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ENGINEERING AND GINNING

Seed Hull Fracture Resistance of Upland and Pima Cotton Cultivars

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ABSTRACT

Seed durability is a current issue of cotton ginners, who have noted smaller and weaker seed, lower seed grades, and increased seed coat fragments within ginned cotton fiber. To better understand the differences in the seed hull properties of Upland (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L) seed, compression testing was conducted to determine the seed hull fracture resistance of both species. Plants were grown in Five Points, CA, U.S., for two years. After roller ginning, seed were conditioned to standard environmental conditions and were compressed until rupture on a material strength tester. Seed of the Pima cultivars generally required greater maximum compressive force and energy to rupture than did the seed of the Upland cultivars. However, when the seed were compressed in a vertical orientation, a few individual Upland cultivars did have compression properties within the range of values observed for the Pima cultivars. Hence, it is possible to find Upland seed with compression properties comparable to or slightly greater than those of some Pima seed. Differences in the data for the two years showed that growing environment affects seed hull strength properties. The results should help clarify some conflicting literature regarding the relative strength attributes of *Gossypium* species seed.

Cottonseed from Pima cultivars (*Gossypium barbadense* L.) are typically cracked prior to feeding dairy cows, whereas cottonseed from Upland cultivars (*G. hirsutum* L.) are often fed whole. Coppock et al. (1985) fed dairy cows fuzzy and acid-delinted Upland seed and showed that the large numbers of kernels from the latter group passed through the animals undigested. Sullivan et al. (1993b) expanded this work with different forms of Pima cottonseed and showed that larger numbers of whole Pima seed, which largely lack linters, also pass through the cows undigested, compared with either whole Upland, cracked Pima, or milled Pima seed. These reports suggest that Pima seed needed to be cracked because of the absence of linters, which allows the seed to pass through the rumen more quickly, resulting in less regurgitation and re-mastication of the tissue, and more intact seed passing through the digestion system. In a second study, Sullivan et al. (1993a) also concluded that Pima seed were weaker (i.e. required less force to break) than Upland seed based on measurement of their seed samples with a hand-held strain gauge. This further supported the conclusion that it was differences in rumen hold-up time that led to the need to crack Pima seed prior to feeding, it could not then be due to the animals having more difficulty masticating the Pima seed.

While reduced retention in the rumen is certainly a component of the need to crack Pima seed, the conclusion that Upland seed were harder or stronger than Pima seed has always raised some questions. This research group has processed many Upland and Pima seed samples for oil extraction, gossypol analysis, or other purposes, and has frequently observed that the Pima seed samples often require more power or longer milling times to crack, dehull, or mill than do Upland seed samples.

A recent study on hull compressive strength illustrates the point. Because of ginner's concerns over the seed of commercial varieties becoming smaller and weaker, a study was undertaken to determine the factors affecting the measurement of seed hull fracture resistance, and the range of resistance among commercial seed samples (Dowd et al., 2019). As part of this study, seeds of 22 cotton cultivars were tested for hull fracture resistance with an Instron material tester fitted with an adapter to allow the seed to be compressed to failure. Included among these samples was one Pima cultivar (DP340) that when analyzed showed among the greatest maximum average rupture force when the seed was compressed vertically, and the maximum average rupture force observed when

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the seed was compressed horizontally. Because the samples used in the prior report were grown in different locations and years, the differences in this dataset could not be solely attributed to genetics or species. Hence, while it suggests that at least some Pima seed may be 'stronger' than Upland seed, especially when force was applied to the radial side of the seed (e.g. as one could expect would be the most likely orientation of seed being chewed by a cow), the study only included a single Pima seed sample.

To try to get a better resolution of this issue and improve our understanding of the differences in the hull strength of cottonseed, and how these differences may correlate with seed quality and processing practices, seed samples were collected and tested for fracture resistance from recently grown Upland and Pima cotton cultivars. To eliminate the complication of environment, all seed were collected from plants grown in a single field location (Five Points, CA). Samples were collected and tested for two harvest years.

MATERIALS AND METHODS

Samples. The collected seed cultivars were all from the Western Regional component of the National Cotton Variety Test or the Regional Breeders Trial Network from 2017 and 2018. In 2017, single pooled samples of eight Upland and seven Pima cultivars were available. In 2018, duplicate plot samples were collected from 12 Upland and eight Pima cultivars. All samples were allowed to equilibrate in an environmentally controlled testing laboratory at 65 ± 2% relative humidity and 21°C (70 ± 1 °F) temperature for three weeks or more prior to mechanical testing, as has been found necessary in prior work (Dowd et al., 2019).

Compression Testing. After acclimatizing to the laboratory conditions, seed were compressed in both vertical and horizontal orientations. Testing was conducted with a Universal Testing Machine (Instron model 5567, Norwood, Mass.) operated with a 1 kN load cell and fitted with an adapter to translate the tensile force into a compressive force. The adapter arms each held an aluminum cylinder with the mating ends having a single shallow dimple to help support the seed. Seed were positioned either parallel to their long axis (vertically) or perpendicular to their long axis (horizontally). The crosshead speed was set at 5 mm/min, a value within the range used in prior seed studies and recommended by American Society for Testing Materials (ASTM) standards for lower tensile strength materials (ASTM, 2018). Samples were subjected to a pre-load force of 10 N at the start of the compression cycle and were then slowly compressed until the seed hull cracked. The instrument's Bluehill 3 software (Instron, Norwood, Mass.) was used to collect the load-compression (i.e., force-distance) data at 0.01 sec intervals.

Twenty-five well-formed undamaged seed were tested for each seed sample. The duplicate field samples available in 2018 were analyzed individually and compared. Only minor differences were observed among the pair of estimated means, and t-tests did not result in significant differences. Hence, the duplicate field lots of each variety were pooled, in effect resulting in 50 individual seed measurements for each cultivar.

Calculations and Statistics. The energy needed to break the seed was calculated by integration of the force-distance data. This area under the curve was calculated numerically using Microsoft Excel. t-Tests were used to determine if there were significant differences between the species. To compare the mechanical properties of the cultivars within each species, ANOVA analysis was run with the Tukey's multiple mean comparison test ($\alpha = 0.05$). Analyses were conducted for each year and on the combined dataset.

Box-Whisker plots were prepared with Grapher (Golden Software, Golden, CO) and the figures were annotated with Adobe Illustrator. Box-Whisker plots allow the data to be represented in quartiles. Hence, the 'whiskers' are not standard deviations of the pooled data but represent the full range of each plotted property.

RESULTS

The compression results for the Upland seed were within the range of results previously reported for a group of Upland cultivars (Dowd et. al., 2019). Vertically compressed Upland cultivars fractured with a range of maximum loads that varied between 69 and 98 N in 2017. In 2018, this range shifted to higher values between 82 and 104 N. In comparison, the Pima varieties compressed vertically over a narrower range but generally greater maximum load values between 95 and 106 N in 2017 and an essentially identical range between 95 to 108 N in 2018. Hence, there appears to be modest overlap of the ranges of the Upland and Pima varieties when compressed vertically.

When compressed horizontally, less force was needed to rupture the seed. The range of maximum compressive load varied between 44 and 62 N for the 2017 Upland cultivars and between 47 and 69 N for the 2018 Upland cultivars. Again, the maximum compression loads for the Pima seed varied over a narrower range but with greater values between 68 and 79 N in 2017 and between 73 and 80 N in 2018. In this orientation, no overlap in the maximum compressive load ranges was observed in either year (Figs. 1 and 2).

t-Tests indicated significant differences in the maximum compression loads for the two species, regardless of the test orientation, or the year, with the Pima cultivars showing greater fracture resistance than the Upland cultivars. The same result was found if the data was pooled over the two years and were tested as a single set. ANOVA testing showed substantial differences among the maximum compressive loads of the Upland varieties, but much less discrimination between the Pima varieties (Tables 1 and 2).

For cottonseed, the elongation to break and the seed rupture energy have previously been reported to correlate significantly with the maximum compression loads, i.e., samples with greater compressive loads tend to also have greater elongation and correspondingly greater rupture energy (Dowd et al., 2019). These correlations were found among individual seed from a single cultivar as well as for the mean values from different cultivars.

Similar trends and t-test differences were observed for both compressive properties. As for the maximum load needed to rupture the seed, t-tests between the maximum extension and the rupture energy were significantly different for the two species, with the Pima seed exhibiting a greater amount of extension and requiring more energy to rupture compared to the Upland seed. The sole exception to this was for the maximum extension of the vertically compressed Upland and Pima cultivars in 2017 (Fig. 1), where no significant difference was indicated by the testing.

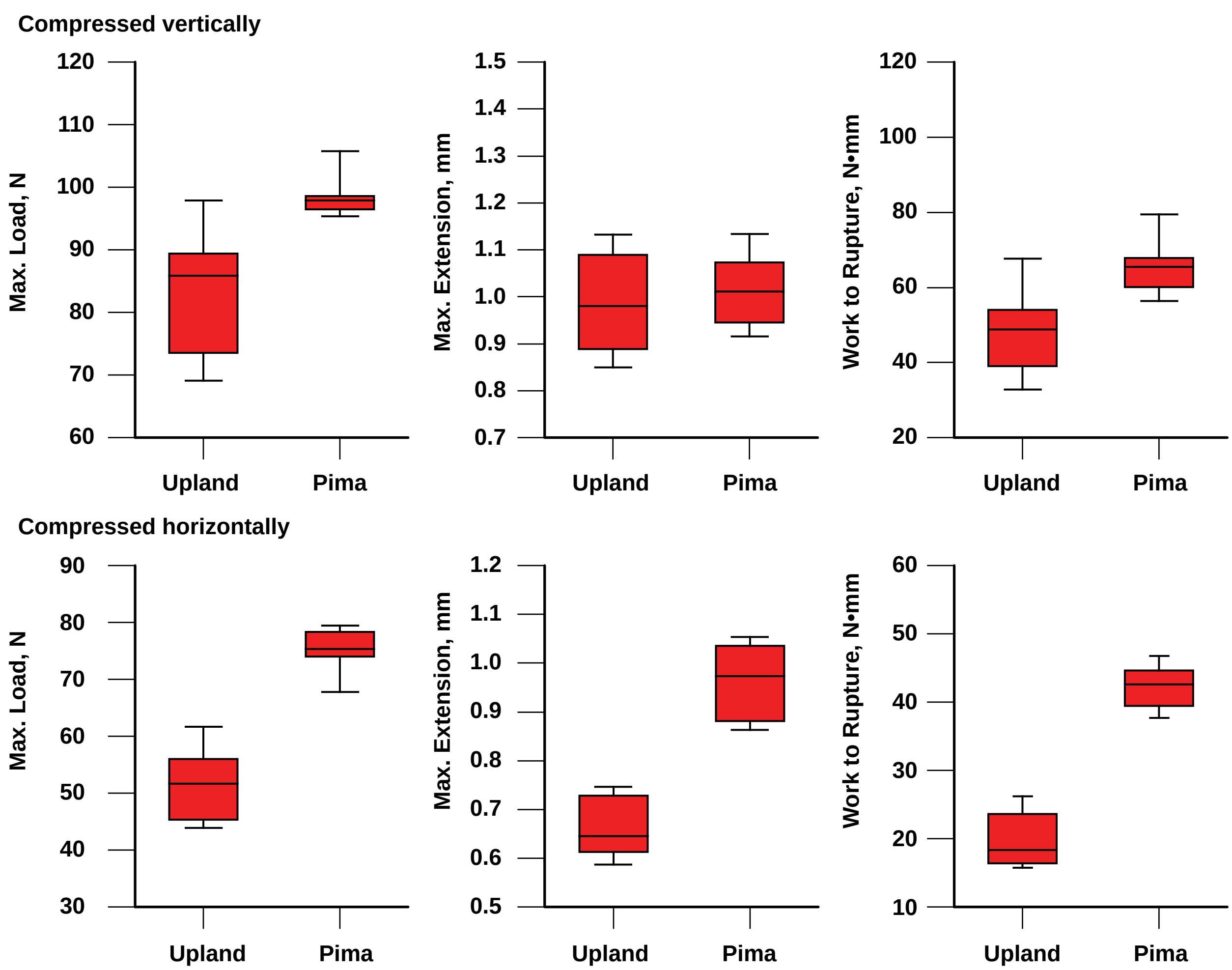

Figure 1. Comparison of the mechanical testing properties of commercial Upland and Pima cottonseed grown in Five Points, CA in 2017.

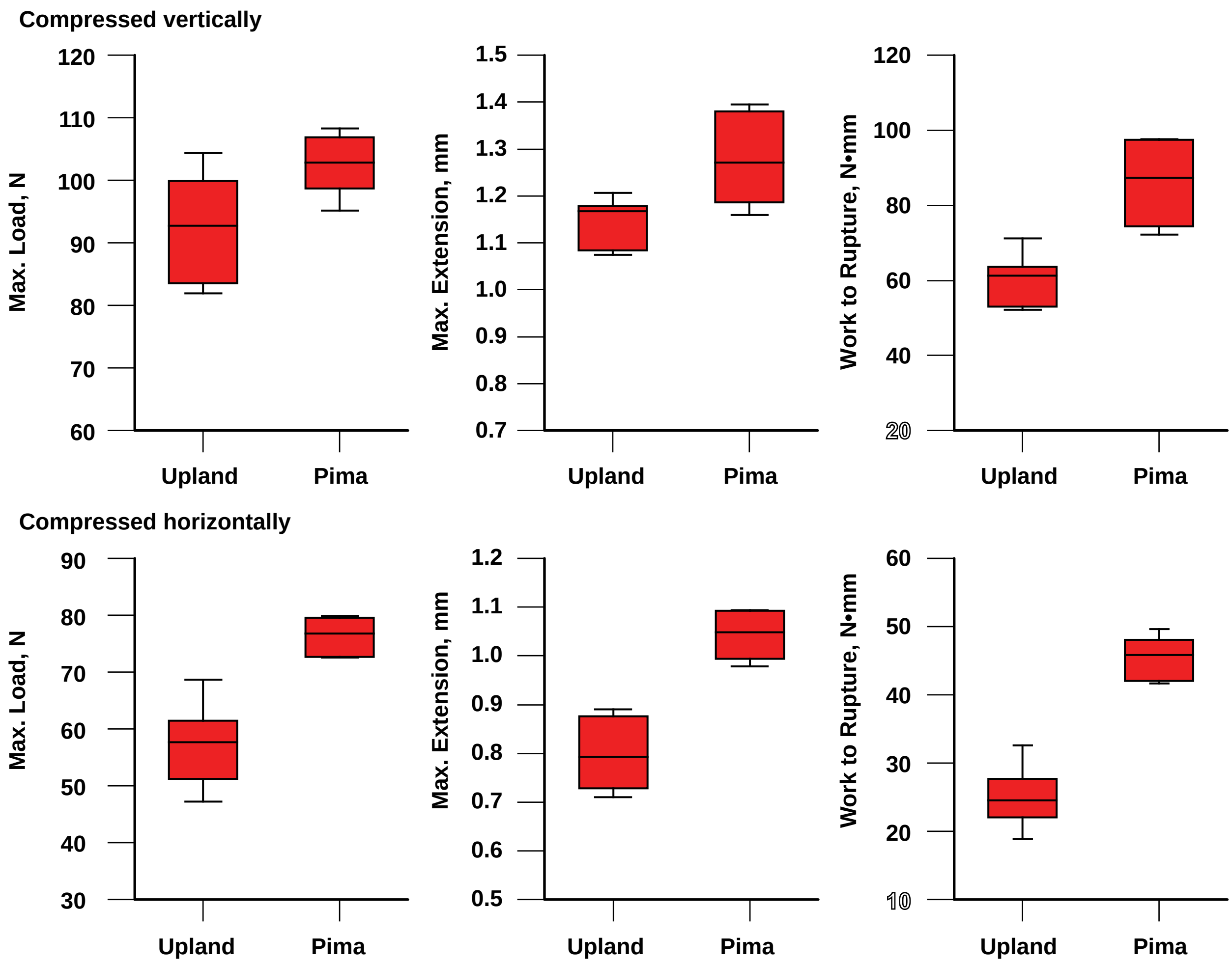

Figure 2. Comparison of the mechanical testing properties of commercial Upland and Pima cottonseed grown in Five Points, CA in 2018.

Table 1. Compressive seed properties of Upland and Pima cotton varieties produced in Five Points, CA in 2017.

Cultivar	Compressive Properties at Seed Rupture[Z]		
	Max. load (N)	Distance (mm)	Energy (N·mm)
		Compressed Vertically	
Upland Cultivars			
DaytonaRF	69.1 ± 11.3 D	0.889 ± 0.231 B	32.8 ± 11.4 D
DG2355B2XF	97.9 ± 8.1 A	1.132 ± 0.378 A	67.6 ± 32.5 A
DP1549B2XF	85.0 ± 6.6 B	0.952 ± 0.156 AB	48.4 ± 13.4 BC
DP1646B2XF	86.8 ± 13.1 B	1.024 ± 0.124 AB	49.9 ± 10.6BC
NG4545B2XF	73.6 ± 17.6 CD	0.850 ± 0.208 B	39.0 ± 15.2 CD
PHY499WRF	89.4 ± 9.4 AB	1.090 ± 0.158 A	54.1 ± 11.7 AB
PHY725RF	83.2 ± 10.6 BC	0.970 ± 0.223 AB	46.4 ± 14.6 BCD
PHY764WRF	86.7 ± 12.4 B	0.991 ± 0.209 AB	49.4 ± 14.2 BC
Pima cultivars			
DP348RF	97.9 ± 16.5	0.949 ± 0.256	60.3 ± 24.6
DP358RF	97.1 ± 13.7	0.916 ± 0.271	60.2 ± 26.3
PHY802RF	98.1 ± 19.0	1.041 ± 0.342	65.4 ± 28.8
PHY805RF	95.4 ± 18.5	1.073 ± 0.306	67.8 ± 29.6
PHY841RF	96.5 ± 13.5	0.954 ± 0.207	56.4 ± 15.8

Table 1. Continued

Cultivar	Compressive Properties at Seed Rupture^Z		
	Max. load (N)	Distance (mm)	Energy (N·mm)
PHY881RF	98.6 ± 17.3	1.011 ± 0.306	65.5 ± 29.5
PHY888RF	105.8 ± 10.4	1.134 ± 0.365	79.4 ± 34.6
		Compressed Horizontally	
Upland Cultivars			
DaytonaRF	44.0 ± 13.8 B	0.652 ± 0.224	15.8 ± 9.2 B
DG2355B2XF	61.7 ± 12.1 A	0.729 ± 0.183	26.2 ± 10.4 A
DP1549B2XF	54.5 ± 14.5 AB	0.638 ± 0.174	19.6 ± 8.8 AB
DP1646B2XF	48.8 ± 12.5 B	0.587 ± 0.154	17.0 ± 8.4 B
NG4545B2XF	45.4 ± 15.0 B	0.613 ± 0.224	16.4 ± 9.8 B
PHY499WRF	54.7 ± 13.5 AB	0.746 ± 0.190	23.6 ± 9.9 AB
PHY725RF	46.7 ± 15.0 B	0.619 ± 0.202	17.1 ± 9.2 B
PHY764WRF	56.0 ± 14.9 AB	0.703 ± 0.223	21.9 ± 12.6 AB
Pima cultivars			
DP348RF	78.4 ± 24.9	1.036 ± 0.333	46.8 ± 24.2
DP358RF	79.4 ± 16.1	0.863 ± 0.256	39.5 ± 16.3
PHY802RF	67.8 ± 22.0	0.901 ± 0.290	37.7 ± 20.4
PHY805RF	74.9 ± 17.1	0.974 ± 0.205	43.5 ± 15.8
PHY841RF	75.4 ± 24.0	1.104 ± 0.326	44.7 ± 23.4
PHY881RF	74.0 ± 18.4	1.054 ± 0.268	42.6 ± 16.0
PHY888RF	77.3 ± 23.4	0.882 ± 0.293	41.7 ± 21.7

[z] Difference letters within a series and orientation indicate a significant difference based on the Tukey multiple mean comparison test ($\alpha = 0.5$). The lack of letters indicates that no significant differences were detected.

Table 2. Compressive seed properties of Upland and Pima cotton varieties produced in Five Points, CA in 2018.

Cultivar	Compressive Properties at Seed Rupture^Z		
	Max. load (N)	Distance (mm)	Energy (N·mm)
		Compressed Vertically	
Upland Cultivars			
DaytonaRF	85.2 ± 13.6 DE	1.090 ± 0.201	51.7 ± 17.3 C
DG2355B2XF	99.9 ± 10.9 AB	1.165 ± 0.255	63.9 ± 19.1 ABC
DP1549B2XF	83.6 ± 10.4 E	1.074 ± 0.317	53.1 ± 23.0 C
DP1646B2XF	94.5 ± 7.4 BC	1.175 ± 0.221	62.5 ± 18.6 ABC
DP1845B3XF	83.3 ± 9.6 E	1.172 ± 0.174	57.0 ± 15.3 BC
FM1830GLT	94.5 ± 14.3 BC	1.170 ± 0.199	61.8 ± 18.4 ABC
FM2334GLT	91.4 ± 13.9 CD	1.084 ± 0.192	56.3 ± 15.4 BC
FM2498GLT	104.3 ± 8.8 A	1.198 ± 0.247	71.3 ± 22.3 A
NG4545B2XF	82.0 ± 12.3 E	1.080 ± 0.231	53.0 ± 18.1 C
PHY499WRF	92.2 ± 9.8 CD	1.179 ± 0.268	63.3 ± 22.8 BC
PHY764WRF	93.3 ± 15.7 BC	1.160 ± 0.278	60.8 ± 26.5 ABC
STV5122GT	102.3 ± 11.3 A	1.207 ± 0.239	69.2 ± 19.3 AB
Pima Cultivars			
DP341RF	102.2 ± 18.6 AB	1.280 ± 0.348 AB	89.9 ± 40.3 AB
DP348RF	95.1 ± 15.7 B	1.186 ± 0.332 AB	74.5 ± 32.3 AB
DP358RF	108.3 ± 14.0 A	1.395 ± 0.390 A	97.4 ± 42.8 A
PHY802RF	100.7 ± 16.1 AB	1.263 ± 0.369 AB	83.4 ± 34.7 AB
PHY805RF	103.5 ± 22.1 AB	1.353 ± 0.429 AB	96.4 ± 49.6 AB

Table 2. Continued

Cultivar	Compressive Properties at Seed Rupture[z]		
	Max. load (N)	Distance (mm)	Energy (N·mm)
PHY841RF	98.7 ± 17.5 B	1.160 ± 0.316 B	72.2 ± 33.9 B
PHY881RF	104.8 ± 14.3 AB	1.380 ± 0.395 AB	97.6 ± 41.5 A
PHY888RF	106.8 ± 19.3 A	1.260 ± 0.421 AB	84.6 ± 45.9 AB
		Compressed Horizontally	
Upland Cultivars			
DaytonaRF	57.8 ± 17.2 BCD	0.728 ± 0.211 CD	23.2 ± 13.4 BCD
DG2355B2XF	65.3 ± 14.9 AB	0.783 ± 0.200 ABCD	27.7 ± 11.8 ABC
DP1549B2XF	61.5 ± 15.1 ABC	0.876 ± 0.216 AB	29.9 ± 13.7 AB
DP1646B2XF	47.7 ± 14.2 E	0.740 ± 0.194 BCD	18.9 ± 9.4 D
DP1845B3XF	54.4 ± 12.4 CDE	0.827 ± 0.232 ABCD	25.0 ± 11.2 BCD
FM1830GLT	51.3 ±13.5 DE	0.862 ± 0.200 ABC	23.2 ± 9.1 BCD
FM2334GLT	54.5 ± 14.9 CDE	0.880 ± 0.237 A	26.1 ± 13.6 ABCD
FM2498GLT	60.5 ± 15.4 ABCD	0.793 ± 0.171 ABCD	26.1 ± 10.4 ABCD
NG4545B2XF	47.2 ± 14.4 E	0.724 ± 0.254 D	19.3 ± 12.0 D
PHY499WRF	68.7 ± 12.3 A	0.890 ± 0.244 A	32.6 ± 13.2 A
PHY764WRF	57.6 ± 13.4 BCD	0.794 ± 0.164 ABCD	24.1 ± 10.1 BCD
STV5122GT	58.6 ± 14.4 BCD	0.711 ± 0.176 CD	22.1 ±9.5 CD
Pima Cultivars			
DP341RF	72.7 ± 19.5	1.093 ± 0.291	49.6 ± 21.0
DP348RF	79.9 ± 20.9	1.044 ± 0.274	46.7 ± 18.8
DP358RF	79.6 ± 19.3	1.061 ± 0.211	47.5 ± 18.4
PHY802RF	78.9 ± 17.9	1.053 ± 0.267	48.1 ± 18.2
PHY805RF	79.2 ± 19.5	1.094 ± 0.282	49.6 ± 20.4
PHY841RF	72.6 ± 21.2	0.999 ± 0.252	41.7 ± 18.5
PHY881RF	74.8 ± 19.1	0.994 ± 0.305	44.4 ± 21.0
PHY888RF	73.5 ± 19.3	0.979 ± 0.259	42.0 ± 20.9

[z] **Difference letters within a series and orientation indicate a significant difference based on the Tukey multiple mean comparison test ($\alpha = 0.5$). The lack of letters indicates that no significant differences were detected.**

ANOVA Tukey testing indicated significant differences existed among the tested Upland cultivars but fewer differences among the Pima cultivars. No significant differences were detected for any of the three compressive properties for the 2017 Pima cultivars regardless of the testing orientation. In 2018, the same result was found when the Pima cultivars were tested horizontally. When tested vertically, however, the Tukey method suggested the tested varieties fell into two classes.

DISCUSSION

Although some overlap was apparent in the ranges of the maximum loads for the vertically cracked Upland and Pima cultivars, i.e., one Upland cultivar (DG 2355 B2RF) was within the range of the Pima cultivars in 2017 and three Upland cultivars were within the Pima range in 2018. Statistical testing indicated that as a species, Pima seed generally required greater force to crack than Upland seed. In contrast, in the weaker horizontal dimension, there was no range overlap observed, i.e., all Pima cultivars tested required more force to crack the seed than did any of the Upland cultivars tested. This result is different from that reported by Sullivan et al (1993a), who tested seed with a hand strain gauge in a horizontal orientation. A couple of factors may have contributed to this difference. First, there was no discussion in this early report of whether the samples were conditioned prior to testing. Because seed moisture (and therefore indirectly atmospheric humidity) significantly impacts the mechanical properties of cottonseed (Kirk and McCleod, 1967; Dowd et al., 2019), bias may have occurred if the seed were not conditioned to the same environmental conditions prior to testing. Second, only single Pima and Upland seed samples were evaluated by Sullivan, and the identities and histories of these seed samples were not discussed. Because growing environment likely influences seed hull properties (Dowd et al., 2019), differences in the two seed's histories may have affected the results. However, while the range of horizontal maximum load values for the two species were not found to overlap in this work or in our prior report (Dowd, et al., 2019), it is always possible that some Upland cultivars could exist that have greater rupture resistance than the commercial cultivars tested in this work. Although exceptions may exist, Pima seed generally have greater fracture resistance than Upland seed, and hull strength cannot be ruled out as a contributing factor in the need to crack Pima seed before use as a dairy feed ingredient.

In many respects, the data from the seed of the two harvested years were similar; however, some differences were apparent. Some Upland cultivars included in both years exhibited greater compressive properties in the second year of the study, while others tested similar in both years. For instance, the average maximum load for the vertically compressed Upland cultivars was 92 N in 2018 compared with 84 N in 2017, and when compressed horizontally, the average maximum load was 57 N in 2018 compared with 51 N in 2017. The yearly differences in maximum loads were more modest for the Pima cultivars. The annual differences were most apparent in the maximum compression distance. For example, the average of the compressive distance for the vertically compressed Upland cultivars was 0.987 mm in 2017 and 1.146 mm in 2018. For the Pima varieties cracked in the same orientation, the average varietal compression distance was 1.011 mm in 2017 and 1.295 mm in 2018. Although smaller in magnitude, similar trends were apparent in the compressive distance when the seed were cracked horizontally. The trends suggest that environmental differences can have an important influence on the seed's mechanical properties and that the environment differences may vary somewhat between species. This aspect of seed structure needs further study.

In summary, recent concerns by ginners and seed processors of weak and low-quality seed is a developing problem that may have multiple environmental and genetic causes. Mechanical compression testing of a series of current Pima and Upland cultivars indicates that Pima seed generally have greater fracture resistance than Upland seed in both the axial (vertical) and radial (horizontal) directions. If Upland seed continues to be difficult to process without significant damage, then it may require that seed hull strength be considered during cultivar development. Because moving traits between the Upland and Pima species of cotton is readily achieved, it may be that Pima germplasm can be used to breed Upland seed with stronger and more durable hulls.

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DISCLAIMER

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