

Fiber, Nitrate, and Protein Content of *Amaranthus* Accessions as Affected by Soil Nitrogen Application and Harvest Date

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Abstract. Six accessions of edible amaranths (*Amaranthus* spp. L.) of varied geographic and genotypic origin were grown in a soil enriched with 0, 50, or 100 kg·ha⁻¹N. Leaves were harvested at 25, 35, 45, 55, and 65 days after germination (DAG) and analyzed for crude protein (CP), neutral detergent fiber (NDF), and NO₃ N. In grain-bearing accessions, leaf CP content increased with N application but declined linearly over harvest dates. In vegetable types, leaf CP levels tended to fluctuate over time. In both types, NDF content declined with N application, whereas response to harvest date varied. Leaf NO₃ increased two-fold in plants from fertilized plots compared to plants from unfertilized plots, but declined rapidly with time. Leaf content of NO₃ did not exceed 239 mmol·kg⁻¹ dry weight with any N fertilization treatment. Edible amaranth appeared to be adapted to soils and climate of the southeastern United States. *A. tricolor* was most susceptible to disease among the accessions evaluated.

Edible amaranths are protein-rich, annual, herbaceous dicotyledons of worldwide distribution (12, 14). Their use in tropical agriculture as a vegetable or as a grain has been well-documented (4, 6, 8, 11). Like many tropical C-4 monocotyledons, such as sorghum (*Sorghum bicolor* (L.) Moench [S. *vulgare* Pers.]) and sugarcane (*Saccharum officinarum* L.), amaranths flourish in warm, humid, sunny environments. Amaranth leaf dry matter is rich in protein and fiber (19). Interest in assessing selected amaranth accessions as a heat-tolerant greens crop for the temperate zones has expanded (2, 10).

The objective of this study was to evaluate several components of leaf dry matter in selected *Amaranthus* accessions from Latin America, Africa, and Southeast Asia. Accessions were selected to reflect the broad geographic and genotypic spectrum of cultivated members of the genus. Results describing the response of selected leaf nutrients to different levels of soil N enrichment and harvest date treatments are presented.

Six amaranth accessions were selected for this study. Three accessions, *A. tricolor* RRC no. 241 (Southeast Asia), *A. dubius* NCRPIS no. 2014 (Surniam), and *A. blitum* RRC no. 313 (Southeast Asia) were "smooth-leaved" vegetable types. *A. cruentus* NCRPIS no. 1964 (Nigeria), RRC no. 1011 (Mexico), and

A. paniculatus RCSC (Latin America) were relatives to the coarse-leaved New World grain types. Seeds were obtained from Rodale Research Center (RRC), Kutztown, Pa., North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, and Redwood City Seed Co. (RCS), Redwood City, Calif.

The experiment was conducted at the Plant Science Field Laboratory in Knoxville, Tenn. in 1984. The experimental design was a randomized complete block with a split-split-plot factorial arrangement of treatments with four replications. Three N fertilizer rates (main plot factor) of 0, 50, and 100 kg·ha⁻¹N were applied as NH₄NO₃ to an Etowah silt loam (Fine-loamy, siliceous, thermic Typic Paleudult) of medium fertility. Whole-leaf tissue samples from six accessions (subplot factor) were obtained during the growing season at 25, 35, 45, 55, and 65 days after germination (DAG).

All plots received a preplant broadcast application of 60 kg·ha⁻¹P and K. Supplemental irrigation was provided as needed.

Amaranth transplants were grown in a greenhouse in 2.5-cm cellular, plasticfoam flats. Thirteen days after germination, seedlings were transferred to the field.

The experimental area was 28 × 40 m with individual blocks separated by a 1.8-m alley. Within row and between row spacings were 30 cm for all accessions. Five sets of four plants each were logged as sample plants. These four plant units comprised the sub-subplot fact. Harvests were randomized so that a different set of four plants were sampled at each of five harvest dates. First samples were taken 25 DAG, when plants were judged to be large enough to yield a sufficient quantity of leaf material for analyses.

Six mature, expanded leaves (excluding petiole) from the upper portion of the main stem were randomly sampled from each of four plants. For *A. blitum*, an accession with small leaves, 20 mature, fully expanded leaves were randomly sampled from each of four plants.

Leaf samples were dried at 70°C for 24 hr and ground in a Wiley mill to pass a 20-mesh screen. Total N concentration of 200-mg sub-samples was measured, after digestion in 18 M H₂SO₄ and 11.9 M H₂O₂, by the indophenol colorimetric method (18). Crude protein (CP) was estimated by multiplying total N by 6.25. Neutral detergent fiber (NDF) was used as an estimate of total cell wall constituents at 25, 45, and 65 DAG (5). Water soluble NO₃ was extracted from two accessions, *A. cruentus* NCRPIS no. 1964 and *A. dubius* NCRPIS no. 2014. Extraction was by addition of 50 ml of boiling deionized H₂O to 500-mg sub-samples and placement on a mechanical shaker for 30 min. After filtering through no. 42 Whatman paper, the eluant was diluted to a constant volume. NO₃ concentration of the eluant was measured by selective high performance liquid ion chromatography.

Data were processed by analysis of variance. Treatment effects on leaf nutrient content were partitioned by polynomial single degree of freedom contrasts. To maintain within-class variance homogeneity, each accession was analyzed separately.

Regression equations calculated to estimate leaf CP concentration as a function of the N fertilizer and harvest date treatment effects are given in Table 1. Overall, soil N enrichment increased leaf CP significantly ($P \leq 0.01$). Generally, leaf CP was enhanced linearly with soil N enrichment. In *A. dubius*, a significant ($P \leq 0.05$) quadratic effect was observed. Significance of the linear response varied, depending mainly on age of the plants at harvest. Analysis of partial regression coefficients (results not shown) demonstrated that leaf CP response to N rate was minimal at harvest dates ≤ 35 days after germination and increased with increasing age of the plant. Hence, differences in leaf CP concentration among the different N applications did not vary appreciably at the early harvest dates.

Among the six amaranth accessions evaluated, significantly ($P \leq 0.05$) different overall mean leaf CP levels were observed (Table 2). Averaged over accessions and harvest dates, the portion of the total variance in leaf CP resulting from the N treatments was 17%. Significant leaf CP increase was expected; however, the magnitude of the response to N among accessions varied. For example, the percentage of leaf CP variance resulting from the N treatments was greatest in *A. dubius* (26%), whereas the overall mean leaf CP level in this accession ranked lowest. The smallest overall percentages of leaf CP variance accounted for by the N treatments were observed in *A. paniculatus* and *A. blitum* (13% and 12%, respectively). On the other hand, overall mean CP levels in these accessions were exceeded

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Table 1. Calculated regression equations estimating accession leaf crude protein (Y) concentration as a function of N fertilization rate (N) and harvest date (H).

Accession	Function ²	R ²
<i>A. cruentus</i> , NCRPIS no. 1964	$Y = 302 + 2.61N - 36.2H + 0.17(N \times H)$	0.86
<i>A. dubius</i>	$Y = 334 + 0.786N - 86.8H + 1.33H^2 - 1.2(N \times H) + 0.786(N \times H)^2 - 1.08(N \times H)^3$	0.83
<i>A. tricolor</i>	$Y = 232 + 0.831N - 31.8H + 2.3H^2$	0.72
<i>A. cruentus</i> , RRC no. 1011	$Y = 294 + 0.759N - 55.8H + 3.35H^2$	0.85
<i>A. paniculatus</i>	$Y = 292 + 0.183N - 33.4H + 0.116(N \times H)$	0.91
<i>A. blitum</i>	$Y = 267 + 0.645N - 80.2H + 32.0H^2 - 4.5H^3$	0.66

²Units for N are kg·ha⁻¹. Units for harvest date are 1 to 5 by 0.1. Each 0.1 increase = 1 per 10-day interval.

Table 2. Main effect means for leaf crude protein and neutral detergent fiber (NDF) concentration of six *Amaranthus* accessions.

Accession	Constituent	
	Crude protein (g·kg ⁻¹)	NDF (g·kg ⁻¹)
<i>A. cruentus</i> NCRPIS no. 1964	245 a ²	296 a
<i>A. paniculatus</i>	228 b	290 a
<i>A. blitum</i>	218 bc	270 b
<i>A. cruentus</i> RRC no. 1011	216 c	257 c
<i>A. tricolor</i>	203 d	255 c
<i>A. dubius</i>	198 d	253 c

²Mean separation in columns by Duncan's multiple range test, $p = 5\%$.

only by *A. cruentus* NCRPIS no. 1964 (Table 2). These results point to a negative correlation between response to N fertilizer and leaf CP levels among amaranth accessions.

Averaged over a harvest period of 65 DAG, leaf CP enrichment from N addition of 50 kg·ha⁻¹/N was 25% above that of the unfertilized controls. Differences in leaf CP increase resulting from soil N enrichment have been reported by other workers (17).

Leaf CP concentration in *A. dubius*, *A. tricolor*, and *A. blitum* diminished as linear ($P \leq 0.01$), quadratic ($P \leq 0.01$), or cubic ($P \leq 0.05$) functions of harvest date (Table 3). In the grain-bearing types, *A. cruentus* NCRPIS no. 1964, *A. cruentus* RRC no. 1011, and *A. paniculatus*, leaf CP decreased linearly ($P \leq 0.01$) with harvest date. No significant cubic effects were demonstrated among the grain-bearing accessions. This differential response may be explained by comparison of the growth habits of vegetable vs. grain types. Vegetable amaranths, particularly the smooth-leaved accessions of commercial prominence in Southeast Asia,

tend to possess an indeterminate growth habit. Thus, prolonging the vegetative state often results in abundant new, succulent, axillary growth during the season, with floral buds arising directly in the leaf axils (2). In time, some of these accessions develop no identifiable main stem, forming rather a capitate mound or bush-like vegetation at maturity. Consequently, levels of leaf N and other nutrients in these accessions fluctuate, depending on physiological and management factors that would tend to suppress bolting and maintain them in the leafy, vegetative state. Grain-bearing amaranths possess a main stem axis that terminates in a large, branched inflorescence. In these accessions, leaf nutrient concentration is likely to follow a more consistent rate of change over time. Determinate crops, such as wheat, fall into this category. Leaf N content of wheat has been showed to decline similarly (7).

Soil N enrichment generally resulted in a decline in leaf NDF concentration, but the magnitude of decline among accessions was not uniform. Leaf NDF declined linearly (P

≤ 0.05) with increasing soil N application levels (Table 3). In two accessions, *A. dubius* and *A. paniculatus*, no significant differences in leaf NDF resulted from the application of N. These results agree with the observed negative correlation between the concentration of plant fiber in *A. gangeticus* and N supply reported by Ramachandra (13). Results presented herein suggest a similar relationship in other species of amaranth.

NDF response to the harvest treatments differed among the accessions (Table 4). In three accessions, *A. cruentus* NCRPIS no. 1964, *A. dubius*, and *A. blitum*, a significant ($P \leq 0.01$) increase in leaf NDF concentration occurred during the 65-day period of observation. In *A. cruentus* RRC no. 1011 and *A. paniculatus*, leaf NDF concentration changed little and, in *A. tricolor*, a significant decline occurred over the period of observation (Table 3).

Although Grubben (6) and others (3,12) have recommended generous fertilization of amaranths for optimum leaf productivity, plants often accumulate considerable NO₃⁻ in stem and leaves under these conditions. Large concentrations of NO₃⁻ are not desirable in food products. Earlier, Schmidt et al. (16) reported two- to three-fold increases in NO₃⁻ content of amaranth leaves after soil N enrichment. NO₃⁻ data reported for *A. cruentus* NCRPIS no. 1964 and *A. dubius* corroborate these observations (Table 5). Increased levels of N fertilization generally resulted in corresponding increased levels of leaf NO₃⁻. Soils enriched with N fertilizer produced plants containing nearly twice the concentration of leaf NO₃⁻ than that in unfertilized plants. The magnitude of differences in leaf

Table 3. Selected components of the analysis of variance for crude protein (CP) and neutral detergent fiber (NDF) of six *Amaranthus* accessions.

Source	Level of significance											
	<i>A. cruentus</i> NCRPIS no. 1964		<i>A. dubius</i>		<i>A. tricolor</i>		<i>A. cruentus</i> RRC no. 1011		<i>A. paniculatus</i>		<i>A. blitum</i>	
	CP	NDF	CP	NDF	CP	NDF	CP	NDF	CP	NDF	CP	NDF
Nitrogen												
Linear	**	**	**	NS	**	**	**	**	**	NS	**	**
Deviation from linear	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS
Harvest date												
Linear	**	**	**	**	**	**	**	NS	**	NS	**	**
Quadratic	NS	NS	NS	NS	NS	**	*	NS	NS	*	**	**
Cubic	NS	NS	**	NS	**	NS	NS	NS	NS	NS	NS	*
Interaction (nitrogen × harvest date)	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS	*

NS,*,**Not significant and significant at $P = 0.05$ and 0.01 , respectively.

Table 4. Neutral detergent fiber (NDF) concentration of six *Amaranthus* accessions grown at three N rates and harvested on three dates.

Accession	Harvest date (days)	NDF (g·kg ⁻¹)		
		N rate (kg·ha ⁻¹)		
		0	50	100
<i>A. cruentus</i> NCRPIS no. 1964	25	278	268	251
	45	323	288	254
	65	372	343	292
<i>A. dubius</i>	25	223	246	223
	45	276	243	263
	65	257	273	271
<i>A. tricolor</i>	25	280	240	249
	45	306	282	258
	65	238	212	210
<i>A. cruentus</i> RRC no. 1011	25	286	238	237
	45	272	272	258
	65	266	260	234
<i>A. paniculatus</i>	25	285	289	251
	45	311	311	297
	65	290	289	282
<i>A. blitum</i>	25	231	232	238
	45	334	296	239
	65	367	289	204

Table 5. Effects of N fertilization rate and harvest date on nitrate concentration of two selected *Amaranthus* accessions.

Accession	N rate (kg·ha ⁻¹)	NO ₃ ⁻ /(mmol·kg ⁻¹)		
		Harvest date		
		25 days	45 days	65 days
<i>A. cruentus</i> NCRPIS no. 1964	0	91	3	0
	50	170	95	4
	100	203	176	57
Significance				
Treatment				
Nitrogen				
Linear		**		
Deviation from linear		NS		
Harvest date				
Linear		**		
Quadratic		NS		
Cubic		NS		
Interaction (nitrogen × harvest)		NS		
<i>A. dubius</i>	0	112	35	0
	50	215	18	0
	100	239	63	0
Significance				
Treatment				
Nitrogen				
Linear		**		
Deviation from linear		NS		
Harvest date				
Linear		**		
Quadratic		**		
Cubic		**		
Interaction (nitrogen × harvest)		**		

NS,**Not significant and significant at the 0.01% level, respectively.

NO₃⁻ content among the different N treatments increased with increasing age of the plants. Leaf content of NO₃⁻ of both species declined linearly ($P \leq 0.01$) in response to soil N fertilization and harvest date. Very low or no detectable levels of NO₃⁻ were observed in upper main stem sampled leaves at later harvest dates. Overall, levels of soluble leaf NO₃⁻ did not exceed 239 mmol·kg⁻¹ dry weight. These levels are comparable to those found in other leafy green vegetables such as spinach, beet, and chard (9).

Edible amaranths in the southeastern United

States appear productive, with potential for improvement in selected accessions with improved cultural management. They offer a nutritious alternative in summer when cool-season green, leafy vegetables are not available. *A. tricolor* seemed susceptible to attack by stem rot fungi (*Fusarium* and *Phytophthora*) and stem borers, with susceptibility at all growth stages being noted.

Soil N enrichment can be expected to enhance the nutritional quality of amaranth greens. Applications up to 100 kg·ha⁻¹ N increased leaf CP concentration and decreased

NDF concentration. Leaf dry matter of young amaranth plants is richest in protein and relatively low in fiber. In NO₃⁻ accumulating plants, such as amaranth, N fertilization is likely to increase leaf NO₃⁻ concentration. Among the treatment combinations analyzed in this study, leaf NO₃⁻ did not attain unusually high levels as compared to other greens crops similarly treated (1, 15). Generally, leaf CP content declined linearly over harvest dates in grain-bearing types, whereas fluctuating CP levels were observed in the Southeast Asian vegetable types. Differences noted in plant growth habit in vegetable vs. grain types probably resulted in the divergence in leaf protein response over time. Prolonging the vegetative state in amaranths by N fertilization may result in considerable fluctuations in the levels of leaf protein, and other nutrients during the growing season.

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Paclobutrazol Suppresses Vegetative Growth of Large Pecan Trees

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Abstract. The desirability of controlling growth of large pecan [*Carya illinoensis* (Wangenh.) C. Koch] trees prompted the evaluation of paclobutrazol (PBZ) for growth suppression. PBZ was applied to 75-year-old 'Stuart' pecan trees via trunk injection (rates of 0, 50, 100, and 200 mg·cm⁻¹ trunk diameter) or as a spray to the orchard floor (rates of 0, 19, 38 and 76 g/tree). Terminal-shoot growth and leaf area were reduced during 4 years after treatment in both studies. In-shell nut yield was reduced the third and fourth years after PBZ injection, but was increased the second year after soil application. PBZ can reduce terminal-shoot growth in large trees, but higher doses may produce a decline of nut production. Chemical name used: β -[(4-chlorophenyl)methyl]- α -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol (paclobutrazol).

Tree size control is a major problem associated with pecan culture. A large portion of the pecans marketed in the United States is produced by large (\geq 60-year-old) trees growing at orchard spacings generally ranging from 12 \times 12 m to 30 \times 30 m. Even at these relatively low densities, pecan trees may encroach upon one another, resulting in an eventual decline of productivity (7). The current practice in such orchards is to reduce tree size and competition by removing trees (2), removing major limbs over a period of several years, or by hedge pruning (10). Tree removal creates a large area of unused orchard space, resulting in a substantial loss in orchard productivity that lasts from several years to decades after thinning (2). The duration of this problem is largely dependent on the prethinning tree spacing. Removal of major limbs and hedging can reduce tree size; however, both methods are expensive (10).

Use of a growth retardant to reduce tree

growth may have potential when trees have nearly filled their allotted space. Paclobutrazol (PBZ) (ICI Americas, Goldsboro, N.C.), a potent inhibitor of gibberellin biosynthesis (3), has been reported to be an effective retardant of vegetative growth of apple (6, 8) and peach (4) trees and pecan seedlings (5, 9) and young pecan trees (1). The effectiveness of paclobutrazol for controlling growth of mature pecan trees under orchard conditions is unknown.

The objectives of this study were to assess the effectiveness of PBZ for controlling vegetative growth of large mature pecan trees and to evaluate the influence of PBZ on nut production and quality.

The influence of PBZ on vegetative growth and nut characteristics of mature trees was assessed on 75-year-old 'Stuart' pecan trees spaced at 18 \times 22 m. Two studies were performed, one involving the application of PBZ via trunk injection and the other to the orchard floor. The injection study consisted of pressure injection (11 kg·cm⁻²) of technical grade (95%) PBZ (dissolved in 100% methanol) at six equally spaced points around the tree trunk \approx 60 cm above soil level. The check consisted of both noninjected trees and trees injected with methanol, the PBZ carrier. PBZ doses used were 50, 100, and 200 mg a.i./cm of trunk diameter (\approx 0, 3.4, 6.8, and 13.6 g a.i./tree) in Oct. 1982 using a total injection volume of 10 ml/cm of trunk

diameter. The average trunk diameter was \approx 66 cm. The experimental design was a randomized complete block with nine single-tree replicates per treatment. Trees were measured annually for terminal shoot growth (20 random shoots per tree sampled at mid-crown), in-shell nut yield (total crop harvested), percent kernel (based on 100 nuts), and leaf characteristics (10 terminals per tree). Trees were managed for optimum fertility and pest control according to Georgia Cooperative Extension Service recommendations (2) and were not irrigated.

Evaluation of PBZ applied to the orchard floor was carried out under the same cultural, tree age, and cultivar conditions as described in the first experiment. PBZ was applied to the orchard floor at 0, 19, 38, or 76 mg/tree using a 50% wettable powder formulation (ICI-GFU029). PBZ was applied in 7 liters of solution using a hand sprayer to the portion of the orchard floor beneath the tree crown in Feb. 1983. The experimental design was a randomized complete block with three single-tree replicates per treatment. The soil type for both experiments was a Norfolk loamy fine sand (siliceous, thermic typic Paleudult). Trees were measured annually as described previously and data analyzed using the SAS statistical package (SAS Institute, Raleigh, N.C.).

Although injection points were equally spaced around the tree trunk in an effort to obtain uniform distribution, some major limbs showed no effects of PBZ for the duration of the study. Closer examination of such limbs revealed that the injection point usually was positioned between the groups of xylem vessels leading to two major limbs, suggesting that PBZ exhibited little lateral movement. The injection points did not appear to be injured by the PBZ carrier (methanol), but developed depressed areas \approx 15 cm in diameter by 4 years after treatment. Visual evaluation of the phloem and cork cambium at these sites indicated that these tissues were alive and appeared healthy, but were depressed due to the differential growth between the tissues at the injection site and adjacent tissues.

Paclobutrazol injection treatments did not influence nut volume or percent kernel (Table 1). In-shell nut yield was unaffected the first 2 years after treatment but declined considerably with increasing PBZ treatment the third and fourth years after injection.

Injected PBZ retarded growth of terminal shoots for 4 years after treatment in 1982

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