Comparative early growth of cotton seedlings expressing a visible true leaf at emergence and normal phenotype seedlings[1, 2]

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ABSTRACT

At emergence, a cotton seedling of a normal phenotype has a poorly developed first true leaf and a photosynthetic area essentially limited to the cotyledons. After emergence, the expansion of the first true leaf and the vegetative development is slow, lengthening the seedling stage, thus increasing vulnerability to biotic and abiotic stresses. Plants of several breeding lines were found to express a visible first true leaf at emergence (VTLE), which may be associated with rapid growth through the seedling stage. The objectives were to compare early growth of seedlings expressing a VTLE with that of seedlings of a normal phenotype, and to compare their growth when cotyledons were removed at different times after emergence. In the first test, plants were rogued for either a VTLE or normal phenotype, and sampled 10, 20 and 30 days after emergence (DAE). Plants with a VTLE had more nodes, photosynthetic area and plant dry weight than plants of a normal phenotype. In the second test, either one or both cotyledons were removed at emergence and at 5-day intervals between 5 and 20 DAE. Removal of both cotyledons was more detrimental than the removal of one. Plant growth increased as the time of cotyledon removal was delayed from emergence. Even with cotyledon removal, plants expressing a VTLE grew more than plants of a normal phenotype. Differences associated with the expression of a VTLE were more conspicuous early in the plant development.

Key words: *Gossypium hirsutum*, cotton seedlings, first true leaf, growth

RESUMEN

Crecimiento comparativo entre plántulas de algodón con la expresión de una hoja visible a la emergencia y plántulas de fenotipo normal

Al momento de la emergencia, las plantas de algodón de fenotipo normal poseen una primera hoja verdadera poco desarrollada por lo que el área fotosintética está esencialmente limitada a los cotiledones. El desarrollo de la primera hoja verdadera y el desarrollo vegetativo son lentos por lo cual la etapa de plántula es relativamente larga, aumentando la vulnerabilidad a fac-

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tores bióticos y abióticos. Varias líneas experimentales han mostrado una hoja verdadera visible al momento de la emergencia (HVVE) que puede estar asociada a un rápido crecimiento durante la etapa de plántula. Los objetivos de este estudio fueron comparar el crecimiento de plantas que expresan una HVVE con plantas de fenotipo normal durante las primeras etapas de desarrollo, y comparar el crecimiento de ambas cuando se removieron los cotiledones. En el primer experimento las plantas se identificaron para la expresión de HVVE o fenotipo normal. Los muestreos se realizaron a los 10, 20 y 30 días después de la emergencia (DDE). Plantas con HVVE mostraron más nudos, mayor área fotosintética y mayor peso seco que plantas de fenotipo normal. En el segundo experimento plantas con HVVE y de fenotipo normal se sometieron a la remoción de uno o ambos cotiledones desde la emergencia hasta 20 DDE a intervalos de 5 días. La remoción de ambos cotiledones redujo más el crecimiento que la remoción de uno. El crecimiento aumentó a medida que la remoción de los cotiledones fue más tardía. Aún con la remoción de los cotiledones, las plantas con una HVVE crecieron más que las plantas de fenotipo normal. Las diferencias asociadas a HVVE fueron más conspicuas en las etapas más tempranas del desarrollo.

INTRODUCTION

Efficient cotton [*Gossypium hirsutum* (L.)] production depends upon adequate germination, emergence and rapid seedling establishment. At emergence, the photosynthetic area of a normal-phenotype cotton seedling is essentially limited to the cotyledons. At this stage the plumule is poorly developed. Consequently, the first true leaf expansion and the early vegetative development is slow (Mauney, 1986). The result is a prolonged seedling stage, which increases the plant's vulnerability to biotic and abiotic stresses. This vulnerability may be reduced in genotypes which can progress through the seedling stage quickly as a result of fast leaf area and nodal development. A well developed true leaf at emergence may increase possibilities for survival and growth if early loss in photosynthetic area occurs.

Plants of several F_3 breeding lines were found to express a visible first true leaf at emergence (VTLE). Seedlings showing a VTLE had an approximately 5-mm-long true leaf after cotyledons separated from the seedcoat and expanded (Figure 1). The breeding lines having seedlings expressing a VTLE were derived from the cross between the breeding line 7823-1-3 and 'Miscot 7803-52' (Bourland and White, 1989). Breeding line 7823-1-3 was a selection from the Texas A & M University Program for the Genetic Improvement of Cotton (Bird, 1982). Seedlings expressing a VTLE continued to be observed after further selfing (F_5 to F_7 generations). The objectives of this study were to compare early growth of seedlings expressing a VTLE with that of seedlings of a normal phenotype, and to compare their growth when cotyledons were removed at different times after emergence.

FIGURE 1. Cotton seedling expressing a visible true leaf at emergence (left) and a seedling of a normal phenotype (right).

MATERIALS AND METHODS

Early seedling growth

The study was conducted under greenhouse conditions at the Arkansas Agricultural Experiment Station in Fayetteville. Average temperature and relative humidity in the greenhouse were 29.6°C and 64.4%, respectively. The experimental cotton lines used for this study were 8304-54-06 (hereafter 54-06), 8304-49-24 (49-24), 8304-59-01 (59-01) and 8304-59-05 (59-05). These genotypes have varying proportions of seedlings that express a VTLE and seedlings of normal phenotype.

Before planting, variation in germination speed was standardized by germinating the seeds in a germinator at 32°C and 90% relative humidity for 24 h. Germinated seeds with radicle length of approximately 0.8 cm were planted 2.5 cm deep in 2,000 cm^3 plastic pots filled with sand. After emergence, half of the pots planted with the same genotype were rogued to have one seedling expressing a VTLE. The other half was rogued to leave one seedling of a normal phenotype. Water-soluble fertilizer solution (20-20-20 formulation with trace elements) was applied to the pots at 7-day intervals starting 10 days after emergence (DAE). Water was applied as needed.

Three plants per treatment were randomly chosen and sampled 10, 20 and 30 DAE. Height, number of main-stem nodes, leaf and cotyledon areas were recorded after harvest. Leaf and cotyledon areas were measured with an electronic area meter. Roots were washed from the sand and separated from non-root material. Dry weight of the plant parts was recorded after the tissues were oven-dried at 34°C to a constant weight. Plant dry weight and a combined photosynthetic area (cotyledon and leaf areas) were used to calculate the relative growth rate (RGR) and the net assimilation rate (NAR). The calculations were performed according to the formulas given by Gardner et al. (1985):

$$RGR = (\ln W2 - \ln W1)/(T2 - T1) = g/g/d;$$
$$NAR = (W2 - W1) / (T2 - T1) \cdot (\ln LA2 - \ln LA1)/(LA2 - LA1) = g/cm^2/d$$

where Ln is the natural logarithm; W, dry weight in grams; T, time in days; and LA, leaf area in cm^2. The data were averaged for the three sampled plants. Treatments were the factorial arrangement of genotypes by the seedling phenotype (with a VTLE or a normal phenotype at emergence). The experiment repetitions were planted 26 March, 29 April, 5 May and 26 May 1991. For each sampling period, the data were analyzed as randomized block with four repetitions. Means were compared by using the least significant difference (LSD) at the 0.05 probability level.

Roots of the first repetition were measured for length with an image analyzer system. Before the measurement, root samples were stained with a toluidine blue solution. The stain was prepared by dissolving 1 g of toluidine blue-O in 100 ml of methyl alcohol as stock, and then diluting 2 ml of stock solution in 100 ml of water. Stained samples were floated in water in glass-bottom trays, divided into sub-samples, and spread to avoid overlapping during measurements. After being measured, the roots were oven-dried and weighed. Root length was regressed on root dry weight. The regression coefficients were evaluated for dependence on the seedling phenotype and on the genotype by seedling phenotype interaction (factors) by using covariance analyses.

Seedling growth after cotyledon removal

Two genotypes, 54-06 and 49-24, were used for this part of the study. Standardization of germination speed, planting, roguing and fertilization were as previously described. Immediately after emergence, either one or both cotyledons were removed. Removal continued at 5-day intervals between 5 and 20 DAE. Controls without cotyledon removal were included for each genotype by seedling phenotype combination.

Treatment combinations were applied to at least six plants per repetition. Two plants per treatment combination were randomly chosen and sampled at 34 and 48 DAE. Growth measurements were as previously described, except that cotyledons were not included. Treatments were the factorial combination of genotypes, seedling phenotype at emergence and cotyledon removal. The experiment repetitions were planted 29 September and 10 December 1990 and 21 February 1991. In the September planting, the average temperature and relative humidity were 32.7°C and 49.1%, respectively. During the December planting the temperature was 28.1°C; relative humidity, 52.2%. In February, temperature and relative humidity were 30.1°C and 47.0%, respectively. For each sampling period, the data were analyzed as randomized block with three repetitions. Means were compared by using the LSD.

RESULTS AND DISCUSSION

Early seedling growth

No significant interactions between the genotypes and seedling phenotype were detected for any of the growth measurements. Therefore, data for the seedling phenotype were averaged across genotypes. At 10, 20 and 30 DAE, plants with a VTLE had more main-stem nodes, leaf area, stem dry weight, and total plant dry weight (Table 1). However, the presence of a VTLE was not associated with an increase in root dry weight. At 10 DAE, plants expressing a VTLE had significantly more cotyledon area and cotyledon dry weight. In cotton, cotyledons are the main body of the embryo (Pollock and Jensen, 1964). This finding suggests that plants expressing a VTLE resulted from seeds with a larger embryo. At 20 DAE, height, leaf dry weight and cotyledon dry weight were not affected by the seedling phenotype. At 30 DAE, plants with a VTLE had significantly larger growth measurements except for cotyledon area and cotyledon dry weight. On the basis of these differences, a fast early seedling development was associated with the expression of a VTLE.

Neither the increase in dry weight relative to the initial weight (measured as RGR) nor the photosynthetic efficiency (measured as NAR) was affected by the genotype, the seedling phenotype, or their interaction. This result suggests that the increased growth associated with a VTLE did not result from the efficiency of the plant to accumulate dry weight. For 10 to 20 DAE, average RGR and NAR were 7×10^{-2}g/g/d and 7.5 x 10^{-4}g/cm^2/d, respectively. For 20 to 30 DAE, RGR was 12×10^{-2}g/g/d and NAR was 2×10^{-3}g/cm^2/d.

Root length increased linearly with an increase in root dry weight (Figure 2). Covariance analyses did not provide evidence for the significant effect of the seedling phenotype on the genotype by seedling

TABLE 1.—*Growth measurements at 10, 20 and 30 days after emergence (DAE) comparing cotton plants from seedlings expressing a visible true leaf at emergence (VTLE) with plants from seedlings of normal phenotype.*

Seedling phenotype	Plant height	Main-stem nodes	Leaf		Cotyledon		Dry weight		
			Area	DW[1]	Area	DW	Stem	Root	Total
	cm	no.	cm^2	g	cm^2	g	g		
					10 DAE				
VTLE	5.4	2.5	3.1	0.15	12.5	0.08	0.03	0.1	0.22
Normal	4.6	2.1	1.4	0.02	11.1	0.07	0.02	0.1	0.10
$LSD_{0.05}$	0.5	0.2	0.7	0.01	0.9	0.01	0.01	NS	0.01
					20 DAE				
VTLE	9.0	5.7	34.7	0.14	16.7	0.08	0.08	0.2	0.44
Normal	6.4	5.1	26.1	0.12	14.5	0.08	0.06	0.1	0.37
$LSD_{0.05}$	NS	0.1	5.4	NS	1.0	NS	0.01	NS	0.05
					30 DAE				
VTLE	17.6	6.7	186.4	0.69	17.7	0.08	0.38	0.5	1.60
Normal	15.9	6.3	134.6	0.50	17.4	0.08	0.28	0.4	1.26
$LSD_{0.05}$	1.5	0.3	32.1	0.13	NS	NS	0.08	NS	0.27

[1]DW is dry weight.

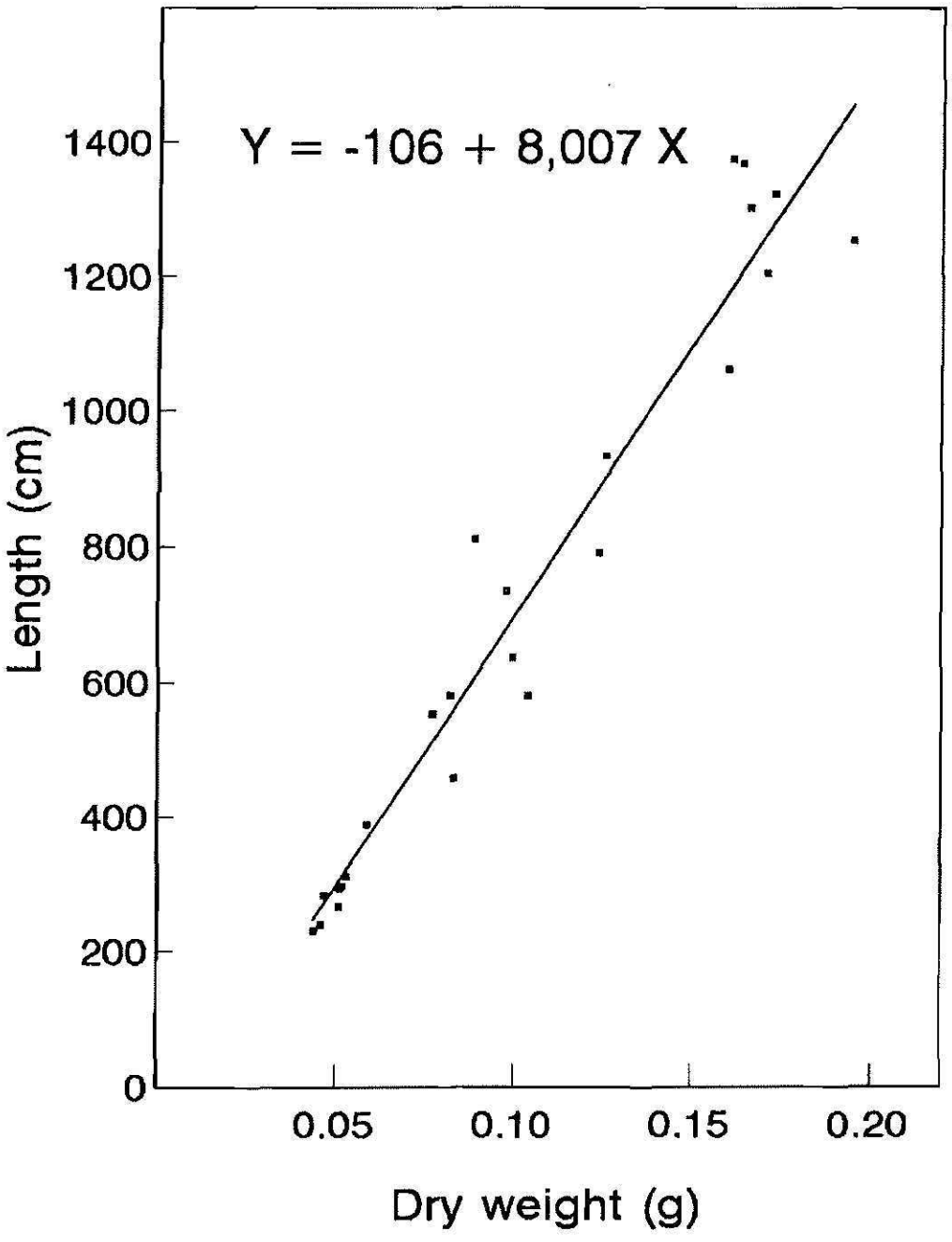

FIGURE 2. Relationship between root length and root dry weight for cotton plants sampled at 10, 20 and 30 days after emergence.

phenotype interaction in this relationship (Table 2). Thus, the root length per unit of dry weight was independent of the expression of true leaf at emergence.

In this part of the study the genotypes expressed significant differences in some of the cotyledon measurements (Table 3). At 10 DAE, cotyledon dry weights of 49-24 and 59-01 were significantly greater

TABLE 2.—*Mean squares in the covariance analyses for the straight line relationship between root length and root dry weight in cotton plants sampled at 10, 20 and 30 days after emergence.*

Factor	Source	DF	Mean square
Seedling phenotype (SP)	Dry weight (DW)	1	3675521.6**
	SP	1	1427.7
	SP × DW	1	2400.0[1]
	Error	20	219724.3
Genotype (G) × Seedling phenotype (SP) [G × SP]	DW	1	3675521.5**
	[G × SP]	7	17262.0
	[G × SP] × DW	7	8419.5[2]
	Error	8	43780.5

**Significant at the 0.01 probability level.

[1]Absence of significance of the SP × DW as a source of variation indicates a non significant effect of the seedling phenotype over the simple linear regression between root dry weight and root length.

[2]Absence of significance of the [G × SP] × DW interaction as source of variation indicates a non significant effect of the genotype by the seedling phenotype interaction over the simple linear regression between root dry weight and root length.

than cotyledon dry weights of 54-06 and 59-05. At 20 and at 30 DAE, the cotyledon area of 49-24 was significantly larger than that of the other genotypes. Genotype 49-24 also had the highest cotyledon dry weight at 30 DAE.

Results of this part of the study associate the expression of a VTLE with significantly larger aerial plant parts regardless of the genotype. Neither root growth nor the efficiency of the plant to accumulate dry weight was affected by the expression of a VTLE.

TABLE 3.—*Cotyledon area and dry weight for cotton genotypes at 10, 20 and 30 days after emergence.*

	Days after emergence					
	10		20		30	
Genotype	Area	Dry weight	Area	Dry weight	Area	Dry weight
	cm^2	g	cm^2	g	cm^2	g
49-24	12.4	0.08	17.4	0.08	20.6	0.09
54-06	11.6	0.07	15.2	0.08	17.5	0.08
59-01	12.1	0.08	14.6	0.08	15.8	0.07
59-05	11.0	0.07	15.3	0.08	16.3	0.08
$LSD_{0.05}$	NS	0.01	1.4	NS	2.8	0.01

Seedling growth after cotyledon removal

No significant interactions involving cotyledon removal treatments were detected for growth measurements at 34 or 48 DAE. Therefore, to illustrate the effect of cotyledon removal on growth, the data were combined across genotypes and seedling phenotype combinations. Removal of both cotyledons at any time after emergence was in general more detrimental than removal of one (Figures 3 and 4). The presence of only one cotyledon at emergence was not always sufficient to supply the needs for maximum growth.

At 34 DAE, there were significant reductions in all growth measurements when one cotyledon was removed at or before 10 DAE (Figure 3). The same response was observed when both cotyledons were removed at or before 15 DAE; the longer the removal of both cotyledons was delayed from emergence, the less the effect on growth reduction. This trend was accentuated for leaf area and for the dry weight measurements. Similar trends have been observed in apple seedlings [*Malus domestica* (Borkh.)] (Jia-Rui et al., 1985) and in black locust [*Robinia pseudoacacia* (L.)] (Marshall and Kolowski, 1974). Cotyledons have been found to be essential for early plant growth and development (Jia-Rui et al., 1985; Lowell and Moore, 1971). Furthermore, in cotton seedlings the cotyledons are both the major storage and the primary photosynthetic organs (Pollock and Jensen, 1964). Thus, the reduction in growth as a consequence of cotyledon removal was expected. A study by Kerby et al. (1988) also indicated that loss of cotyledons significantly reduces growth of Pima cotton [*Gossypium barbadense* (L.)]. In our study, removal of both cotyledons at emergence reduced leaf area, root dry weight, shoot dry weight and plant dry weight to less than 20% of that of the control plants (Figure 3).

When growth measurements were taken 48 DAE, the reduction in growth associated with cotyledon removal was not as dramatic as that for 34 DAE (Figures 3 and 4). Measurements at 48 DAE showed that removal of both cotyledons at or before 10 DAE reduced plant growth. But removal of both cotyledons at 15 DAE or one cotyledon after 5 DAE generally did not cause significant reduction in growth (Figure 4). Also observed at 48 DAE was the tendency for increased growth commensurate with the longer time delay between emergence and removal.

Seedling phenotype at emergence

The seedling phenotype at emergence did not interact significantly with genotypes nor with cotyledon removal for growth measurements at 34 DAE. Thus, growth measurements were averaged across genotypes and cotyledon removals. Even under the stress caused by

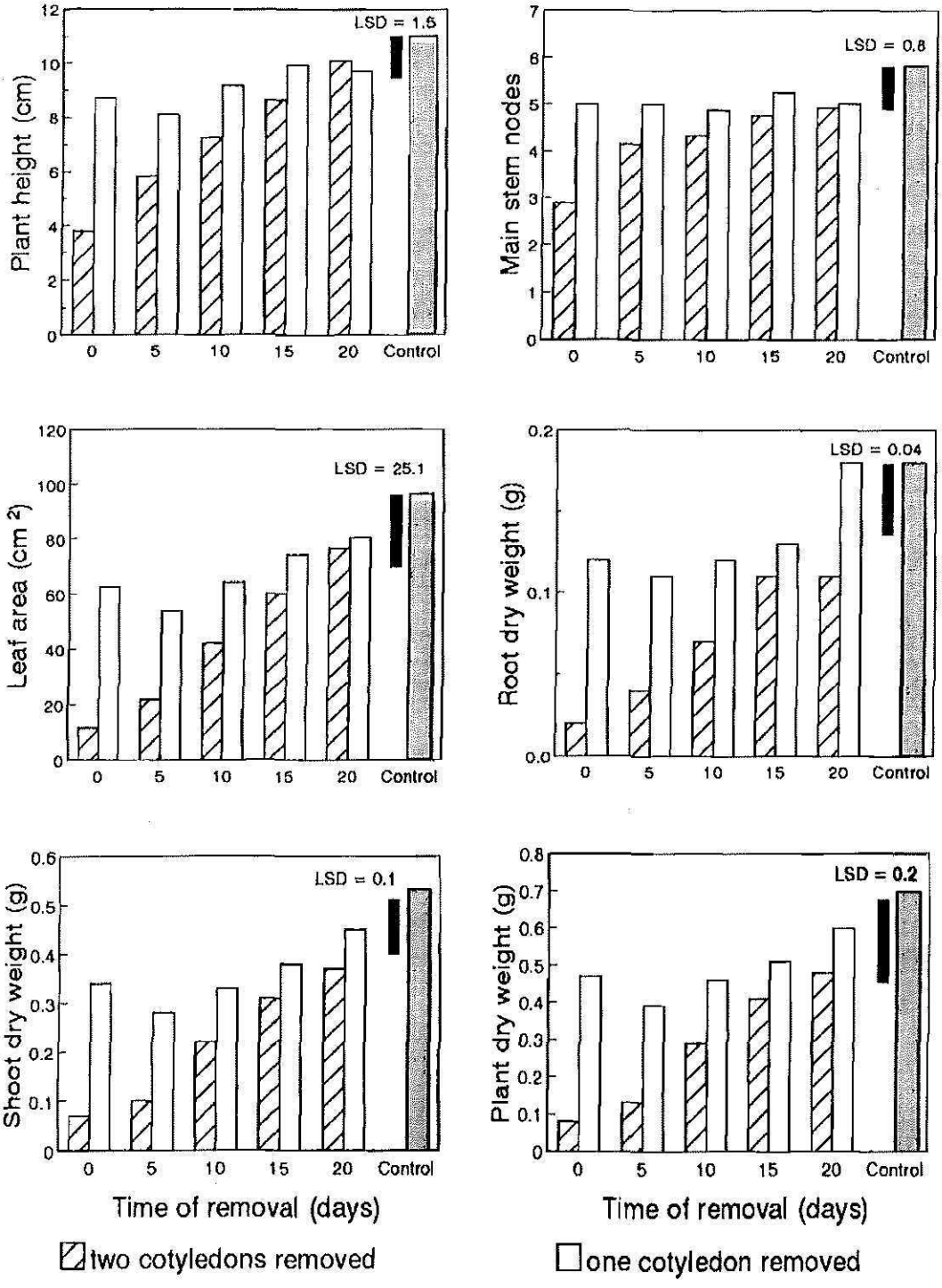

FIGURE 3. Effects of cotyledon removal on plant growth at 34 days after emergence. The vertical bar in black, to the left of the control, represents the LSD value at the 0.05 probability level.

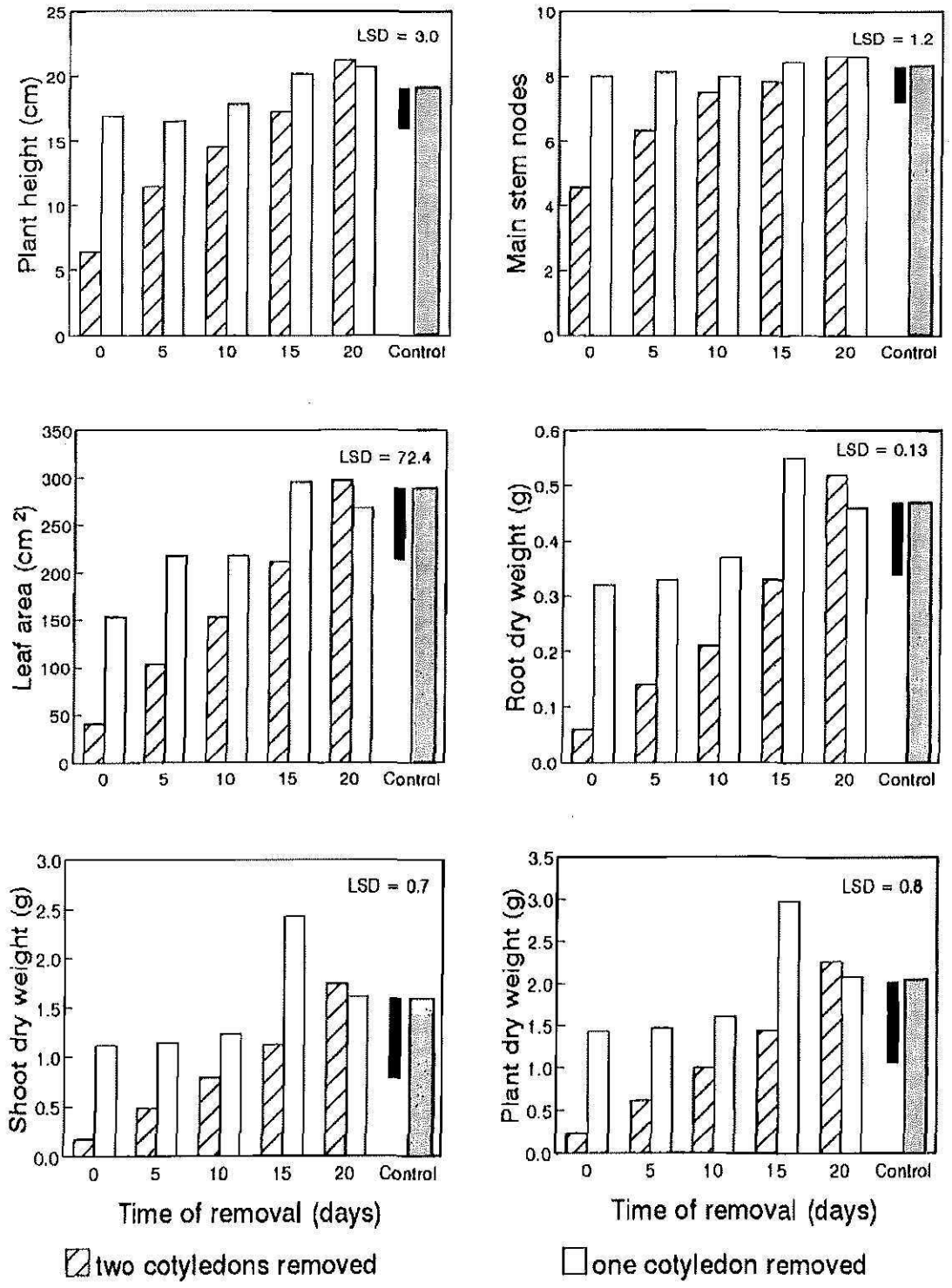

FIGURE 4. Effects of cotyledon removal on plant growth at 48 days after emergence. The vertical bar in black, to the left of the control, represents the LSD value at the 0.05 probability level.

cotyledon removal, the expression of a VTLE was associated with taller plants, more leaf area, more shoot and total plant dry weight and more main-stem nodes (Table 4). However, root dry weight was not affected by the expression of a VTLE. At 34 DAE, genotypes resulted in a significant source of variation for plant height; 49-4 (8.8 cm) was significantly taller than 54-06 (7.9 cm).

For plants sampled at 48 DAE, the presence of a VTLE was associated with significantly more shoot and total plant dry weight regardless of the genotype (Table 4). However, the genotype by seedling phenotype interaction was significant for plant height, number of main-stem nodes, leaf area and root dry weight. The expression of a VTLE was associated with taller plants, more root dry weight and main-stem nodes in the genotype 49-24 but not in 54-06. This result shows that differences may exist among the genotypes relative to the growth associated with the VTLE expression. Even under cotyledon removal, plants with a VTLE were larger, thus having already attained greater development when the early photosynthetic area loss occurred.

TABLE 4.—*Growth measurements at 34 and 48 days after emergence (DAE) comparing cotton plants from seedlings expressing a visible true leaf at emergence (VTLE) with plants from seedlings of normal phenotype.*

Factor	Plant height	Main-stem nodes	Leaf area	Dry weight		
				Shoot	Root	Total
	cm	no.	cm^2	g		
	34 DAE					
Seedling phenotype						
VTLE	9.0	5.1	65.8	0.35	0.12	0.47
Normal	7.7	4.3	50.7	0.26	0.10	0.36
$LSD_{0.05}$	0.6	0.4	10.7	0.05	NS	0.07
	48 DAE					
VTLE	18.3	8.1	245.4	1.50	0.40	1.90
Normal	14.6	7.2	171.1	0.92	0.28	1.20
$LSD_{0.05}$	—	—	—	0.32	—	0.36
Genotype × Seedling phenotype						
54-06 VTLE	15.2	7.4	189.0	1.24	0.31	1.52
54-06 Normal	14.2	7.3	174.6	0.83	0.27	1.15
49-24 VTLE	22.3	9.1	319.7	1.56	0.52	2.40
49-24 Normal	15.2	7.0	167.1	0.86	0.30	1.26
$LSD_{0.05}$	1.9	0.7	43.9	—	0.08	—

The inconspicuous nature of the cotton plumule at emergence may have contributed to the limited attention paid to this structure in seedling vigor studies. Results of this study suggest that a well developed true leaf at emergence may result in a faster seedling growth. Thus, the expression of a VTLE arises as a potential trait for cotton improvement.

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