–50

* If seed moistures are below or above this range, test should probably be repeated.

and the seed would be aged at a lower temperature for approximately one hour. Tomes et al. (1988) also reported that the most consistent temperatures in the inner chamber boxes could be maintained by allowing some air space between boxes on each shelf placed in the outer chamber.

SUMMARY

Sufficient evidence has now accumulated to show that repeatable vigor results can be achieved with the AA test. To maintain this uniformity however, seed analysts must take certain precautions that may not be required when running a warm standard germination test. These precautions are:

1. Use water-jacketed type of accelerated aging chamber (if available).
2. Precisely monitor aging temperature and maintain it $\pm 0.3^{\circ}\text{C}$ at the desired temperature.
3. Weigh (do not count) seeds and place a constant weight of seeds in the inner chamber (see Table 6).
4. Do not open the door of outer aging chamber during aging period.
5. Prevent water (from condensation) from dripping onto lids of inner chamber boxes.
6. Record time at start of aging period and remove seed from outer chamber at the exact number of hours specified and plant within ± 1.0 hour after removal.
7. Measure seed moisture of check sample after aging (see Table 6).

I'm convinced that the future of accelerated aging as a vigor test may be good for wide range of crop species. Expanded use of this test will

require research to identify the best aging temperature and time of separate seed lots by vigor level. The amount of seed required as well as the moisture at the end of the aging period must also be determined for each species. This information is provided in Table 6 for the species that have been evaluated to date in our laboratory. This list will be expanded in future years and a final recommended list will be updated and published by the AOSA Vigor Seed Testing Committee.

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