COMMUN. SOIL SCI. PLANT ANAL., 30(19&20), 2801–2810 (1999)

Sample Size Influences Estimation of Cotton Dry Matter, Nitrogen Accumulation, and Lint Yield[1]

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ABSTRACT

Accurate estimates of cotton (*Gossypium hirsutum*) dry matter accumulation and nitrogen content are important for both production and environmental reasons. One of the important factors in estimate accuracy is sample size. The objective of this investigation was to determine the cotton sample size necessary for acceptable estimates of cotton dry matter, lint yield, and shoot N per 100 kg of lint ratio (NLR) values. Three cotton cultivars (DeltaPine 90, DeltaPine 5415, and Stoneville 474) were planted on 13 May 1997 in an Eunola loamy sand (fine-loamy, siliceous, thermic Aquic Hapludult) in 9.3-m^2 subplots of a split-split plot design. Split plots were four sampling dates. Split-split plots were four sampling techniques [a) four randomly selected plants (4RP), b) 0.3 meter of row (0.3-m), c) one meter of row (1-m), and d) two meters of row (2-m)]. Each entire subplot was harvested on each sampling

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date after sampling by the four techniques. Shoot dry matter for the whole plot was 7.2 Mg ha^{-1}, and lint yield was 1.46 Mg ha^{-1}. Cotton shoot dry matter and NLRs were significantly overestimated by both the 4RP and 0.3-m techniques, but not by the 1- and 2-m techniques. The NLRs of cultivar subplots varied with cultivar from 9.1 to 11.4. The earliest maturing cultivar, DeltaPine 90, had the lowest NLR and the latest maturing cultivar, Stoneville 474, had the highest NLR. Accurate estimates of cotton dry matter accumulation and N content will likely require 1-m samples, and 2-m samples should further improve precision. The NLRs were similar to data (NLR <15) that suggest 1.6 Mg ha^{-1} (3-bale/acre) cotton lint yields can be achieved with less than 250 kg ha^{-1} of shoot-accumulated N.

INTRODUCTION

Acquisition of reliable estimates of crop parameters requires a balance among the precision and accuracy required, resources available, and sample size. Common techniques of estimating crop parameters involve two basic techniques: 1) the random selection of several plants and multiplying by an estimated plant population per hectare or 2) selection of a random portion of row and dividing by the represented fraction of a hectare. When these techniques are employed, the resources necessary to acquire and analyze samples increase with the number of plants or length of row sampled. Thus, there is a preference for the smallest acceptable sample size. The smallest sample size for soybean (*Gylcine max* L.) dry matter was one meter of row (Hunt et al., 1987). For estimation of dry matter accumulated in 20-m^2 whole plots, we found that neither a 4-random-plant (4RP) nor a one-foot-of-row (0.3-m) technique produced acceptable precision or accuracy. Both of these techniques gave upwardly biased estimates with large variation. However, simply increasing the sample size to one meter of row (1-m) gave good precision and unbiased estimates. Additionally, neither precision nor accuracy was significantly improved by using a two-meter-of-row sample (2-m).

One of the historical crop parameters for soil and water management of cotton is the shoot N per 100 kg of lint ratio (NLR). It has been used for estimating the shoot N necessary to produce high yielding cotton. Early investigations of NLRs gave much needed insight into cotton growth, but nearly all of the studies were based on small samples. Fraps (1919), for instance, determined the NLRs varied from 15 to 79; he used only one representative plant per experiment station field. From these ratios, he recommended 25 as the most representative value. He also compared his mean ratio (32) to the earlier USDA publication on cotton growth that had a ratio 40% lower (McBryde and Beal, 1896). Later, Olson and Bledsoe (1942) conducted studies on three soils in Georgia with samples that ranged from 5 to 10 mature plants. Their NLRs for the three soils were 17, 29, and 42. However, the cotton on the soil with a NLR of 17 was the only one with a lint yield >0.54

Mg ha^{-1} (one bale/acre). Lower NLRs have been reported for most modern cotton production systems. Bassett et al. (1970) determined that high yielding, irrigated cotton in California had a NLR of 10; he used 12 selected plants from 10 treatments. Halevy (1976) in Israel found cotton with a lint yield of 1.70 Mg ha^{-1} to have a NLR of 13.5; he used 1 meter of row samples. Unruh and Silvertooth (1996a, 1996b) compared upland cotton and pima cotton (*G. barbadense* L.) under irrigation in the southwestern United States using one meter samples. Their data indicated NLR values of 15.1 and 20.7 for the upland and pima cotton, respectively. Hunt et al. (1998) found even lower NLRs (6.8 to 9.2) for cotton with a lint yield of 1.36 to 1.23 Mg ha^{-1} lint when it was watered and fertilized using buried microirrigation in South Carolina; we sampled 1 meter of row. Our nonirrigated cotton yielded 1.08 Mg ha^{-1} lint and had a NLR of 11.2. Modern cotton cultivars in Alabama were reported to have NLRs of 19.9 by Mullins and Burmester (1990), but they used samples as small as 0.3 m of row. In light of these findings over time and location, the objective of this investigation was to determine the cotton sample size necessary for acceptable estimates of cotton dry matter, lint yield, and NLR values.

MATERIALS AND METHODS

The experiment was conducted on an Eunola loamy sand (fine-loamy, siliceous, thermic Aquic Hapludult), which is typical of the eastern Coastal Plain. It was located on the Clemson University Pee Dee Research and Education Center near Florence, SC, at latitude 34°18', longitude 79°44', and an elevation of 37 m above sea level. It utilized a split-split plot design with cultivars arrayed as the whole plot treatments. Cotton cultivars (DeltaPine 90, DeltaPine 5415, and Stoneville 474) gave a range of maturity; Stoneville 474 had the earliest and DeltaPine 90 had the latest. Four split-plot treatments (four sampling dates) were 9.3 m^2 in area. The split-split plot treatments were four sampling techniques (Figure 1). They were a) four randomly selected plants (4RP), b) 0.3 meter of row (0.3-m), c) one meter of row (1-m), and d) two meters of row (2-m). Samples obtained by these techniques were compared to the entire subplot. Each of the subsamples was nested in the next largest sample. The entire subplot included the material from the four sampling techniques as well as the remainder of the subplot.

Fertilization consisted of 92 and 75 kg ha^{-1} of N and K, respectively. Nitrogen was applied as 34% N-ammonium nitrate. Pesticide applications followed standard practices. Field cultivation was used to supplement chemical weed control. Cotton was planted on 13 May 1997 at the rate of 13 seeds m^{-1} of row. Each subplot contained four rows that were 3.0 m long and 0.97 m apart. Cotton dry matter was sampled on 100, 114, and 129 days after planting, and seed cotton yields were taken 174 days after planting. The 0.3-m end of each row was excluded from sampling. On each sampling date, the four sampling techniques were used

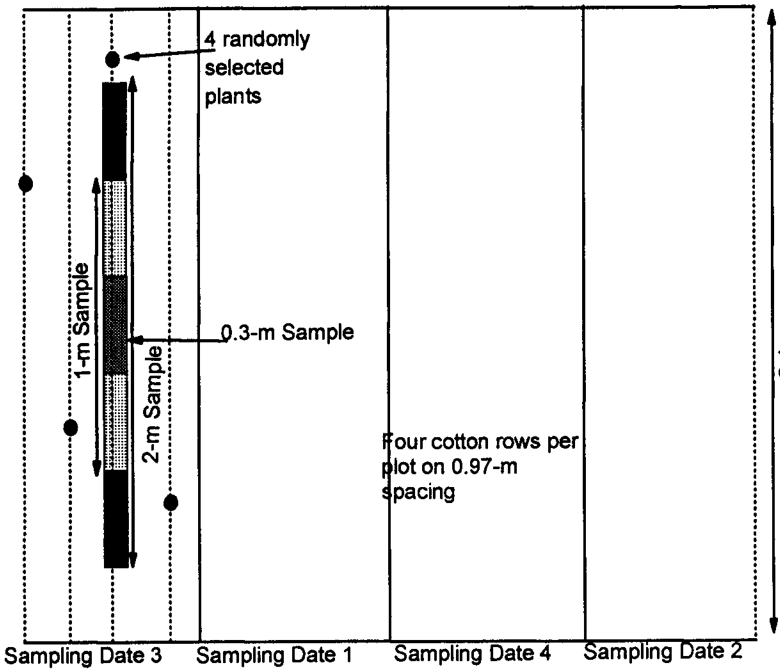

FIGURE 1. Schematic of subplot sampling techniques for one cultivar and one replication.

on the third row of each subplot. The remaining three rows were then harvested and combined with the third row to obtain the entire 9.3-m^2 subplot measure. A 2.4-m measuring rod was placed 0.3 m from the end of the row, and the 0.3-m sample was collected from the center of the 2.4-m measured row (Figure 1). The 1-m sample contained the 0.3-m sample plus 0.35 m on each side. Similarly, the 2-m sample contained the 1-m sample and 0.5 m on each side. This procedure was followed in each subplot. The four randomly selected plants were obtained in three of the rows by randomly placing a meter stick in each of the four rows in the subplot and harvesting the plant nearest to the 1-m mark. In the row used for spatial samples, the plant nearest the end of the 2.4-m rod was selected. Seed cotton was harvested manually with the same sampling techniques used for cotton dry matter. The cotton was not irrigated, but 556 mm of rain fell during the growing season. There were 158 frost-free days and 1,856 seasonally accumulated heat units.

Cotton dry matter samples and seed cotton samples were dried at 70°C for 48 h and measured for dry weight. Seed cotton from the 1-m sampling technique was

TABLE 1. Cotton shoot dry matter as estimated by four techniques.

Sampling technique	Shoot weight Mg ha^{-1}	CV† %	REMS†
4 Random plants	8.92	33	2.93
0.3-meter	8.08	28	2.25
1-meter	7.51	32	2.43
2-meter	7.58	25	1.86
Whole plot‡	7.17	18	1.30
$LSD_{0.05}$	0.90		

†CV=coefficient of variation, RMSE=root mean square error.

‡Whole plot was 9.3 m^2 of row (4 rows on 0.97-m spacing with 4.2-m length).

processed using a small saw gin to separate the seed and lint. After drying, the lint was weighed. The ginned cotton seed was acid-delinted, dried, and weighed. Cotton dry matter samples and delinted cotton seed samples from the 1-m sampling technique were separately analyzed for total N content using a LECO CN2000 Carbon/Nitrogen Analyzer (LECO Corp., St. Joseph, MI). It was assumed that the sampling technique would not alter the estimate of plant N, and the concentration of the 1-m sample was used for all samples. The N content in the cotton dry matter and delinted cotton seed for each sampling technique was calculated by multiplying shoot dry matter or delinted cotton seed weight by the percent N. The lint yield for each sampling technique was calculated by multiplying the seed cotton yield by the lint/seed cotton ratio.

Data were analyzed using SAS (1990). Sample means were calculated by the Means procedure; coefficient of variation (CV), root of mean square error (RMSE), and least significant difference (LSD) were calculated by the General Linear Model procedure. Only the date by technique interaction was significant for dry matter accumulation, and none of the interactions for accumulated N were significant. Therefore, data were pooled across cultivar and sampling date for discussion of dry matter and N accumulation. Since we were not only interested in the precision of measure, we measured for inherent over- or under-estimation; i.e., bias. Estimate of bias, standard error of bias, t-test, and sign test were calculated by the Univariate procedure. Sampling techniques were compared in pair-wise comparisons.

RESULTS AND DISCUSSION

As Hunt et al. (1987) found with soybean, cotton shoot dry matter was overestimated by both the 4RP and the 0.3-m techniques (Table 1). These two

TABLE 2. Bias of cotton shoot dry matter estimated by four sampling techniques.†

	4RP vs. WP	0.3-m vs. WP	1-m vs. WP	2-m vs. WP
Estimate of bias (Mg ha^{-1})	1.75	0.91	0.34	0.41
S.E. of bias	0.56	0.58	0.42	0.29
P-value, t-yest	0.01	0.08	0.38	0.11
P-value, sign test	0.01	0.13	0.68	0.19

†4RP=4 random plants; WP=whole 9.3 m^2 plot; 0.3-m=0.3 m of row; 1-m=1 m of row; 2-m=2 m of row.

techniques gave shoot dry matter estimates that were $\geq$13% higher ($LSD_{0.05}$) than the whole plot mean of 7.2 Mg ha^{-1}. The problems of the 4RP and 0.3-m techniques were even more clearly seen in the estimates of bias, which were 1.75 and 0.91 Mg ha^{-1}, respectively (Table 2). Further, when compared by the t-test and the sign test, data obtained with the 4RP technique were highly significantly different from those of the whole plot ($P \geq 0.01$), and the 0.3-m technique was marginally significantly different ($P \geq 0.08$).

Again, as with soybean, the whole plot shoot dry matter of cotton was not significantly overestimated ($LSD_{0.05}$) by either the 1-m or 2-m technique, which both estimated 7.5 Mg ha^{-1}. Their estimates of bias were <0.41 Mg ha^{-1}, which was less than half those of the 4RP and the 0.3-m techniques. When compared by the t-test and the sign test, neither of these techniques was significantly different from the whole plot ($P \geq 0.10$). This was true even though the 2-m technique had a lower RMSE and the associated greater ability to detect differences. The 2-m technique was the most precise sampling technique; its CV and the RMSE values were 25% and 1.86, respectively. These values compared well to the CV and the RMSE values of the whole plot, which were 18% and 1.30, respectively. Nonetheless, data from this study showed cotton dry matter accumulation to be more variable than found by Hunt et al. (1987) in a similar study of soybean. The dual problems of bias and variation signify that at least a 1-m sample is needed to eliminate sampling bias, and a 2-m sample may be needed for precision in the sampling of cotton shoot dry matter.

The 1-m and 2-m samples were also adequate for estimation of accumulated shoot N (Table 3). This was true even though these estimates were more variable and had less precision than dry matter because of variation in N content among cultivars and sampling dates. Shoot N estimates by the 1-m and the 2-m techniques vs. the whole plot were >153 vs. 144 kg N ha^{-1}, respectively. As with the dry matter, estimates of shoot N by the 4RP and the 0.3-m techniques were not adequate. They were sufficiently large (>170 kg N ha^{-1}) to be significantly different from those of the whole plot by the LSD test at the 0.05 level.

TABLE 3. Nitrogen accumulated in cotton shoots as estimated by four techniques.

Sampling technique	Cotton nitrogen kg ha^{-1}	CV† %	REMS†
4 Random plants	170	33	56
0.3-meter	172	29	51
1-meter	155	42	64
2-meter	153	33	50
Whole plot‡	144	25	36
$LSD_{0.05}$	22		

†CV=coefficient of variation, REMS=root error mean square.
‡Whole plot was 9.3 m^2 of row.

These shoot dry matter and N values can be considered typical of a good growing year in the eastern Coastal Plain; the lint yield of the three cultivars in their 9.3-m subplots ranged from 1.35 to 1.57 Mg ha^{-1} (Table 4). The cultivars ranged in earliness from ST 474 to DP 90. The latest, DP 90, yielded the least. Cotton lint yield was overestimated by the 4RP technique and underestimated by the 1-m technique (Table 5). There were additional problems of high variability for the smaller samples. The CV values were very large (>49%) for the 4RP and the 0.3-m techniques. The CV for the whole plot was 10%, and CVs for the 1-m and 2-m techniques were <18%. Thus, the lint yield data also indicated that a 2-m sample was desirable.

TABLE 4. Cotton lint yield and NLRs for three cultivars as measured in 9.3-m^2 subplots.

Cultivar†	Yield Mg ha^{-1}‡	NLR§
DP 90	1.35	9.1
DP 5415	1.57	10.0
ST 474	1.46	11.4
Mean	1.46	10.1
$LSD_{0.05}$	0.12	2.2

†From earliest to latest maturity range, the cultivars were ST 474, DP 5415, and DP 90, respectively.
‡Bale/acre=0.54 Mg ha^{-1}.
§NLR=nitrogen per 100 kg of lint ratio.

TABLE 5. Cotton lint yield for the whole plot and estimated from subsamples.

Sampling technique	Lint yield Mg ha^{-1}	CV† %	REMS†
4 Random plants	1.95	59	1.14
0.3-meter	1.47	49	0.72
1-meter	1.30	16	0.20
2-meter	1.47	18	0.26
Whole plot‡	1.46	10	0.15
$LSD_{0.05}$	0.12		

†CV=coefficient of variation, REMS=root error mean square.
‡Whole plot was 9.3 m^2 of row.

For the comparison of shoot-accumulated N to yield, we used the whole plot yields to determine the NLR values because of their lower variation. We also used only the later two sampling dates for shoot N because the shoots in the earliest date had not reached the maximum N accumulation level. The NLRs decreased with earliness from ST 474 with a value of 11.4 to DP 90 with a value of 9.1. The whole plot NLR mean was 10.1 (Table 6). It had a CV of 21% and a RMSE of 2.09. The 4RP technique gave a highly significant overestimate of the whole plot ratio (12.0), and the 0.3-m technique was marginally different with an estimate of 11.8. The estimate of bias for NLR by the 4RP and the 0.3-m samples was nearly three times greater than the 1- and 2-m techniques >1.73 vs. <0.64 (Table 7). The NLR of the 1- and 2-m techniques (10.7) was not significantly

TABLE 6. Ratio of shoot nitrogen to 100 kg of cotton lint.

Sampling technique	NLR†	CV† %	REMS†
4 Random plants	12.0	29	3.53
0.3-meter	11.8	28	3.25
1-meter	10.7	36	3.87
2-meter	10.7	28	2.94
Whole plot‡	10.1	21	2.09
$LSD_{0.05}$	1.6		

†NLR=shoot N per 100 kg of cotton lint, CV=coefficient of variation, REMS=root error mean square.
‡Whole plot was 9.3 m^2 of row.

TABLE 7. Bias of cotton shoot nitrogen to lint ratios estimated by four sampling techniques.

Statistic	4RP vs. WP	0.3-m vs. WP	1-m vs. WP	2-m vs. WP
Estimate of bias	1.98	1.73	0.64	0.62
S.E. of bias	0.48	0.61	0.50	0.32
P-value, t-test	0.01	0.07	0.41	0.22
P-value, sign test	0.01	0.08	0.63	0.29

different from the whole plot. As in the estimates of shoot dry matter, a 1-m sample would seem to be necessary, and a 2-m sample would likely be desirable to reduce both the bias and the variation.

These NLR values, although lower than those reported by Olson and Bledsoe (1942) and Mullins and Burmester (1990), are similar to data (NLR <15) that suggest 3-bale cotton can be achieved with less than 250 kg ha^{-1} of shoot-accumulated N (Bassett et al., 1970; Unruh and Silvertooth, 1996a, 1996b; Hunt et al., 1998). Further, our whole plot values have relatively low CVs, are from high yielding cotton, and reflect a good conversion of shoot dry matter into lint.

CONCLUSIONS

1. At least 1 m of row sample was needed to minimize sample bias from estimates of cotton dry matter, lint, and NLR values.
2. A 2-m sample may be needed for improved precision in the estimation of differences ($P \geq 0.05$) for these parameters.
3. The low NLRs (<15) of this investigation and other investigations that used at least a 1-m sample indicate that high lint yields in cotton often require less that 250 kg ha^{-1} of shoot accumulated N.

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