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C. C. Baskin

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COTTONSEED QUALITY CONTROL ASSURANCE

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Charles C. Baskin¹

Introduction

The indeterminate fruiting habit of the cotton plant makes the production of seeds with a high germination and high physiological quality difficult. This is much more of a problem in the humid areas where rainfall during harvest may be frequent or sparse than in the arid areas where rainfall during harvest is very infrequent. Field exposure of seeds in open bolls can result in very poor quality seeds. Even in arid areas, it is desirable to harvest with a minimum amount of field exposure.

Field Selection

Production of good quality cottonseeds begins in the field before planting. Field selection is important from several standpoints. One factor is weed problems. Field selection should include consideration of the weed situation (history). Weeds contribute to problems in several ways:

- (1) They reduce yield.
- (2) They can interfere with harvest. Often weeds will remain green when cotton has been defoliated and is ready for harvest. The green material can affect the moisture content of seeds, particularly when seed cotton is moduled and held for several weeks before ginning. Moisture will move from green plant material to seed cotton and can cause heating which can reduce seed quality.
- (3) Weed seeds, cocklebur in particular, are very difficult to remove from cottonseeds. Acid delinting will allow removal using a gravity table. However, it is best not to have cocklebur in cottonseeds. Where cocklebur plants are few, pulling them out by hand is a good practice. Even though this may be expensive, it could prevent cocklebur contamination of cottonseeds.

¹Agronomist and Professor, Seed Technology Laboratory, Miss. State University, Miss. State, MS. Field selection is important from several other standpoints. One of the primary things to consider is harvesting. Uniformity of the field is reflected in uniformity of crop maturity. Non-uniform soil types, low spots, etc., make the crop maturity uneven. This may mean some areas will open earlier or later than others. There may be a few "green spots" at harvest time which create the same problems as green weedy spots from a green plant material standpoint. Uniformity of cotton plant maturity and boll opening at harvest can greatly enhance the harvest of good quality seeds.

Defoliation

Defoliation improves drying conditions and makes harvesting easier. When harvesting for seeds, timing of application of defoliant is much more critical than when lint is the only consideration. Defoliating too early will greatly increase the amount of immature Actually cottonseeds are capable of germination at 22 to 25 seeds. days after bloom opening, but will produce weak seedlings at this stage of maturity. Seed germination decreases as the age of the boll decreases and the quality of the immature seeds is lower. In general, bolls that are 40 days old or older contain seeds that are mature enough to be strong and high germinating at the time of defoliation. When bolls have accumulated a minimum of 750 DD, 60's is a good point to check maturity. Do not let this be the only basis for deciding to defoliate. Cutting bolls that are questionable in maturity may help make decisions about timing defoliation. The seedcoats may be quite thin, but if they appear as a thin brown line around the seeds, the boll is mature enough that fiber quality will not be reduced by defoliation. One rule of thumb is to apply defoliant when 60% of the bolls are open. However, this percentage may be lowered to 50% if good management practices have been followed throughout the season, 80% of the bolls are set on the first two positions, and there is a fairly even distribution of fruiting branches with bolls set. Defoliants are most effective when applied in the early morning or late afternoon when humidity is high, winds calm, and temperature above 60°F.

Timing Harvest

The longer cotton stays in the field after bolls have opened, the more seeds are exposed to weathering. Weathering lowers seed quality. The bolls lower on the plant open first before plants have been defoliated. Temperatures are usually quite high at this time and relative humidity is high in the plant canopy. These two factors accelerate seed deterioration. Seeds from bolls on the lower part of the plant are generally low in quality if they germinate at all. Seeds from bolls in the middle part of the plant are exposed to weathering

for less time, therefore, are generally better quality than seeds from bolls from the lower part of the plant. Seeds from bolls from the upper part of the plant are more likely to be somewhat immature and will generally be lower in quality than seeds from mid-plant bolls.

The detrimental effects of field exposure have been well documented. Caldwell and Parker (1961), studying environmental effects in the Mississippi Delta, found that seeds germinated 79% one week after boll opening, 64.5% three weeks after opening, and 54% six weeks after opening. Relative humidity and/or ambient temperature are high in the Delta. The same trends were reported by Texas A&M University in USDA Production Research Report No. 135. This report also noted temperature/humidity relationship. When temperatures were in the 80-85°F range and relative humidity did not exceed 75%, there was virtually no change in germination after 28 days field exposure. However, when relative humidity exceeded 75%, germination declined significantly after 21 days field exposure.

Seed Cotton Moisture Content and Storage

Seed cotton should be dry - 12% moisture or less - at picking. It makes no difference if cotton is to be on trailers for a short period of time or moduled. Dry seeds and seed cotton are a must for good seed quality. Seeds at 12% or less moisture are less likely to be damaged by pickers and gin saws.

Covers should be available for trailers. In the event of rain, cotton should be protected as much as possible. Covering of modules is a necessity to prevent damage from rain. Modules must be placed in well drained areas until they can be ginned.

Monitoring temperatures in modules is necessary if seeds are to be saved. If temperatures exceed $100^{\circ}F$, the value of the seeds for planting becomes questionable.

The single, most important factor affecting seed quality in storage of both seed cotton and cottonseeds is moisture. Other factors contribute to quality; however, their effect is superimposed on the effect of moisture, thus other factors modulate, but never supersede the important effect of moisture (<u>Altschul</u>, 1948). The indeterminate fruiting habit of the cotton plant results in variation of maturity of bolls, thus there is a wide variation in seed moisture content. Irregular maturity of plants within fields, green material from cotton plants, and weeds all contribute to problems with moisture. Moisture will migrate from damp lint, green material (plant parts) and other seeds to drier seeds, thus, what might appear to be dry seed cotton may heat, causing seed damage and loss of viability. The variation in seed cotton moisture as related to days after boll opening was determined by Sorenson and Wilkes (1973) and is presented in Table 1.

The presence of high moisture foreign material is much more of a problem in the southeastern areas where plant growth is irregular, re-growth after defoliation sometimes occurs, and green weeds may be present at harvest. Sorenson and Wilkes (1973) measured moisture transfer from foreign material at different moisture contents and in different quantities to seed cotton and cottonseeds (Table 2). This study clearly demonstrates the moisture problems that can be created when seed cotton contains green material.

Seed cotton is quite often stored in trailers for several days after picking. Heating can be a problem. Griffin and McCaskill (1964) observed temperatures up to 140° F when seed moisture content was 16.4%. They concluded that trailer storage was not safe unless cottonseed moisture was 10% or less. Cooling and drying in trailers are not practical.

A USDA/ARS sponsored study (USDA 1965) concluded that seed cotton at low moisture content could be safely stored in the field on well drained sites without covers in the High Plains (West Texas) but in areas where even limited rainfall occurred, seed germination was drastically reduced. Numerous other studies where seed cotton was stored in mechanically packed modules on pallets have reached essentially the same conclusion - seed cotton at 10% or less moisture with little or no green foreign material can be stored at densities of 12 to 14 lbs. per cubic foot without any problems provided adequate protection is provided from precipitation and the storage site is well drained. (Baskin, 1976, Roberts, et al. 1974).

Picker Adjustments

Adjust pickers according to the manufacturers' recommendations. Check adjustments regularly to make sure machines are operating properly.

Adjust doffers to within 1/64 of an inch of the spindles and never let them touch the spindles.

Align picker bars by the use of a machine. One bar not in height alignment makes it impossible to adjust doffers correctly.

Adjust moistener pads to clean the complete spindle. Use only enough water or water and wetting agent to keep spindles clean. Too much or too little water will result in poor doffing.

		Days After Boll Opening									
Component		 0	4	8	12	16	20				
		R	ange in	Moisture	Content	Percen	t				
Seeds		10-22	11-21	10-16	10-13	8-13	8-12				
Locks		10-21	12-20	10-12	10-17	8-11	7-11				
Bolls		14-17	11-18	10-12	10-12	9-11	8-11				
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Table 1. Variation in moisture content of individual seeds, composite samples of seeds in individual locks and individual bolls at 0, 4, 12, 16 and 20 days after boll opening.¹

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	Foreign	Moistur	Moisture Content			
Moisture Content of	Material	Seed cotton		Cottonseeds		of Foreign
Foreign Material at Start of Storage ¹	1n Stored Seed Cotton	Start of Storage	End of Storage	Start of Storage	End of Storage	Material at End of Storage
			;	κ		
	5	8.0	11.5	9.1	12.7	19.5
80	10	8.0	15.4	9.1	17.2	29.5
	15	8.0	18.5	9.1	21.2	24.5
	20	8.0	20.9	9.1	24.5	46.2
	25	8.0	24.6	9.1	28.0	49.4
	5	6.9	9.1	7.8	10.2	14.0
	10	6.8	10.4	7.6	12.8	19.5
65	15	7.0	14.4	7.8	15.7	25.6
	20	7.2	16.4	7.8	18.5	30.0
	25	7.0	18.2	7.7	20.6	31.1
	5	6.9	8.6	7.8	9.7	13.8
	10	6.9	10.0	7.7	11.4	16.6
46	15	7.0	11.2	7.8	12.7	18.8
	20	7.1	12.5	7.8	14.4	21.7
	25	6.8	12.8	7.7	14.6	22.7

Table 2. Effect of storing seed cotton with different amounts of foreign material (stems, leaves, and other plant parts) on changes in moisture content of the seed cotton and seeds.

¹Seed cotton samples containing foreign material with an initial moisture content of 80% were stored for 42 days. Samples containing foreign material with initial moisture contents of 65 and 46% were stored for 28 days.

After Sorrenson and Wilkes. 1973.

Use minimum pressure on pressure plates for the first picking to leave green cotton on stalks. Green seeds are more susceptible to impact damage, which reduces quality.

Keep belts tight at all times. When replacing belts, use proper sizes. Never speed up fans by changing pulleys. Operate picker drums at recommended speeds. Check speed of the picker head anytime the power unit of the picker has changes in carburetor setting or governor linkage made.

Ground Speed: Synchronize ground speed and head speed for best picking efficiency. This practice reduces mechanical damage and results in highest lint quality.

Keep Pickers Clean: Check conveying systems several times daily. Keep doors and fan housings free of mud and trash. Reducing the size of openings in the conveying system increases velocity of air movement. This reduces picker efficiency and increases seed damage. Check spindles at each dumping to remove any buildup on them.

Ginning

Gin as soon as possible after picking. Adjust gin speeds and gin saws to keep mechanical damage to seedcoats to 12% or less. Cut and cracked seedcoats lower germination of seeds, but even more important, when seeds are acid delinted, acid will enter these openings and damage the embryos. Embryos of severely mechanically damaged seeds will be so badly acid burned that they will not germinate.

Drying and Aeration

Drying cottonseeds is not practiced except for small quantities for research purposes. The general practice is to harvest cotton as dry as possible, measure seed moisture content after ginning and if it is not acceptable for storage, send the seeds to the oil mill. Seed moisture contents of 10 to 12% or less are usually acceptable. Whether 10 or 12% is a maximum acceptable level depends on the preferences of the individual and, to some extent, the capacity of aeration in storage. With proper aeration, cottonseeds at 12% moisture can be safely stored.

Cooling cottonseeds as they come from the gin, then maintaining the seed mass under conditions that will prevent hot spots and moisture migration, is necessary to prevent loss of quality. The most economical means of maintaining cottonseeds at safe storage temperatures is by aeration (Cherry, 1976). Aeration systems to be used for planting seeds should be designed for planting seeds. High air flow rates are necessary for planting seeds. These may be as high as 20 ft³. min/ton but should not be less than 10 ft³. min/ton as compared to 2 ft³ min/ton for oil mill storage (Smith, 1975).

The general practice in cottonseed aeration is to draw air down through the seeds because the natural tendency for air movement is upward from the warm seeds to the cool upper surface is in part offset. The warm, moist exhaust air in early aeration is expelled in the lower part of the storage bin and possible condensation at the cooler upper surface is prevented. Also the operator can detect any offensive odors in the exhaust air (Cherry, 1976). Smith (1975) suggests a manifold aeration system for planting seeds. The advantages of this system are:

- Aeration can be started as soon as enough seeds are placed in storage to cover one or two ducts to a sufficient depth.
 - (2) As additional seeds are in storage and more ducts are covered, additional slide gates can be opened.
- (3) If an area is not cooling sufficiently or a hot spot develops, all gates can be closed except the one in the trouble spot and all of the air can be directed through that area.
- (4) Adjustments can also be made for selective removal of cottonseeds from the storage area.

After gin heat has been removed, temperatures of 85° F or lower at a relative humidity of 80% or less are recommended for aeration. A final seed mass temperature of 50 to 55° F is desirable for planting seeds.

Some drying may occur during aeration; however, the rate of moisture removal at these air flow rates is too slow and is insufficient for drying high moisture cottonseeds.

Evaluating Seed Quality

There are numerous tests for evaluating seed quality. Some tests employ speed for a quick estimate while others may be more accurate but require more time and labor.

The cutting test is for a very quick estimate of germination of gin run cottonseeds. With experience, a fairly good estimation of germination can be obtained. The tools for the cutting test are a cutting bar or some other means of holding the seeds in place and a cutting instrument. Randomly select 100 seeds from the sample, place in the bar, cut and evaluate the cut seeds. The immature seeds are readily recognized as are the very dark colored seeds. The dark seeds are not germinable. The good seeds have a whitish to yellowish green color. When seeds appear brown to brownish-yellow, the quality is poor and they probably won't germinate. The difficulty in evaluation is with seeds that are marginal.

<u>Visual mechanical damage</u> should also be used in evaluating seed quality, especially where seeds are to be acid delinted. Cut or cracked seedcoats will allow the entry of acid which can severely damage the embryos or even kill the seeds. Hand acid delinting a sample of gin run seeds and randomly selecting 200 to 400 seeds for evaluation is generally adequate. Some type of magnification and a good light is necessary for evaluation.

Damaged seeds may be classified as to the severity of damage. This is important because the more severe the damage, the greater the chance the seeds are of no value. The following classification is suggested:

No damage - seeds with completely intact seedcoats.

- Pinhole damage seeds with only one or two small (pinhole) punctures in seedcoats.
- Minor damage seeds with seedcoats cracked or cut, but not severely; damage primarily to the chalazal end or on sides.
- Major damage seeds with large cuts or ruptures in the seedcoats, part of the seedcoat missing, cotyledons exposed, or damage to the radical end of the seeds.

Seedcoat maturity is also an important characteristic in seed quality. Immature seeds are not as high in quality as mature seeds. There are differences in the color of mature seeds depending on variety. Seedcoat color will range from brown to black. Thickness of seedcoat will also vary with variety. One must be familiar with the color of the mature seeds of the variety in question. Even then, slightly immature seeds may be difficult to detect; however, the real problem is the grossly immature seeds with the white or very light seedcoats. These can be visually separated in a hand acid delinted sample. The number of immature seeds in the sample selected for mechanical damage evaluation will generally be satisfactory for an estimate of the number of immature seeds. Maturity is also detected in the cutting test. This, combined with seedcoat evaluation, should give a good estimate of maturity of the sample in question.

The standard germination test is basic in an evaluation program. Everything else revolves around germination. If germination is not up to the standards set, then nothing else matters. Germination may be acceptable, however, and the seeds still not suitable for conditioning or for marketing. The standard germination tests should be conducted according to the Association of Official Seed Analysts, Rules for Testing Seed. Copies of the Rules are available from the Secretary-Treasurer of the AOSA.

The tetrazolium test is an enzyme reaction in which live tissues stain red and dead tissues do not stain. It can be used to estimate both seed germination and vigor and can be a very useful tool in determining the nature and extent of seed quality problems during harvesting, conditioning, storage, and distribution.

The basis for viability and vigor evaluation involves the location, identification and appraisal of sound, weak or dead embryo tissues as these tissues relate to seedling development, overall strength of the developing seedling and the possible influence on length of life of the seeds in storage. The analyst must have a knowledge of seedling structure, what constitutes normal and abnormal seedlings and what parts of the embryo develop into the respective seedling structures.

In the preparation of seeds for staining, the seedcoat and the membrane surrounding the embryo must be removed before placing them in the tetrazolium solution. It is necessary to condition seeds by softening the seedcoats before attempting to remove them.

Gin run (fuzzy) seeds should be soaked in water for 12 to 18 hrs. (overnight). Place the sample to be tested in a container and cover with water and let it stand at room temperature until ready for seedcoat removal. Seeds that have been flame delinted or acid delinted are best conditioned by rolling them in wet germination towels for 12 to 18 hrs. (overnight). Soaking acid delinted seeds in water may result in an enormously low estimation of germination if there is an acid residue on the seedcoats. This does not appear to be a problem when seeds are rolled in towels.

The removal of the seedcoat and membrane is best accomplished using sharp, pointed tweezers. Hold the seed between the thumb and index finger and remove the seedcoat beginning at the chalazal end. As the seedcoat is removed, drop the embryo into a container of water. This will help loosen the membrane. When seedcoat removal is completed, remove the membrane, taking care not to break the radicle or otherwise damage the seed with the tweezers. Place the seed back in water.

When membrane removal is completed, drain water from seeds, cover with tetrazolium solution and place in an oven at 40° C for 1 hour, then drain the tetrazolium solution from the seeds and rinse two to three times in cold water. Cover seeds with water. If evaluation is not made immediately, or you have several samples to evaluate, seeds should be placed in an ordinary refrigerator. They may be held up to 24 hrs. under refrigeration, if necessary.

Evaluation should be done under magnification and good light. Combination fluorescent light and magnifying lenses are available and are suggested. In the evaluation process, the embryos must be thoroughly examined, especially the radicle. The cotyledons should be parted with tweezers in order to see the entire radicle. Cutting the embryo with a single edge razor blade can be helpful in evaluation. This allows you to "see" inside the embryo, if necessary.

The most desirable color for cottonseeds is a dark pink to light red. Darker seeds may also be germinable. Germinable seeds are completely stained; have dead or weak tissue over less than one-third of the cotyledonal area; have slight, small dead areas over the cotyledons or the chalazal end; radicle tip only may be dead. The radicle tip in a good seed may be quite dark because this is an area of high metabolic activity and the small amount of tissue allows deeper penetration of the tetrazolium solution resulting in a dark appearance. This should not be mistaken for weak tissue.

Non-germinable seeds include those with one-third or more of the radicle unstained; those with more than one-third of the cotyledonal tissue unstained; those stained very dark red to purplish red; those stained grayish red or milky in appearance; and, those completely unstained. The very dark or milky seeds may also be somewhat soft and flaccid. Immature seeds may stain a desirable color, but may be somewhat soft and flaccid and the cotyledons are beginning to unfold or are nearly unfolded. If these seeds germinate, they will be weak and of little value.

The conductivity test is being used by some seed companies. This test measures the electrical conductivity of water in which seeds have been soaked. As seeds deteriorate, cell membranes begin to break down. This allows cellular contents to "leak." The electrical conductivity of the steep water from deteriorated seeds will produce a different reading than that from high quality seeds.

Mechanical damage to a seed will also result in more material being leached into the steep water, thus a seed with a damaged seedcoat would give a reading similar to that of a deteriorated seed without mechanical damage. Seed treatment materials, fungicides and insecticides, may also affect the amount of materials in steep water, thus affecting conductivity readings.

The equipment for measuring conductivity is highly specialized and sophisticated. Machines capable of measuring conductivity of as many as 100 cells at a time with digital readout and printout are available. Specific instructions for seed preparation, operation and interpretation for specific models come with the instruments.

Research by Hopper (1981) indicated that conductivity was related to germination, emergence potential and early seedling growth.

Free fat acidity is used quite extensively as an index of seed quality. This test is based on the breakdown of fats and oils to fatty acids and glycerol as seed deterioration progresses. Free fatty acids usually build up under high temperatures and high seed moisture conditions. Once seeds are dry - 10% moisture or less - free fatty acids will not increase. The level might actually decrease slightly -0.1% or so - when stored under cool, dry conditions. Deterioration, however, is not always accompanied by an increase in free fatty acids.

A seedlot may germinate poorly or not at all and be well below 1.0% in free fatty acidity. Free fat acidity is best used to indicate variation among seedlots, or to detect variation in seed quality and possible problem lots. The 1.0% level of free fatty acids is the most common acceptable level for seeds; however, as has already been mentioned, seeds well below 1.0% may be of poor quality. On the other hand, seeds at 1.5% free fatty acid may be acceptable for planting. There may be times when most of the seeds available are above 1.0% free fatty acid and there is no choice but to save these.

An example of when to use free fat acidity is: if seeds are being checked as they are transported to a warehouse for storage and free fat acidity is regularly in the range of 0.5 to 0.8% and suddenly samples begin to run 1.2 to 1.5%, these seeds should be rejected or at least stored in a separate area until they can be further evaluated. Certainly seeds that are high in free fatty acids should not be carried over to the next planting season.

The solvent method of extracting oil is not practical for most seed companies; however, there are hydraulic presses available that will extract small quantities of oil. These are quite suitable for most seed companies. In addition to an oil press, other equipment and supplies are needed.

Low temperature germination, referred to as the Cool Germination Test, can also be used as an indication of physiological quality.

As indicated by its name, this test subjects seeds to cool, wet conditions and is intended to represent cool, wet field conditions. One major difference, however, is the absence of the soil borne organisms that cause seedling rot and seedling diseases. The test does measure differences in seedlots and provides information of value and has a place in a seed program.

This test will require the maintenance of a constant 18C $(64.4^{\circ}F) \pm 0.5^{\circ}C$. Maintaining constant temperature is essential. If the temperature is allowed to fluctuate up to $20^{\circ}C$ ($68^{\circ}F$), much of the effects of the test will be lost.

Preparation of seeds for testing is the same as for a standard germination test; seeds are rolled in moist germination towels. Generally, 200 seeds from each lot are tested in four replications of 50 seeds each. Rolled towels are placed on end (upright) in a wire or plastic mesh container or plastic crisper with a cover. If a dry cabinet is used, cover containers to prevent towels from drying during the test.

Only one count is made on the seventh day. All normal seedlings having a combined root/hypocotyl length of 1 1/2 inches or longer are counted. The root/hypocotyl measurement is made from the tip of the radicle (primary root) to the point of attachment to the cotyledons. These are considered the vigorous or strong seedlings. This test should be used only on seeds that have been delinted and treated with a fungicide. It should not be used for gin run seeds.

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