

BREEDING AND GENETICS

Estimating Seed Surface Area of Cottonseed

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ABSTRACT

Seed surface area (SSA) may be estimated by WinSeedle Image Analysis System, transformations of seed volume (Hodson method) or by seed index. If fuzzy seed index were related to SSA, SSA could be estimated without delinting seed. Our objective was to compare different methods of estimating SSA using fuzzy, acid- and flame-delinted seed. Seed were produced at two Arkansas locations and included eight diverse genotypes. After ginning, 100-fuzzy seed samples from a total of 10 replicates of field plots were randomly selected and weighed. Samples were flame-delinted, and then weighed, volume displacement determined, and scanned by digital analysis. The same seeds were then acid-delinted, weighed and SSA recalculated in the same manner. Location by genotype and genotype by delinting method interactions were significant for seed index, Hodson SSA and WinSeedle SSA. Volume means were significant for all main effects. Variation among genotypes was relatively similar for each parameter. Lowest correlations were found associated with volume of flame-delinted seed. Otherwise, seed index of fuzzy and acid-delinted seed were highly correlated with seed volume and both SSA measurements. Fuzzy seed index explained more variation in WinSeedle SSA than any other parameter. A regression equation may be used to transform fuzzy seed index to estimated SSA. The strong relationships of fuzzy seed index to delinted seed index, seed volume and the WinSeedle SSA measurements indicate that it can be used as an indicator of SSA when estimating fiber density.

Seed surface area (SSA) estimates have been a component of cotton breeding selection indices for many years. Lint frequency (Hodson, 1920) and lint

density index (Mason, 1951) utilized SSA estimates as a means of determining fiber weight produced on a given area of seed surface. Breaux (1954) found that lint frequency and lint density index were positively correlated with lint percentage, and lint percentage was positively correlated with lint yield. He suggested that the lint percentage measurement offered increased accuracy and convenience over SSA estimates. Since it was correlated with yield and was easy to measure, lint percentage became favored over lint frequency or lint density by many breeding programs. However, selection based upon lint percentage has led to decreased seed size and concerns of decreased lint yield stability (Lewis 2001).

Use of fiber density as a selection criterion has the potential of improving yield stability. Fiber density standardizes seed size by dividing the number of fibers per seed by the estimated SSA. By standardizing seed size, yield might be improved and stabilized by focusing on a specific yield component. However, standardizing seed size area requires an accurate and easily derived estimate of SSA.

The non-uniform, conical shape of cotton seed hinders direct measurement of SSA. Hodson (1920) established a method of estimating SSA based on volumetric displacement of ethanol by acid-delinted cottonseed. He then established SSA estimation by assuming the cotton seed have a uniform shape of hemispherical cone. A ratio of largest seed diameter to length was used to establish a table to estimate SSA for seed possessing a range of volumes (Table 1). However, seed size of many modern cultivars is outside the range of conversion tables provided by Hodson, and thus requires extrapolation of values. Culp and Harrell (1975) observed that seed size of cultivars has declined over time and noted that some older methods of SSA estimations may no longer be valid. Additionally, volumetric displacement is a cumbersome process that may not be practical for breeding programs with a small labor source.

Bourland and Bird (1983) described a strong correlation ($r = 0.97$) between cotton seed weight (seed index) and volumetric displacement of acid-delinted seed. This finding suggested that cotton seed weight may be substituted for volumetric displacement.

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Thurman (1953) linked seed index to SSA with a minimum correlation of 0.91 among five F_2 populations. Thurman's work did not specify if the seed were fuzzy or delinted. A relationship between seed index and SSA exists, but the relationship between fuzzy seed index and SSA needs further examination.

New technology may provide improved, direct means of estimating SSA. Cranmer et al. (2005) utilized the WinSeedle 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada) to measure SSA of cotton seed while investigating the influence of fibers per seed on cotton yield stability. The WinSeedle program uses scanned images of seed to calculate the maximum length and width of each seed in the sample tray. SSA is estimated by any one of five methods. This study utilized the round object method, which assumes that seed have a circular cross-section. The seed length was then multiplied by the seed cross-section perimeter to estimate SSA. At present, image analysis using WinSeedle program appears to be the most direct and practical method available for estimating SSA of cotton seed.

Previous work with the Hodson and WinSeedle methods described above utilized the acid-delinting method for removing linters from cottonseed coats. Acid-delinting may be accomplished by either a wet acid or dry acid method. Wet acid method, which utilizes either concentrated or dilute sulfuric acid, requires subjecting cottonseed to an acid bath followed by subsequent neutralizing and drying. With the dry acid method, fuzzy seed are placed in a sealed chamber, and then gaseous hydrochloric acid is pumped into the chamber and agitated. Subsequently, the seed are neutralized, but do not require drying. Since air and seed moisture increases the heat generated by hydrochloric acid, the dry acid method is seldom used in areas having high relative humidity. As cottonseeds imbibe solvent and are handled by any of these methods, the physical properties of the seed coat may be altered. Each method requires specialized equipment and considerable time and expense. In addition, some cottonseed may be lost by acid damage or during handling in the delinting process. Thus, the integrity of a sample may be compromised

Flame-delinting was sometimes used prior to the development of acid-delinting methods. Fuzzy seed are simply dropped through a tube having a controlled flame. The fuzz is burned, leaving charred singe on the seed surface. Flame-delinting is relatively quick and inexpensive, but uniform, acceptable delinting is difficult to achieve. However, flame-delinting might

be an acceptable alternative to acid-delinting for the determination of SSA.

A SSA estimate of cottonseed is needed to estimate density of fibers on the seed surface, which may be an important trait for improving yield and yield stability. The WinSeedle method is assumed to be the most direct, accurate measure of SSA now available, but it requires considerable time to process a single sample. To be practically used in a breeding program, the method for estimating SSA must be relatively quick so a large number of samples can be handled. Optimally, SSA can be estimated on fuzzy seed so that delinting will not be required. The hypothesis of this test was that SSA of cottonseed can be estimated by measurements of weight or volume with or without delinting the seed. The objective of this study was to evaluate different methods of estimating SSA of cottonseed using fuzzy, acid (wet concentrated method) and flame-delinted seed with SSA estimated by the WinSeedle direct scanning method.

METHODS AND MATERIALS

Eight regionally adapted Upland cotton genotypes (used as parents in associated genetic study) which exhibited diversity for seed index and lint index were chosen and planted into a Sharkey silty clay soil (very-fine, smectitic, thermic Chromic Epiaquerts) at the Northeast Research and Extension Center near Keiser, AR, on April 30, 2007 and a Hebert silt loam soil (fine-silty, mixed, thermic Aeric Ochraqulf) at the Southeast Branch Experiment Station near Rohwer, AR, on May 15, 2007. The eight genotypes included 'DP393' (PVP 20040026), DX25105N (breeding genotype developed by Syngenta Seeds, Inc. and evaluated in Arkansas Cotton Variety Tests in 2004 through 2006), Arkot 9203-03 (Bourland and Jones, 2006a), Arkot 9208 (Bourland and Jones, 2006b), Arkot 9314 (Bourland and Jones, 2007b), Arkot 9506 (Bourland and Jones, 2007a), Arkot S23-2 (Bourland et al., 2006), and Arkot 9108 (Bourland and Jones, 2005). The eight genotypes were originally classified by seed index as low (DP393, Arkot 9506 and Arkot S23-2), medium (DX25105N and Arkot 9203-03), high (Arkot 9208 and Arkot 9108) or very high (Arkot 9314).

Plots at each location were two rows 12.2 m long on 0.96 m centers. The genotypes were planted using a RCBD with four replications at Rohwer and six replications at Keiser. Plots at each site were furrow-irrigated and cultural inputs were based on University

of Arkansas recommendations for cotton production and applied as detailed by Bourland et al. (2008).

After all bolls had opened, a 50-boll sample was collected from each plot by hand-harvesting all bolls from consecutive plants. The samples were ginned on a laboratory saw gin, and fuzzy seed samples of 100 seed were randomly taken from each of the 10 replicates of field plots over two locations. After collecting data on fuzzy seed, the seed were flame-delinted by placing the fuzzy seed in a tea-strainer and burning the linters with a flame provided by a propane burner. Only burnt char remained on the seed after flame-delinting. After collecting data on the flame-delinted seed, the remaining burnt char on the seed were removed by acid-delinting. The seed were acid-delinted by immersing the seed in concentrated sulfuric acid followed by neutralizing the acid with lime and drying the seed. The resulting acid-delinted seed were void of any fiber or char and were assumed to be the same as seed produced by acid-delinting of fuzzy seed.

Seed index (weight of 100-seed), volume and SSA were determined for the fuzzy, flame-delinted, and acid-delinted samples. Volume was determined by placing 100 seeds in a 50 cc graduated cylinder and covering the seed with ethanol from a 100 cc burette until a volume of 40 ml was achieved in the graduated cylinder. The amount of ethanol displaced by the seed was subtracted from the 40 ml cylinder volume to determine the volumetric displacement of the seed sample. However, due to air-pockets imbedded in fuzzy fibers, an accurate measure of volume of fuzzy seed samples could not be obtained. The SSA was then extrapolated using Table I from the publication by Hodson (1920). Extrapolation involved converting the Hodson table values (Table 1) from cm to mm and plotting a linear curve ($y = 43.79x - 128.11$) through the points. This equation was used to extrapolate SSA for cotton seed involved in the experiment.

For the WinSeedle scanning method, the 100-seed samples were placed into a tray so that seed were neither touching one another nor the sides of the tray. The tray was placed into a scanner modified to accommodate the tray and a scanned image was acquired. The image was analyzed by WinSeedle Pro software and SSA estimates were calculated based upon the round object method, where seed length was multiplied by the seed cross-section perimeter. Since calculation required a well-defined perimeter of the object being scanned, image analyses of fuzzy seed samples were not obtained.

Table 1. Seed surface area estimation based on diameter and length of cottonseed from Hodson (1920).

Diameter	Length	Seed Surface Area
cm	cm	cm ²
0.5000	0.8775	0.9236
0.5100	0.8951	0.9604
0.5200	0.9126	0.9889
0.5300	0.9302	1.0372
0.5400	0.9477	1.0772
0.5500	0.9653	1.1169
0.5600	0.9828	1.1585
0.5700	1.0004	1.1987
0.5800	1.0179	1.2427
0.5900	1.0355	1.2852
0.6000	1.0530	1.3299
0.6100	1.0706	1.3738
0.6200	1.0881	1.4190
0.6300	1.1057	1.4655
0.6400	1.1232	1.5120
0.6500	1.1408	1.5610
0.6600	1.1583	1.6090
0.6700	1.1759	1.6575
0.6800	1.1934	1.7069

Seed index, volume, Hodson SSA, and WinSeedle SSA were each analyzed as a split-split-plot with location as the whole plot (fixed effect), genotype (fixed effect) as the subplot, and delinting method (fixed effect) as the sub-subplot. Treatment means were calculated across replicates (random effect) at each and separated using Fisher's protected LSD at the 0.05 significance level. Regression analyses using WinSeedle SSA as the dependent variable and each of the other parameters as the independent variable were conducted. All data were analyzed using the PROC GLM or PROC REG procedure in SAS Version 9.1 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Of the seven parameters measured on the seed (Table 2), the WinSeedle scanning of acid-delinted provided the most direct measure of SSA, and was assumed to be the most accurate. However, this estimation of SSA required seed samples to be acid-delinted (approximate 10 minutes even for a

small sample), then scanned (approximately three minutes per sample). Obviously, the time required to obtain WinSeedle SSA of acid-delinted seed hinders its use on the large number of samples that are generally associated with a cotton breeding program. Although counting fuzzy seed requires more time than counting delinted seed, seed index of fuzzy seed is the most time efficient since it does not require delinting of the seed. In addition, loss of seed or seed fragments during delinting may compromise the integrity of seed samples. Of the two delinting methods, acid-delinting requires more time than flame-delinting since acid-delinted seed must be neutralized and dried. Once seed are delinted, seed volume measurement requires more time than simply weighing the seed. Based on these observations, the seven parameters listed in Table 2 are arranged in order of the expected amount of time required to make the measurements. Techniques listed higher in the table would be more acceptable for use in a cotton breeding program.

Location by genotype as well as genotype by delinting interactions were observed for seed index, Hodson SSA and WinSeedle SSA (Table 3). These particular genotypes were selected for variability in lint yield, lint percent, seed index and lint index, so genotypic influence would be expected (Table 4, 5 and 6). Mean square error values revealed the greatest variability among effects was delinting method followed by genotype and location. All other effects exhibited similar mean square error values. Volume was only significant for main effects.

Location by genotype mean values for seed index, Hodson SSA and WinSeedle SSA were similar across either location for a given genotype (Table 4). DP393 was more affected by the location by genotype interaction than any other genotype. Location was significant for DP393 across seed index, Hodson SSA and WinSeedle SSA. Location was also a significant factor for DX25105N.

Table 2. Prediction of WinSeedle seed surface area (SSA) by other seed parameters².

Independent variable (x)	Regression equation	R ²
Seed index of fuzzy seed	SSA = 35.74 + 6.59x	0.63
Seed index of flame delinted seed	SSA = 41.95 + 6.62x	0.56
Volume of flame delinted seed	SSA = 72.37 + 2.77x	0.30
Hodson SSA of flame- delinted seed	SSA = 32.98 + 0.56x	0.48
Seed index of acid-delinted seed	SSA = 41.63 + 7.11x	0.57
Volume of acid-delinted seed	SSA = 60.75 + 4.06x	0.48
Hodson SSA of acid-delinted seed	SSA = 38.92 + 0.70x	0.59

² Parameters are listed in order from least to most time expected to make measurement.

Table 3. Probabilities of F values associated sources of variation for four seed parameters².

Source	df	Volume	Seed index	Hodson SSA	WinSeedle SSA
Location (L)	1	0.0263	<0.0001	0.0295	0.9268
Genotype (G)	7	<0.0001	<0.0001	<0.0001	<0.0001
L x G	7	0.9938	<0.0001	<0.0001	0.0004
Delinting method (D)	1	<0.0001	<0.0001	<0.0001	<0.0001
L x D	1	0.9166	0.3033	0.6773	0.1867
G x D	7	0.3880	0.0318	<0.0001	<0.0001
L x G x D	7	0.7670	0.6389	0.0990	0.2613

² Seed of eight cotton genotypes were obtained from field tests at two Arkansas locations (total of 10 replications) in 2007. Seed index (weight per 100 seed) was determined for fuzzy, flame-delinted and acid-delinted seed. Volume and seed surface area (SSA) were determined on delinted seed. Hodson SSA was estimated by converting seed volume using table in Hodson (1920). WinSeedle SSA was estimated by scanning seed using WinSeedle 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada)

Table 4. Location by genotype interaction mean values for seed index, Hodson seed surface area (SSA) and Seedle SSA¹.

Genotype	Seed index (g) by location		Hodson SSA (mm ²) by location		WinSeedle SSA (mm ²) by location	
	Keiser	Rohwer	Keiser	Rohwer	Keiser	Rohwer
Arkot 9314	10.2	10.6	124.3	120.1	138.8	133.9
Arkot 9208	9.4	9.5	110.8	113.0	122.8	124.2
Arkot 9108	9.2	9.8	118.8	117.1	120.3	117.7
Arkot 9506	9.0	9.3	110.3	111.7	118.1	117.5
DX25105N	8.7	9.6	109.1	114.9	119.1	124.0
DP393	8.6	9.5	99.2	108.2	118.0	124.2
Arkot 9203-03	8.6	8.9	103.7	105.2	114.6	113.2
Arkot S23-2	8.5	8.3	106.0	103.7	110.1	108.1
LSD 0.05 ^y	0.8		5.0		5.2	
LSD 0.05 ^x	0.8		4.8		5.1	

^z Seed of eight cotton genotypes were obtained from field tests at two Arkansas locations (total of 10 replications) in 2007. Seed index (weight per 100 seed) was determined for fuzzy, flame-delinted and acid-delinted seed. Volume and seed surface area (SSA) were determined on delinted seed. Hodson SSA was estimated by converting seed volume using table in Hodson (1920). WinSeedle SSA was estimated by scanning seed using WinSeedle 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada).

^y LSD for comparing two delinting methods of same genotype.

^x LSD for comparing two genotype means at the same or different locations.

Table 5. Genotype by delinting method interaction mean values for seed index, Hodson seed surface area (SSA) and WinSeedle SSA^z.

Genotype	Seed index (g) by delinting method			Hodson SSA (mm ²) by delinting method		WinSeedle SSA (mm ²) by delinting method	
	Fuzzy	Flame	Acid	Flame	Acid	Flame	Acid
Arkot 9314	11.8	10.8	9.9	143.3	102.0	156.4	117.2
Arkot 9208	10.8	9.7	9.1	131.3	92.1	140.7	106.0
Arkot 9108	10.8	9.7	9.2	132.8	103.5	131.9	106.6
Arkot 9506	10.3	9.5	8.8	128.6	93.1	134.2	101.5
DX25105N	10.3	9.3	8.8	124.6	98.3	133.1	109.0
DP393	10.2	9.2	8.6	122.3	83.4	138.2	102.8
Arkot 9203-03	10.0	9.1	8.4	122.5	86.1	129.3	98.7
Arkot S23-2	9.5	8.6	8.1	120.0	89.9	122.0	96.5
LSD 0.05 ^y	0.2			3.1		3.2	
LSD 0.05 ^x	0.6			4.2		4.4	

^z Seed of eight cotton genotypes were obtained from field tests at two Arkansas locations (total of 10 replications) in 2007. Seed index (weight per 100 seed) was determined for fuzzy, flame-delinted and acid-delinted seed. Volume and seed surface area (SSA) were determined on delinted seed. Hodson SSA was estimated by converting seed volume using table in Hodson (1920). WinSeedle SSA was estimated by scanning seed using WinSeedle 2003b Image Analysis System (Regent Instruments, Inc., Quebec, Canada).

^y LSD for comparing two delinting methods of same genotype.

^x LSD for comparing two genotype means at the same or different delinting methods.

The genotype by delinting method interaction data highlighted the influence of delinting method for seed index, Hodson SSA and WinSeedle SSA (Table 5). For each parameter a significant difference was observed across delinting methods for every genotype. Differences across genotypes

within a given delinting method were observed; however, the trend was similar regardless of delinting method or parameter evaluated. For each trait (Table 4, 5 and 6), Arkot 9314 expressed the highest value, while Arkot S23-2 and Arkot 9203-03 expressed the lowest, or equal to lowest,

values. DP393 appeared to have the greatest deviation in their relative ranks over the parameters. DX25105N had a high volume relative to its seed index, which suggests that its seed density was relatively low. Efficiency of flame-delinting is associated with the amount and texture of linters on the fuzzy seed and the degree that seed are exposed to the flame. The acid-delinted method removes all linters and provides a smooth seed coat. Since char remained on the seed after flame-delinting, the flame-delinted seed were expected to have higher seed index, volume and SSA (Tables 5 and 6). Flame-delinted seed were about 7% heavier (Table 5) and had a 7% greater volume (Table 6) than acid-delinted seed. SSA estimated by WinSeedle was 6 and 12% higher than SSA estimated by Hodson for

flame- and acid-delinted seed, respectively (Table 5). Sporadic fragments and/or non-uniformity of char may have increased the images estimated by WinSeedle and skewed its measurement of SSA of the flame-delinted seed. However, the large variation in SSA estimated by the two methods for acid-delinted seed was not expected.

All of the traits tended to be positively correlated (Table 7). All correlation coefficients that included volume of flame-delinted seed as one variable were less than 0.50. The residue char on the flame-delinted seed, which often entrapped small air bubbles, likely contributed to error when measuring volume. The cleaner, more uniform delinting associated with acid-delinting method likely provided high relationships with all other parameters.

Table 6. Location by genotype by delinting method means for seed volume^z.

Genotype	Seed volume (ml) at Keiser by delinting method		Seed volume (ml) at Rohwer by delinting method	
	Flame	Acid	Flame	Acid
Arkot 9314	12.7	12.5	13.1	12.7
Arkot 9208	12.1	10.4	11.6	11.2
Arkot 9108	12.1	11.3	12.2	11.7
Arkot 9506	11.0	10.5	11.3	11.0
DX25105N	12.0	11.2	13.5	11.2
DP393	10.6	10.2	11.2	11.2
Arkot 9203-03	11.4	9.6	11.9	10.1
Arkot S23-2	10.2	9.7	11.3	9.7
LSD 0.05	1.4	2.0	2.2	2.1

^z Seed of eight cotton genotypes were obtained from field tests at two Arkansas locations (total of 10 replications) in 2007. Seed volume (ml 100 seed⁻¹) was determined on flame and delinted seed.

Table 7. Correlation coefficients between measurements of seed index (SI), volume (VOL), and seed surface area by Hodson (Hod) and WinSeedle (Win) methods for acid and flame-delinted seed at Keiser and Rohwer, AR in 2007^z.

Variable 1	Variable 2	Acid-delinted seed by location		Flame-delinted by location	
		Keiser	Rohwer	Keiser	Rohwer
Seed index	Seed Volume	0.80	0.88	0.31	0.35
Seed index	Hodson SSA	0.61	0.69	0.75	0.79
Seed index	WinSeedle SSA	0.69	0.86	0.78	0.81
Seed Volume	Hodson SSA	0.58	0.70	0.48	0.14
Seed Volume	WinSeedle SSA	0.65	0.79	0.46	0.15
Hodson SSA	WinSeedle SSA	0.76	0.79	0.86	0.88
Fuzzy seed index	Seed index	0.87	0.96	0.94	0.98
Fuzzy seed index	Seed volume	0.71	0.88	0.37	0.38
Fuzzy seed index	Hodson SSA	0.67	0.66	0.76	0.79
Fuzzy seed index	WinSeedle SSA	0.75	0.88	0.74	0.82

^z Correlation coefficients greater than 0.28 and 0.35 differ significantly ($P = 0.05$) from zero at Keiser and Rohwer, respectively.

Although the two SSA estimation methods utilized different formulas to calculate SSA, the correlation coefficients between the two methods indicated a good relationship between the two (Table 7). These correlations suggest that either method would be acceptable. Practically, time requirements between the two methods were similar, but the WinSeedle method was not dependant on human measurement or data recording and should be less subject to error.

As found by Bourland and Bird (1983), seed index and volume of acid-delinted seed were highly correlated (Table 7). Similarly seed index and volume were highly correlated with both SSA measurements on acid-delinted seed. These relationships have been the underlying assumptions for using fuzzy seed index to estimate seed volume and SSA in calculations of yield components by University of Arkansas Cotton Breeding Program. The strong correlation of fuzzy seed index with acid- and flame-seed index suggests that delinting of seed is not necessary to obtain a useable seed index. Thus, these findings confirm the use of fuzzy seed index to estimate seed volume and SSA.

Fuzzy seed index explained more variation in WinSeedle SSA than any other parameter (Table 2). This would further increase the confidence in using fuzzy seed index in calculations of yield components. The regression equation could then be used to estimate WinSeedle SSA using fuzzy seed index. Flame seed index, which requires the second least time to measure, might also be used. Volume of flame-delinted seed explained the least variation in WinSeedle SSA and would be the least acceptable method.

CONCLUSION

The challenge of incorporating SSA into an index has always been the time and consistency of the SSA estimate. These data provided similar trends across genotypes for seed index, Hodson SSA, WinSeedle SSA and volume, irrespective of the delinting method. Data associated with the acid-delinting method exhibited the lowest values due to a lack of lint or char and were assumed to be more precise. However this delinting method was the most time consuming method and was only considered superior for WinSeedle SSA estimates. The WinSeedle SSA estimate was considered the most precise, but showed no time advantage when compared to other methods. The fuzzy seed index method was more closely correlated to the WinSeedle SSA method than any of the other eight seed parameters tested.

The wide array of phenotypic values across the selected commercially acceptable genotypes, coupled with the two distinctly different growing environments should allow for adoption of these findings across multiple cotton breeding programs. Continued advancements in technology will hopefully lead to an efficient direct measurement of SSA. Until then the fuzzy seed index method provides a satisfactory alternative method.

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