Subtropical Plant Science 59:38-55.2007

Dynamics of Free Amino Acid Accumulations in Cotton Leaves Measured on Different Timelines After Irrigation

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ABSTRACT

Accumulations of 16 free amino acids (FAAs) in cotton, *Gossypium hirsutum* L., leaves in potted greenhouse plants were recorded across three timelines following irrigation. The timelines, 15-minute, 12-hour, and daily sampling intervals, revealed the dynamic response of each FAA, and showed different trends in concentrations and percentages of total FAAs. Some, but not all, FAAs were correlated with changes in water potential after irrigation. The lack of correlation of some FAAs with water potential during each timeline suggests that metabolic, or biosynthetic, regulation is involved, not just dilution or concentration effects brought about by rehydration and dehydration. The 15-minute timeline revealed a decrease in levels of eight FAAs, and summed detectable free essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) that occurred within the first 45-60 minutes after irrigation. The dilution of those FAAs indicates that physiological and chemical factors other than those that govern FAA synthesis or metabolism are responsible for the initial uptake of water. Despite an increase in water potential 48 hours after irrigation, three FAAs did not increase, but the other 13 FAAs accumulated in a pattern roughly similar to that of water potential over 48 h and 7 d. Only eight FAAs were correlated with water potential in the days timeline (some that were not significantly correlated with water potential increased after a short lag). Possible relationships of irrigation and water-deficit stress, via their effects on summed FAAs essential to herbivorous insect growth and development, are discussed. Our results have utility in assessing the stress status of irrigated cotton that can affect herbivorous insects.

Additional Index Words: Drought, *Gossypium hirsutum,* osmoregulation; water deficit

Water deficit stress in plants induces changes in accumulations of free amino acids (FAAs) and other soluble organic compounds (Bussis and Heineke, 1998). In cotton, *Gossypium hirsutum* L., free proline and free arginine accumulate more than other FAAs in response to water and light deficit, respectively, and both have been used to identify the stress response of cotton plants to competition with weeds, and coverage by white kaolin particle film sprays (Showler, 2002). Although various hypotheses to explain FAA accumulation patterns in plants under conditions of water deficit have been suggested (Barnett and Naylor, 1966; Rhodes et al., 1986), plant osmoregulation using organic solutes appears to be one mechanism for plants, especially 'drought adapted' plants like cotton, to retain water (Golan-Goldhirsh et al., 1989; Bussis and Heineke, 1998). Despite high soil water potential in the main root zone of cotton plants under conditions of water deficit, substantial plant evapotranspiration rates are maintained (Jordan and Ritchie, 1971) in association with elevated concentrations of free proline in the leaves (Janagouar et al., 1983). Osmotic adjustment in cotton leaves under conditions of water deficit is greater than that of roots because of the need to maintain leaf turgor (Oosterhuis and Wullshleger, 1987).

The molecular response to salt- and drought-induced osmotic stress in plants involves several interconnected pathways that produce and transmit signals and stress-responsive metabolites (Ingram and Bartels, 1996; Zhu, 2002). Gene transcripts associated with proteins involved in signaling can undergo rapid, transient up- or down-regulation within minutes of the imposition of stress (Seki et al., 2001). Plants tightly regulate levels of proline and other metabolites involved in tolerance to water deficit stress, while other metabolites vary more passively as a result of water dilution effects. In the model plant *Arabidopsis thaliana* (L.) Heynh, the gene encoding the rate-limiting enzyme that synthesizes proline from glutamine is strongly up-regulated by both short (2 h) (Oono et al., 2003) and prolonged (10 d) (Kishor et al.,

1995) periods of water deficit. Conversely, the catabolic enzyme proline dehydrogenase is strongly suppressed by 2 h of water deficit (Reymond et al., 2000) and is induced within 2 h of re-hydration (Oono et al., 2003). Although FAAs as a whole often increase after water deficit (Showler, 2002; Moran and Showler, 2005), little is known about the responses of broad FAA profiles to re-hydration following water deficit across different timelines.

Amino acids essential to insect growth and development are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, although there are variations across different insect species (Vanderzant, 1958; Nation, 2002). If accumulations of one or more of them change as a result of water deficit stress, the nutritional suitability of the host plant could change (Showler, 2002). The purpose of this study was to examine the effects of irrigation on selected cotton plant FAA levels, as a measure of drought stress, across three different timelines: minutes, hours, and days.

MATERIALS AND METHODS

The study was conducted in a greenhouse where temperature was 29.5 – 32.0 °C, RH 35 – 45%, under normal summer daylight hours at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, Hidalgo County, Texas (26° N latitude, 98° W longitude). Cotton plants (var. DP-50, commonly grown in Texas) were grown in 7.6-liter pots with Sunshine mix No. 1 nursery potting soil (~75% sphagnum peat moss, perlite, dolomitic limestone, and gypsum; Sungro Horticulture, Bellevue, Washington) planted with five cotton seeds on the same day. Seedlings were thinned to three per pot, and 200 ml of Peters Professional (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) water-soluble general purpose 20-20-20 N-P-K fertilizer at 15.8 g/ liter of reverse osmosis water was applied to each pot at the two true leaf stage. All pots were maintained in 3-cm tall plastic dishes that were kept filled with reverse osmosis water. The dishes were removed nine days before sampling began to induce water stress. Five plants, however, were kept well watered in the dishes.

At the eight true, fully-expanded leaf stage, one gram of leaf tissue (fresh wt) was excised from the second fully expanded leaf from the top of five separate plants for FAA analysis. At the same time that each leaf tissue sample was taken, one fully expanded leaf per plant was excised and it's water potential measured with a Model 610 (PMS Instrument Co., Corvallis, Oregon) pressure bomb. Cotton plants at the eight true leaf stage that had been deprived of water for 9 d were visibly wilted. Five well watered and five water-deficit stressed plants were sampled for FAAs and water potentials immediately before the pots were irrigated once to saturation with two liters of reverse osmosis water. Five separate cotton plants were sampled for FAAs and water potentials every 15 min for 6 h (minutes timeline), at 12 h intervals for 48 h (hours timeline) from the same group of plants, and at daily intervals for 7 d (days timeline) after irrigation from a different group of plants a month later in the same greenhouse and ambient temperatures as the minutes- and hours-sampled plants Initial sampling began for the days timeline when the potting soil was more moist than in the other sampling timelines. Once sampled, individual plants were not sampled again to avoid potential effects of destructive sampling on plant physiochemistry. The well watered plants were sampled only once, the FAA values compared with the FAA values obtained for the plants undergoing water stress. The same well watered plant data were used for the minutes and hours timelines, other well watered plants were used for the days timeline.

FAA analysis. Each 1-g leaf tissue sample was homogenized with 10 ml 0.1 N HCl using a Virtishear homogenizer (Virtis, Gardiner, New York) (n = 5). At least 5 g homogenate from each sample was placed in separate 10-ml tubes and centrifuged at 10,000 rpm for 30 min. Samples were stored at –80°C.

One milliliter of supernatant from each sample was filtered through a 0.5-μl filter fitted to a 5-ml plastic syringe. Samples were placed in the autosampler of an Agilent 1100 Series (Agilent Technologies, Atlanta, Georgia) reversed-phase high-performance liquid chromatograph (HPLC) with a binary pump delivering solvent A [1.36 g sodium acetate trihydrate + 500 ml purified HPLC grade water + 90 μl triethylamine (TEA) + sufficient 1-2% acetic acid to bring the pH to 7.2] and solvent B [1.36 g sodium acetate trihydrate + 100 ml purified HPLC grade water (1-2% acetic acid added to this mixture to bring the pH to 7.2) + 200 ml acetonitrile + 200 ml methanol] at 100 and 1.0 ml/min on a Zorbax Eclipse AAA 4.6 × 150 mm 3.5 μ column (Agilent Technologies). Absorbance at 262 and 338 nm were monitored on a variable wavelength detector for 26 min per sample. The autosampler measured and mixed 5 μl sodium borate buffer (0.4 N, pH 10.2 in water), 1.0 μl 9-fluorenylmethylchloroformate (FMOC), and 1.0 μl ophthalaldehyde (OPA) derivitizing agents, and 1.0 μl of sample, then injected 2 μl for chromatographic separation of FAAs. The gradient for solvent B changed from 0 to 57% at 36.2 min, 100 at 37.2 min, and back to 0 at 46.1 min; each analysis took 48 min. Identification and quantification of 17 derivitized FAAs (alanine, arginine, aspartic acid, cysteine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine) were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using Agilent software. Despite the dilution of the samples with HCl, quantities of FAAs were calculated per µl of leaf extract.

Statistical analyses. Differences between FAA accumulations and water potentials over time were calculated using one-way ANOVA after log(x+1) transformation (to ensure normality of the data) (repeated measures analysis was not appropriate because plants had to be destructively sampled at each sample time, and were discarded) and means were separated using Tukey's HSD (Analytical Software, 1998). Percentage FAA content values were computed using total detectable FAAs in the denominator, then arcsine-square root-transformed before *t* test comparison of values for the final time in each timeline to initial values. Lines of best fit were calculated (SAS Institute, 1999) for FAA accumulations and water potential over hours and days, but the minutes timeline data did not conform to a single line of best fit, so equations for that timeline are not presented. Correlations between means for water potential and FAA accumulations at the minutes and days timelines were determined using Pearson's correlation coefficient (Analytical Software, 1998; Zar, 1998). Data points for FAAs and water potential were too limited for correlations to be detected at the hours timeline. Comparisons between well watered FAA levels to concentrations in re-hydrated plants and 6 h, 48 h, and day 7 were made using two-sample *t* tests (Analytical Software, 1998).

RESULTS

All of the amino acids in the standard were detected in cotton leaf extract, excluding free cysteine (Table 1). The HPLC was able to detect all of the free essential amino acids, excluding tryptophan (Table 1). Changes in water potential and FAAs over the three timelines adhered to one of six illustrative patterns (Fig. 1A–F). Four selected FAAs, free essential amino acids, and water potentials are shown for the minutes (Fig. 2A-F), hours (Fig. 3A-F), and days (Fig. 4A-F) timelines. Two of the four individual amino acids in Fig. 2-4, aspartic acid and glutamate, were chosen because they are abundant and building blocks for the synthesis of many other amino acids. Phenylalanine was selected because it was sufficiently abundant to illustrate responses associated with re-hydration and drying, and proline because it is used as an indicator of water-deficit stress. Dehydration after irrigation resulted in visible wilting of leaves in the days timeline only, and permanent damage to leaves, such as crinkling, abscission, and desiccation were not observed.

Minutes. Three temporal patterns governed mean FAA accumulations measured at 15-min intervals for 0 - 6 h. In the first pattern (Fig. 1A), relevant for glutamate, significant differences in FAA levels over time were not detected (Table 1, Fig. 2B). In the second pattern (Fig. 1B), levels of seven FAAs (aspartic acid [Fig. 2A], histidine, isoleucine, leucine, lysine, methionine, and phenylalanine [Fig. 2C]) declined by as much as ≈90% over the 6 h. The third pattern, observed for the remaining eight FAAs, summed free essential amino acids (Fig. 2E), and total FAAs, consisted of a significant decline of 65–90% in the first 45–60 min after irrigation, followed by a consistently observed increase to a level that remained constant for the rest of the observation period (Fig. 1C).

By 6 h, accumulations of free arginine, aspartic acid (Fig. 2A), glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine (Fig. 2C), proline (Fig. 2D), serine, threonine, tyrosine, valine, summed free essential amino acids (Fig. 2E), and total FAAs were 35% (serine) – 100% (methionine) lower than pre-irrigation levels (Table 1). The free alanine level at 6 h was nearest its concentration at pre-irrigation (Table 1). At 6 h, free alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine (Fig. 2C), proline (Fig. 2D), serine, threonine, tyrosine, and valine were at their lowest concentrations in the 0-360 minutes timeline but they were still 22% (serine) – 95% (tyrosine) greater than in the cotton grown in water-saturated soil (Table 1). Summed free essential amino acids (Fig. 2E) and total FAAs were 85% and 34% lower, respectively, lower in cotton leaves grown on well watered soil than on soil that had been dehydrated and then irrigated 6 h before.

The response of water potential to irrigation followed pattern 3 (Fig. 1C) with an initial decrease within the first 60 min after irrigation, then an increase between 60 and 90 min to a level that remained stable thereafter (Fig. 2F). At the 6-h sampling time, water potential was 23% lower than the pre-irrigation level, but 49% greater than in plants grown in water-saturated soil (Table 1). Positive correlations were detected between water potential and levels of 12 FAAs, summed free essential amino acids, and total FAAs (Table 2). At 6 h, 11 FAAs [alanine, arginine, glycine, histidine, isoleucine, lysine, phenylalanine (Fig. 2C), proline (Fig. 2D), threonine, tyrosine, and valine], summed free essential amino acids (Fig. 2E), and total FAAs were 33 (total FAAs) – 95% (tyrosine) lower ($P < 0.05$) in leaves of well watered plants (Table 1). Levels of five FAAs (aspartic acid, glutamate, leucine, and serine) were not statistically different in the 6-h cotton from well watered cotton, and water potential was 49% higher in the plants 6 h after re-hydration than in the well watered treatment (Table 1). Methionine was not detected at 6 h after re-

Table 1. Mean (±SE) picomoles free amino acids/ml leaf extract, and bar water potential , of cotton leaves before (0 h) and 6 h after irrigation, and of plants kept in continuously well irrigated soil.

FAAs and water potential	0h	6h	F[a]	P	Well watered[b]	Ratio well watered:6 h	*t*[d]	*P*
Alanine	734.6 ± 34.6	717.2 ± 83.5	4.25	<0.001	173.0 ± 23.8	0.24	8.26	<0.001
Arginine	4,545.4 ± 582.1	865.8 ± 349.8	6.50	<0.001	65.8 ± 16.7	0.08	5.53	0.001
Aspartic acid	5,841.8 ± 579.4	2,007.9 ± 268.7	3.67	<0.001	2,664.2 ± 292.9	1.33	1.74	0.120
Glutamate	2,329.6 ± 215.7	1,905.9 ± 163.4	0.83	0.441	1,877.3 ± 47.3	0.98	0.03	0.973
Glycine	1,240.8 ± 144.8	305.2 ± 69.5	4.70	<0.001	57.4 ± 2.0	0.19	7.49	<0.001
Histidine	114.2 ± 12.9	74.6 ± 2.6	2.87	<0.001	21.0 ± 1.1	0.28	20.78	<0.001
Isoleucine	544.0 ± 61.6	155.4 ± 29.2	3.25	<0.001	17.6 ± 7.6	0.11	3.43	0.009
Leucine	309.8 ± 33.1	36.2 ± 15.5	3.28	<0.001	14.0 ± 5.7	0.39	0.43	0.680
Lysine	1,207.0 ± 137.7	149.2 ± 45.6	3.52	<0.001	15.0 ± 6.5	0.10	3.40	0.009
Methionine	71.4 ± 8.5	0	9.62	<0.001	3.0 ± 1.2	--	28.32	<0.001
Phenylalanine	1,392.8 ± 131.4	322.4 ± 93.3	4.04	<0.001	47.0 ± 15.8	0.15	2.80	0.023
Proline	1,869.0 ± 191.8	659.8 ± 103.0	3.85	<0.001	69.0 ± 10.2	0.10	10.53	<0.001
Serine	675.2 ± 71.5	437.8 ± 93.0	2.51	<0.001	342.7 ± 24.5	0.78	0.87	0.409
Threonine	537.2 ± 52.5	285.6 ± 35.6	3.04	<0.001	73.8 ± 13.1	0.26	5.54	<0.001
Tyrosine	336.8 ± 13.4	116.4 ± 26.7	6.40	<0.001	5.6 ± 5.6	0.05	5.60	<0.001
Valine	531.6 ± 58.6	134.0 ± 33.2	2.60	<0.001	30.4 ± 3.6	0.23	4.82	0.001
Free essential amino acids	9,253.4 ± 495.8	2,023.2 ± 583.5	5.62	<0.001	287.6 ± 25.8	0.14	6.56	<0.001
Total FAAs	22,279.3 ± 1,206.2	8,213.3 ± 954.8	2.55	<0.001	5,486.8 ± 304.1	0.67	2.92	0.019
Water potential[c]	19.7 ± 1.7	15.2 ± 0.3	13.19	<0.001	7.7 ± 0.3	0.51	17.48	<0.001

[a] df = 25, 129; measurements taken at 15-min intervals from 0 to 6 h after irrigation were used in the ANOVA calculations.
[b] Not reflected by ANOVA values in this table, compares only the well watered cotton to 6-h cotton.
[c] --, denominator = 0.
[d] Two-sample *t* test comparing values at 6 h with values for well watered cotton, df = 8.
[e] U

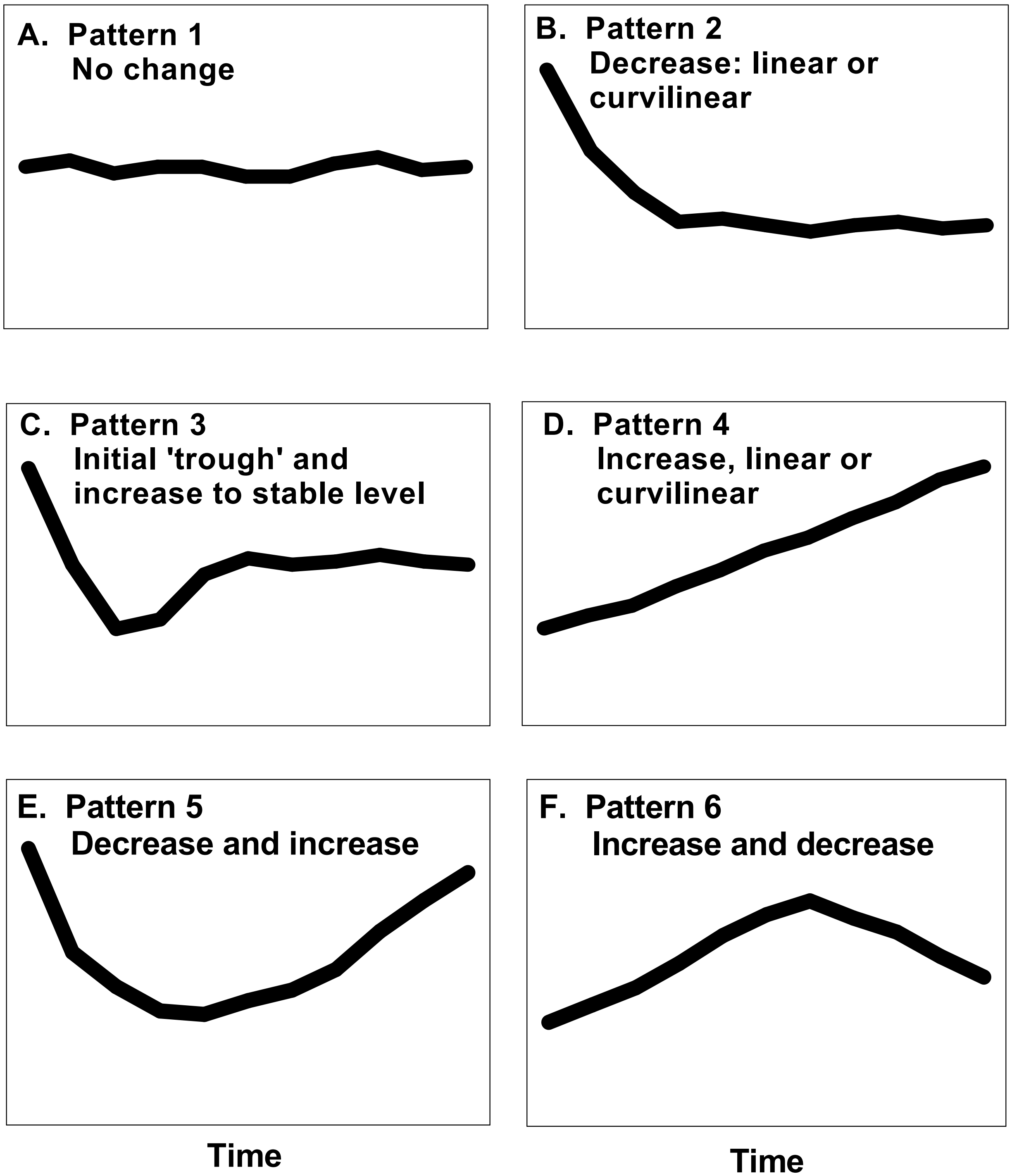


Fig. 1. Six illustrative patterns associated with accumulations of 16 FAAs in cotton leaves during 0 – 360 minutes, 0 – 48 hours, and 0 – 7 days following irrigation of potted greenhouse plants.

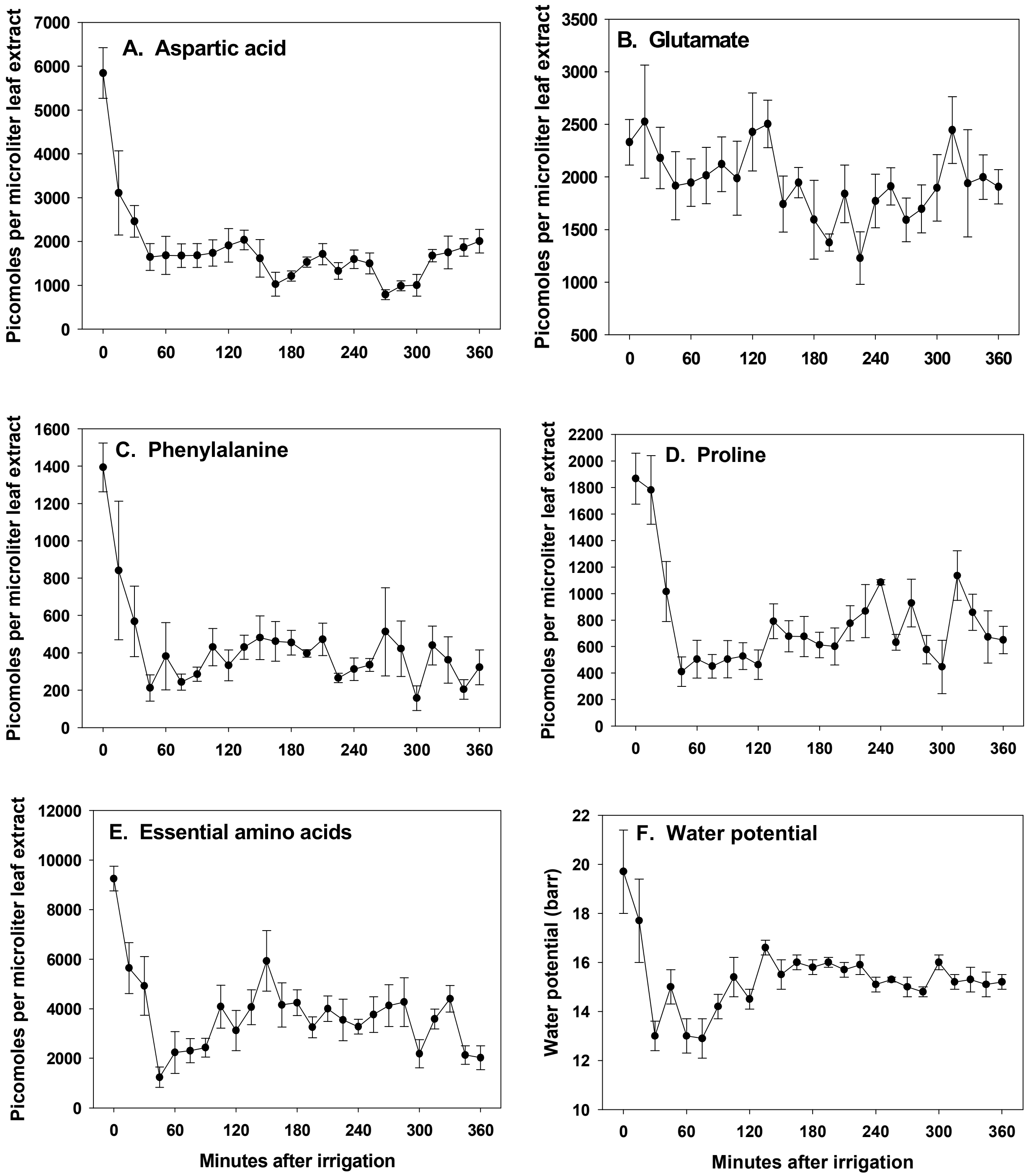


Fig. 2. Mean (±SE) concentrations of four selected free amino acids, free essential amino acids, and water potential in cotton leaves at 15-minute intervals beginning at the time of irrigation to six hours after irrigation (n = 5); A, aspartic acid; B, glutamate; C, phenylalanine; D, proline; E, essential amino acids, and F, water potential.

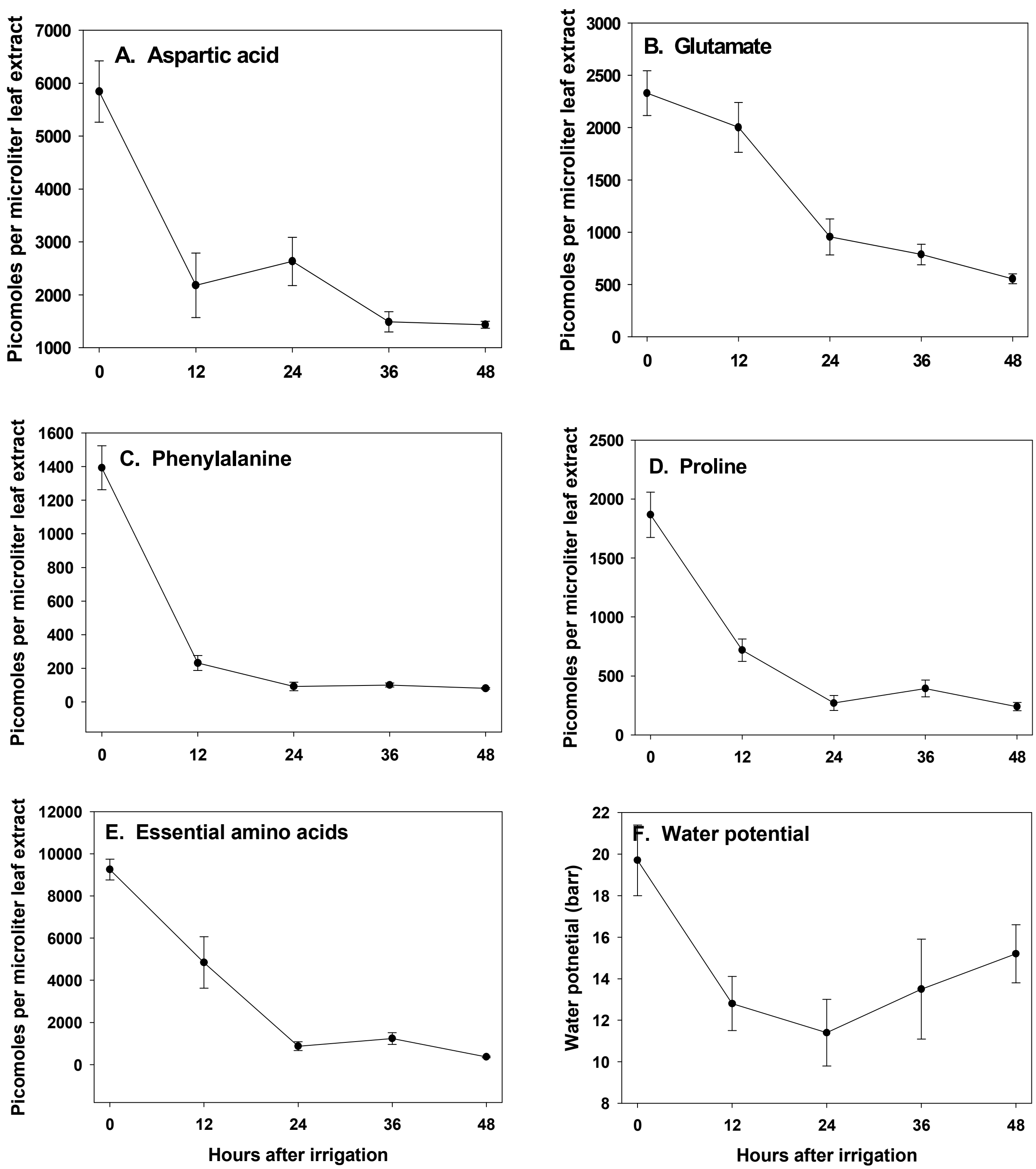
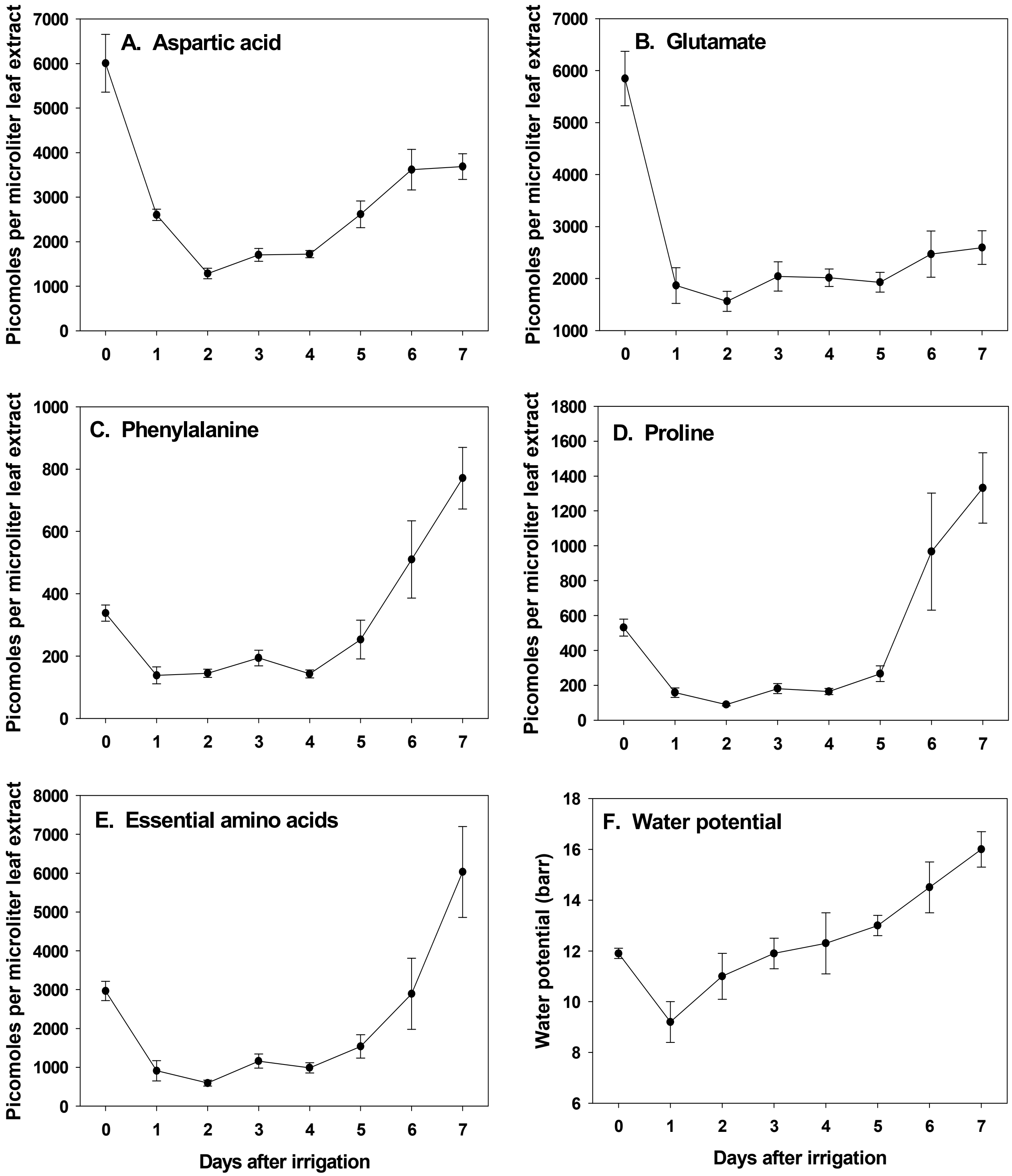


Fig. 3. Mean (±SE) concentrations of four selected free amino acids, free essential amino acids, and water potential in cotton leaves at 12-hour intervals beginning at the time of irrigation to 48 hours after irrigation (*n* = 5); A, aspartic acid; B, glutamate; C, phenylalanine; D, proline; E, essential amino acids, and F, water potential.



.**Fig. 4.** Mean (±SE) concentrations of four selected free amino acids, free essential amino acids, and water potential in cotton leaves at daily intervals beginning at the time of irrigation to seven days after irrigation (n = 5); (A) aspartic acid, (B) glutamate, (C) phenylalanine, (D) proline, (E) essential amino acids, and (F) water potential.

Table 2. Correlations of individual free amino acids with water potential at 15-min intervals from 0 to 6 h, and at 1-d intervals from 0 to 6 h.

	0-6 h 15-min intervals		0-7 d 1-d intervals	
FAA	R	*P*	R	*P*
Alanine	0.30	NS	0.62	NS
Arginine	0.61	<0.01	0.05	NS
Aspartic acid	0.58	<0.01	0.17	NS
Glutamate	0.14	NS	0.09	NS
Glycine	0.73	<0.01	0.82	<0.05
Histidine	0.19	NS	0.05	NS
Isoleucine	0.62	<0.01	0.81	<0.05
Leucine	0.58	<0.01	0.79	<0.05
Lysine	0.64	<0.01	0.76	<0.05
Methionine	0.41	<0.05	0.67	NS
Phenylalanine	0.67	<0.01	0.75	<0.05
Proline	0.65	<0.01	0.75	<0.05
Serine	0.60	<0.01	0.16	NS
Threonine	0.65	<0.01	0.47	NS
Tyrosine	0.55	<0.01	0.76	<0.05
Valine	0.16	NS	0.80	<0.05
Essential AAs[c]	0.65	<0.01	0.70	<0.05
Total FAAs	0.68	0.001	0.41	NS

NS, not significant ($P > 0.05$).
[a] Correlations were analyzed using means, df = 23.
[b] Correlations were analyzed using means, df = 6.
[c] Summed free essential amino acids.

hydration, and the average level in the well watered treatment was only 3 pmoles/µl of leaf extract.

Percentages of total FAAs increased throughout the 0-6 h observation period. Likewise, percent free alanine, glutamate, and histidine increased from pre-irrigation to 6 h after irrigation by 2.8-, 2.2-, and 1.8-fold, respectively (Table 3, Fig. 1D). In contrast, percentages of free isoleucine, leucine, and lysine declined by 30% each, summed essential free amino acids declined by 60%, and methionine declined to zero (Table 3, Fig. 1B). Percentages of the other 10 FAAs did not change within the minutes timeline.

Hours. Histidine was the only FAA whose concentration did not vary (Table 4, Fig. 1A). The other 15 FAAs, summed free essential amino acids, and total FAAs gradually declined from 38 (alanine) to 100% (isoleucine, leucine, and methionine) (Table 4, Fig. 1B, Fig. 3A-E) over 48 h. The equation explaining variation in individual FAA concentrations (y) across time (a) were determined as y = a + bx for serine and threonine; $y = a + bx + cx^2 + dx^3$ for alanine, histidine, and methionine; y = a + b × exp(-bx) for the other 11 FAAs (Table 5).

At 48 h, alanine, arginine, glycine, lysine, phenylalanine (Fig. 3C), proline (Fig. 3D), serine, tyrosine, and summed free essential amino acids (Fig. 3E) were 22 (arginine) – 72% (proline) lower ($P < 0.05$) in leaves of plants grown in well watered soil (Table 4). Aspartic acid (Fig. 3A), glutamate (Fig. 3B), and total FAAs, however, were actually 46%, 70%, and 38% greater, respectively, in the well watered treatment (Table 4). Isoleucine, leucine, and methionine were not detected 48 h after irrigation, but were present in low amounts in well watered plants (Table 4). Water potential declined 40% by 24 h, and then increased gradually until water potential at 48 h was only 23% less than before irrigation (Fig. 1E, 3F).

Differences in percentages of free amino acids, excluding glycine, methionine, proline, and threonine, were found between pre-irrigation and 48-hr-post-irrigation accumulations (Table 3). Percentages of alanine, glutamate, histidine, and serine increased by 1.6- to 4-fold within 48 h (Table 3, Fig. 1D), but percentages of arginine, aspartic acid, lysine, phenylalanine, tyrosine, valine, and summed free essential amino acids declined by 38% to 88% (Table 3, Fig. 1B). Free isoleucine, leucine, and methionine were not detectable at 48 h (Table 3) and percentages of three other FAAs (glycine, proline, and threonine) did not vary over 0-48 h.

Days. Over 7 d, changes in FAA accumulations occurred in two different patterns, each involving significant ($P < 0.05$) differences (Table 6). Free alanine, glutamate (Fig. 4B), and serine concentrations declined such that, by the seventh day, levels were ≤ 50% of the pre-irrigation concentrations (Table 6, Fig. 1B). The second pattern, observed for the other 13 FAAs (Fig. 4A, C, D), summed free essential amino acids (Fig. 4E), and total FAAs involved an initial 47-94% decline for 2 d followed by increases beginning on days 4-5 that, by day 7, reached 0.6- to 13.2-fold pre-irrigation levels (Table 6, Fig. 1E). Five of the 13 FAAs in the second pattern (i.e., free arginine, aspartic acid [Fig. 4A], methionine, proline [Fig. 4D], and threonine) and total FAAs remained at relatively low levels for 3–4 d before increasing. Summed free essential amino acids were 2-fold more abundant on day 7 than at pre-irrigation (Table 6, Fig. 4E), but total FAAs were 27% less concentrated (Table 6). Water potential conformed to the second pattern (Fig. 1E). A 23% decline was observed on day 1, but water potential gradually increased to 1.3-fold the pre-irrigation level by day 7 (Fig. 4F).

Correlations, $R = 0.70–0.82$, were detected between FAA concentrations and water potential for free glycine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine, valine, and summed free essential amino acids (Table 2). The equation explaining effect of time on FAA concentrations was determined as y = a × exp(bx) for isoleucine, y = a + b × exp(-cx) + dx

Table 3. Mean percentages (±SE) of individual FAAs, of total FAA composition, in leaves from potted greenhouse cotton plants before (pre-irrigation) and 6 and 48 h after irrigation with two liters of water.

FAA[a]	Pre-irrigation	Post-irrigation	*t*[b]	*P*	*Post-irrigation*	*t*[b]	*P*
Alanine	3.3 ± 0.2	9.2 ± 1.0	6.91	<0.001	13.3 ± 2.2	6.18	<0.001
Arginine	20.4 ± 2.6	0.6 ± 4.3	2.91	0.020	2.5 ± 0.2	9.16	<0.001
Aspartic acid	26.2 ± 2.6	24.5 ± 3.3	0.45	0.667	16.3 ± 1.4	3.56	0.007
Glutamate	10.5 ± 1.0	23.2 ± 2.0	6.30	<0.001	42.2 ± 2.0	4.79	0.001
Glycine	5.6 ± 0.6	3.7 ± 0.8	1.84	0.102	5.2 ± 1.1	0.45	0.667
Histidine	0.5 ± 0.5	0.9 ± 0.3	1.12	0.293	2.1 ± 0.5	4.42	0.002
Isoleucine	2.4 ± 0.3	1.9 ± 0.4	1.26	0.243	0	8.53	<0.001
Leucine	1.4 ± 0.1	0.4 ± 0.2	2.97	0.018	0	6.38	<0.001
Lysine	5.4 ± 0.6	1.8 ± 0.6	4.31	0.003	0.9 ± 0.3	5.77	<0.001
Methionine	0.3 ± 0.3	0	1.14	0.265	0	1.14	0.265
Phenylalanine	6.2 ± 0.6	3.9 ± 1.1	1.95	0.086	2.4 ± 0.2	7.18	<0.001
Proline	8.4 ± 0.9	7.9 ± 1.3	0.37	0.243	7.0 ± 1.0	1.03	0.334
Serine	3.0 ± 0.3	5.3 ± 1.1	2.16	0.063	4.8 ± 0.7	2.46	0.039
Threonine	2.4 ± 0.2	3.5 ± 0.4	2.23	0.056	2.2 ± 0.3	0.70	0.501
Tyrosine	1.5 ± 0.6	1.4 ± 0.3	0.49	0.634	0.5 ± 0.3	2.77	0.024
Valine	2.4 ± 0.3	1.6 ± 0.4	1.66	0.136	0.8 ± 0.7	6.81	<0.001
Essential AAs[c]	41.5 ± 2.2	4.7 ± 7.1	2.33	0.048	10.8 ± 1.1	12.90	<0.001

[a] Picomoles/ml leaf extract.
[b] df = 1, 8; comparison of pre-irrigation to 6- and 48-h.
[c] Summed free essential amino acids.

for histidine, and $y = a + bx + cx^2$ for the other 14 FAAs and water potential (Table 7).

Fourteen FAAs, summed free essential amino acids, and total FAAs were 2 (serine) – 50 times (tyrosine) greater on day 7 than in cotton leaves grown on well watered soil (Table 6). Water potential on day 7 was 1.9-fold that of the well watered cotton plants (Table 6). Aspartic acid and glutamate levels on day 7 were not different from those in well watered plants.

Changes in percentages of individual FAA over the 7-d experimental period occurred in five different patterns. No changes in percentage free methionine and threonine were detected (Table 8, Fig. 1A). In the second pattern, free glutamate, histidine, and serine decreased within a day or two of irrigation and continued to decline (Fig. 1B). Percentage concentrations for those FAAs were 4.3- and 1.9-fold, respectively, greater on day 0 than on day 7 (Table 8). Percentages free glycine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine, and valine showed the third pattern, increasing from 3- (proline) to 18-fold (lysine) (Table 8, Fig. 1D). Percentages of summed free essential amino acids also occurred in the third pattern (Fig. 1D), with an increase of 2.5-fold by day 7 (Table 8). The fourth pattern (arginine and aspartic acid) was characterized by an initial decline, followed by an increase on days 4 or 5 to a greater level by day 7 (Table 8, Fig. 1E). The fifth pattern, shown by percentage free alanine involved an initial increase, then a decline by day 7 to a level 40% of the pre-irrigation level (Table 8, Fig. 1F).

DISCUSSION

Minutes. The data show that the return of FAAs to levels in well watered plants required more than 6 h after re-hydration, since at 6 h levels of 15 FAAs were still higher in the re-hydrated plants than in well watered plants, and none of them fell below levels observed in well watered plants. Despite variability, accumulations of free glutamate did not change statistically during the first 6 h of the study, free glutamate was not correlated with water potential, and the percentage of glutamate increased during the first hour after irrigation. Free glutamate might have an important role in water uptake when water becomes

Table 4. Mean (±SE) picomoles free amino acids/ml leaf extract, and bar water potential, of potted greenhouse-grown cotton plants before (0 d) and 48 h after irrigation using 2 liters of water, and of plants kepts in continuously well irrigated soil.

FAAs and water potential	0 d	48 h	F[a]	*P*	Well watered[b]	Ratio well watered:48 h[c]	*t*[d]	*P*
Alanine	734.6 ± 34.6	453.2 ± 73.5	9.30	<0.001	173.0 ± 23.8	0.38	4.71	0.002
Arginine	4,545.4 ± 582.1	84.6 ± 8.2	25.07	<0.001	65.8 ± 16.7	0.77	1.34	0.216
Aspartic acid	5,841.8 ± 579.4	1,443.7 ± 66.7	9.91	<0.001	2,664.2 ± 292.9	1.84	5.58	0.001
Glutamate	2,329.6 ± 215.7	553.7 ± 47.9	14.51	<0.001	1,877.3 ± 47.3	3.39	13.57	<0.001
Glycine	1,240.8 ± 144.8	175.0 ± 37.4	33.91	<0.001	57.4 ± 2.0	0.33	5.24	0.001
Histidine	114.2 ± 12.9	71.4 ± 16.3	1.32	0.283	21.0 ± 1.1	0.29	5.43	0.001
Isoleucine	544.0 ± 61.6	0	11.31	<0.001	17.6 ± 7.6	--	14.64	<0.001
Leucine	309.8 ± 33.1	0	14.19	<0.001	14.0 ± 5.7	--	12.28	0.001
Lysine	1,207.0 ± 137.7	30.0 ± 9.1	6.84	<0.001	15.0 ± 6.5	0.50	0.86	0.414
Methionine	71.4 ± 8.5	0	9.94	<0.001	3.0 ± 1.2	--	9.78	<0.001
Phenylalanine	1,392.8 ± 131.4	80.4 ± 5.3	7.26	<0.001	47.0 ± 15.8	0.58	1.44	0.799
Proline	1,869.0 ± 191.8	246.7 ± 41.1	44.77	<0.001	69.0 ± 10.2	0.28	5.87	<0.001
Serine	675.2 ± 71.5	161.8 ± 22.4	8.65	<0.001	342.7 ± 24.5	2.11	4.91	0.001
Threonine	537.2 ± 52.5	73.4 ± 10.3	19.62	<0.001	73.8 ± 13.1	1.01	0.14	0.890
Tyrosine	336.8 ± 13.4	17.6 ± 11.0	9.64	<0.001	5.6 ± 5.6	0.32	0.73	0.483
Valine	531.6 ± 58.6	27.4 ± 2.4	7.84	<0.001	30.4 ± 3.6	1.11	0.53	0.608
Free essential amino acids	9,253.4 ± 495.8	367.2 ± 37.0	20.18	<0.001	287.6 ± 25.8	0.78	1.84	0.102
Total FAAs	22,279.3 ± 906.2	3,401.6 ± 162.0	14.73	<0.001	5,486.8 ± 304.1	1.61	6.82	<0.001
Water potential[e]	19.7 ± 1.7	15.2 ± 1.4	34.68	<0.001	7.7 ± 0.3	0.22	6.59	<0.001

[a] df = 5, 29; measurements taken at 12-h intervals from 0 to 48 h after irrigation were used in the ANOVA calculations.
[b] Not reflected by ANOVA values in this table, compares only the well watered cotton to 48-h cotton.
[c] --, denominator = 0.
[d] Two-sample t test comparing values at 48 h with values for well watered cotton, df = 1, 8.
[e] Unit of measurement is bar.

available, but it is likely that other mechanisms, including evapotranspiration (Karam et al., 2005) and changes in other organic solutes (Showler and Moran, 2003) are more important to osmoregulation during initial re-hydration.

The correlations between water potential and levels of 12 FAAs in the minutes timeline suggest that changes in water potential were early triggers signaling changes in FAA accumulations. The six FAAs that steadily declined (pattern 2, Fig. 1B) did not exactly reflect the decline/partial-recovery pattern (pattern 3, Fig. 1C) associated with water potential. Only three of eight FAAs in pattern 3 (isoleucine, leucine, and lysine) (Fig. 1C) were positively correlated with water potential. However, the 'trough' observed during the first two hours after irrigation for the eight pattern 3 FAAs, summed free essential amino acids, and total FAAs mirrored that of water potential.

Despite the changes observed in absolute FAA concentrations, percentages of free aspartic acid, glycine, histidine, isoleucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine were stable, suggesting that FAA proportions were not important in osmotic regulation during the first six hours after irrigation. Free proline, commonly associated with drought stress in plants (Gzik, 1996; Showler, 2002), increases in *A. thaliana* within the first hour of irrigation and then declines sharply (Oono et al., 2003), but this early proline 'spike' was not observed in cotton.

Hours. Irrigation was associated with declines in concentrations of 15 FAAs by 48 h, but the exact pattern of decline varied, as illustrated by the equations of the lines of best fit. Regardless of the specific pattern, 13 of the FAAs (arginine, aspartic acid, glutamate, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine) declined mostly within the first 24 h, after

Table 5. Examples of lines of best fit[a] for free amino acids and water potential in leaves of potted cotton plants measured at 12-h intervals from 0 to 48 h after irrigation.

FAAs and water Potential	a Coeff. ± SE	a t^b	b Coeff. ± SE	b t^b	c Coeff. ± SE	c t^b	d Coeff. ± SE	d t^b	R^2	*P*
y = a + bx[c]										
Serine	23.65 ± 3.26	7.26**	-3,5.20 ± 1.11	1.82	-	-	-	-	0.52	NS
y = a + bx + cx² + dx³ [d]										
Alanine	743.92 ± 77.39	9.61**	-54.86 ± 16.41	3.34*	2.86 ± 0.87	3.29*	-0.038 ± 0.012	3.22*	0.93	<0.001
y = a ´ exp(-bx)[e]										
Aspartic acid	5,342.66 ± 813.27	6.57*	0.04 ± 0.01	3.33*	-	-	-	-	0.83	0.032
Glutamate	2,442.12 ± 209.53	11.66*	0.03 ± 0.01	5.63*	-	-	-	-	0.94	0.007
Phenyl-alanine	1,389.87 ± 75.61	18.38**	0.14 ± 0.02	6.07*	-	-	-	-	0.99	<0.001
Proline	1,592.42 ± 118.32	13.46**	0.12 ± 0.02	4.40*	-	-	-	-	0.98	0.010
y = a + bx + cx²										
Water potential[f]	19.77 ± 0.70	28.25**	-0.58 ± 0.07	8.36*	0.01 ± 0.01	7.33	-	-	0.97	0.026

[a] *n* = 5.
[b] *, $P < 0.05$; **, $P < 0.001$.
[c] Other FAA conforming to this line of best fit was threonine.
[d] Other FAAs conforming to this line of best fit were histidine and methionine.
[e] Other FAAs conforming to this line of best fit were arginine, glycine, isoleucine, lysine, tyrosine, and valine.
[f] Unit of measurement is bar.

which the rate of decline either slowed or stopped. Although histidine was ≈60% lower at 48 h than at before re-hydration, a difference in concentration over time was not detected, and the 48 h level was ≈3-fold greater than in well watered cotton leaves. Methionine declined to zero by 12 h after re-hydration, and despite an increase over zero at 24 and 36 h, the level returned to zero by 48 h. The variability of methionine over time in the hours timeline resulted in its nonconformity to the tested lines of best fit. Similarly, alanine declined sharply during the first six hours after re-hydration and stayed low until a strong increase was observed at 36 h, followed by another strong decline by 48 h, though still greater than in well watered cotton.

After irrigation, levels of alanine, arginine, glycine, histidine, proline, and total FAAs were still elevated above levels in well watered plants 48 hours after re-hydration because of a lack of change after six hours (histidine), or pattern 2 (Fig. 1B) involving decline from pre-irrigation by 48 hours (alanine, arginine, aspartic acid, glutamate, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, tyrosine, threonine, valine, essential amino acids, and total FAAs), indicating dilution resulting from water uptake, reduced FAA production, or increased loss to fixation in protein and other large molecules, or to breakdown. Six FAAs (aspartic acid, glutamate,

Table 6. Mean (±SE) picomoles free amino acids/ml leaf extract, and bar water potential, of potten greenhouse-grown cotton plants before (0 d) and 7 d after irrigation using 2 liters of water, and of plants kept in continuously well irrigated soil.

FAAs and water potential	0d	7d	F[a]	*P*	Well watered[b]	Ratio well watered: 7d	*t*[c]	*P*
Alanine	3,129.1 ± 322.2	1,557.9 ± 336.5	6.29	<0.001	173.0 ± 23.8	0.11	8.19	<0.001
Arginine	1,181.4 ± 262.5	2,961.4 ± 1027.0	13.68	<0.001	65.8 ± 16.7	0.02	9.62	<0.001
Aspartic acid	6,005.6 ± 650.5	3,686.0 ± 1,107.1	6.92	<0.001	2,664.2 ± 292.9	0.72	0.61	0.557
Glutamate	5,847.4 ± 524.0	2,596.7 ± 423.4	7.49	<0.001	1,877.3 ± 47.3	0.72	1.76	0.117
Glycine	145.1 ± 13.2	717.1 ± 82.5	12.07	<0.001	57.4 ± 2.0	0.08	20.15	<0.001
Histidine	610.4 ± 102.8	125.4 ± 18.4	5.57	<0.001	21.0 ± 1.1	0.17	11.60	<0.001
Isoleucine	70.4 ± 6.4	454.9 ± 92.1	8.86	<0.001	17.6 ± 7.6	0.04	4.64	0.002
Leucine	62.3 ± 4.8	228.1 ± 41.3	4.95	<0.001	14.0 ± 5.7	0.06	4.28	0.003
Lysine	46.1 ± 6.2	608.1 ± 105.5	15.26	<0.001	15.0 ± 6.5	0.02	7.43	<0.001
Methionine	46.3 ± 4.4	51.1 ± 9.4	3.38	0.044	3.0 ± 1.2	0.06	5.78	<0.001
Phenylalanine	338.0 ± 26.1	770.8 ± 98.7	11.27	<0.001	47.0 ± 15.8	0.06	4.11	0.003
Proline	530.8 ± 49.4	1,331.6 ± 201.6	16.20	<0.001	69.0 ± 10.2	0.05	14.46	<0.001
Serine	1,693.1 ± 156.0	729.5 ± 180.8	6.61	<0.001	342.7 ± 24.5	0.47	2.43	0.041
Threonine	543.3 ± 35.6	462.0 ± 95.8	6.78	<0.001	73.8 ± 13.1	0.16	5.88	<0.001
Tyrosine	90.3 ± 2.6	281.4 ± 52.9	8.40	<0.001	5.6 ± 5.6	0.02	6.99	<0.001
Valine	67.8 ± 5.0	371.6 ± 33.2	12.67	<0.001	30.4 ± 3.6	0.08	12.25	<0.001
Free essential amino acids	2,966.0 ± 250.9	6,033.4 ± 1,371.5	14.79	<0.001	287.6 ± 25.8	0.05	12.12	<0.001
Total FAAs	20,408.3 ± 637.9	16,934.1 ± 3,119.5	10.53	<0.001	5,486.8 ± 304.	0.32	5.56	<0.001
Water potential[d]	11.9 ± 0.2	14.7 ± 0.5	8.03	<0.001	7.7 ± 0.3	0.52	11.46	<0.001

[a] df = 7, 39; measurements taken at 24-h intervals from 0 to 7 d after irrigation were used in the ANOVA calculations.
[b] Not reflected by ANOVA values in this table, compares only the well watered cotton to 7-d cotton.
[c] Two-sample t test comparing values on day 7 with values for well watered cotton, df = 1, 8.
[d] Unit of measurement is bar.

isoleucine, leucine, lysine, and serine) declined to levels below that of well watered plants by 48 hours and levels of six other FAAs were not different from well watered plants (arginine, lysine, phenylalanine, threonine, tyrosine, and valine). Water uptake may occur too rapidly for some FAAs to reach stable concentrations. For example, three FAAs (isoleucine, leucine, and methionine) were either diluted beyond the detection limits of our instrumentation, or production and accumulation in the leaves ceased. We suspect the former because continuously well watered plants had low but detectable levels of each of these three FAAs.

FAAs and water potential declined in tandem for the first 24 h, but only some FAAs responded to the subsequent increase in water potential between 24 and 48 h, suggesting that differences in water potential and proximal effects on solute dilution and concentration do not fully explain the changes in FAA concentrations during the hours timeline. The decline of glutamate and aspartic acid, basic building blocks for many other FAAs, to levels below those in well watered plants by 48 h suggests that repression of biosynthetic enzymes or induction of breakdown enzymes occurred. In *A. thaliana*, transcription of glutamate dehydrogenase is induced within 5 h of re-hydration, while glycine dehydrogenase gene expression is repressed (Oono et al., 2003). In this study, glutamate declined most sharply between 6 and 48 h after re-hydration, while glycine declined most rapidly during the first 6 h, suggesting that changes in gene expression in cotton could have enhanced dilution of glutamate beyond 6 h, while counteracting further dilution of glycine. Similar dynamics are possible for other individual FAAs. In *A. thaliana*, re-hydration induces proline dehydrogenase expression and represses proline-5-carboxylate synthetase. These changes contribute to a decline of proline concentrations within 24 h after re-hydration (Oono et al. 2003). In cotton, proline declined but levels were

Table 7. Examples of lines of best fit[a] for free amino acids and water potential in leaves of potted cotton plants measured at 1-d intervals from 0 to7 d after irrigation.

FAAs and water Potential	a Coeff. ± SE	t^b	b Coeff. ± SE	t^b	c Coeff. ± SE	t^b	d Coeff. ± SE	t^b	R^2	*P*
$y = a + bx + cx^2$ [c]										
Aspartic acid	5,087.16 ± 742.20	6.86**	-1,930.44 ± 495.32	3.98*	269.32 ± 68.04	3.96*	-	-	0.67	0.020
Glutamate	4,680.59 ± 826.49	5.66*	-1,584.16 ± 551.57	2.87*	194.57 ± 75.76	2.57*	-	-	0.49	0.070
Phenylalanine	326.80 ± 44.41	7.36**	168.94 ± 29.63	5.70*	32.91 ± 4.07	8.08**	-	-	0.95	<0.001
Proline	511.10 ± 98.89	5.17*	-352.73 ± 66.01	5.34*	67.67 ± 9.07	7.47**	-	-	0.93	<0.001
Water potential	11.42 ± 0.70	9.93**	-1.80 ± 0.47	2.37	0.26 ± 0.06	4.07*	-	-	0.87	0.003
$y = a \acute{} \exp(bx)$										
Isoleucine	28.67 ± 10.17	2.82*	0.40 ± 0.06	7.05**	-	-	-	-	0.93	<0.001
$y = a + b \acute{} \exp(-cx) + dx$										
Histidine	597.10 ± 35.06	9.87**	4.02 ± 2.87	1.40	14.42 ± 5.52	2.61*	13.26 ± 9.34	0.50	0.99	<0.001

[a] *n* = 5.
[b] *n* = 5.
[c] *, *P* < 0.05; **, *P* < 0.001.

still elevated above those in well watered plants after 48 h and proportional proline did not change over 48 h. The pronounced increase in proportional glutamate content over 48 h may, as in the minutes timeline, serve to aid in water regulation over this period instead of proline. Although all of the FAAs, excluding histidine, declined by 48 h, individual relationships to levels detected in well watered cotton differed. In the instances of the FAAs that declined to levels greater than or less than well watered levels, the difference might be explained by the fact that wetting was transient in the hours timeline, indicated by the decline and rise in water potential, as compared to conditions in the minutes timeline.

Days. Water potential showed a two-day period of decline followed by five days of increase as the soil dried. In spite of this re-hydration–dehydration cycle, alanine, glutamate, and serine showed no increases after 48 h through day 7. All of the other FAAs, and summed free essential amino acid accumulations changed in a pattern roughly similar to that of water potential but only eight of them, and summed free essential amino acids, were correlated with water potential. The remaining five FAAs lagged 2-3 d behind water potential before increasing, explaining their lack of correlation. Levels of all 16 FAAs were higher 7 d after re-hydration than in leaves of well watered plants, and 13 FAAs were substantially greater (ratio of well watered:7-d level < 0.20), indicating the renewal of dehydration effects on those FAAs over this timeline.

Despite the relatively low (as compared to the minutes and hours timelines) water potentials in the days timeline, and concentrations of arginine, glycine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine, valine, and essential amino acids were greater 7 d after re-hydration than they were after 9 d of initial dehydration, when the study began. It is possible that this first 9-d dehydration event ‘primed’ the plant to respond more strongly over the 5 d of drying that began 2 d after irrigation. However, water potential was also higher after 7 d than it was immediately before irrigation, and all of the FAAs listed above were correlated to water potential. Alanine, aspartic acid, glutamate, histidine, methionine, serine, threonine, and total FAA levels did not follow this pattern, declining over the entire 7-d period or showing little change (methionine). The

Table 8. Mean percentages (±SE) of FAAs, as compared to total FAAs detected, in leaves from potted greenhouse cotton plants before (pre-irrigation) and 7 d after irrigation with two liters of water.

FAA[a]	Pre-irrigation	7-d Post-irrigation	t^b	*P*
Alanine	15.3 ± 1.6	9.2 ± 2.0	2.38	0.044
Arginine	5.8 ± 1.2	17.5 ± 6.0	2.23	0.056
Aspartic acid	29.4 ± 3.2	21.8 ± 6.5	1.19	0.267
Glutamate	28.6 ± 2.6	15.3 ± 2.5	3.72	0.006
Glycine	0.7 ± 0.6	4.2 ± 0.5	9.50	<0.001
Histidine	3.0 ± 0.5	0.7 ± 0.1	5.55	<0.001
Isoleucine	0.3 ± 0.1	2.7 ± 0.5	5.93	<0.001
Leucine	0.3 ± 0.1	1.4 ± 0.2	5.32	<0.001
Lysine	0.2 ± 0.1	3.6 ± 0.6	8.19	<0.001
Methion	0.2 ± 0.1	0.3 ± 0.1	1.27	0.240
Phenylalanine	1.7 ± 0.1	5.6 ± 0.6	5.83	<0.001
Proline	2.6 ± 0.2	7.9 ± 1.2	5.26	<0.001
Serine	8.3 ± 0.8	4.3 ± 1.1	2.97	0.018
Threonine	2.7 ± 0.2	2.7 ± 0.6	0.07	0.949
Tyrosine	0.4 ± 0.1	1.7 ± 0.3	4.94	0.001
Valine	0.3 ± 0.1	2.2 ± 0.3	7.81	< 0.001
Essential AAs[c]	14.5 ± 1.2	35.6 ± 8.1	2.77	< 0.024

[a] Picomoles/ml leaf extract.
[b] df = 1, 7.
[c] Summed free essential amino acids.

heightened response of some FAAs to the second dehydration thus might not have required increased synthesis of aspartic acid and glutamate building blocks, but instead may have occurred through interconversion of FAAs.

Percentage concentrations of arginine, glutamate, glycine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine, valine, and essential amino acids all showed increases over seven days, while percentages of alanine, glutamate, histidine, and serine declined below pre-irrigation percentages. Our study demonstrated that relative FAA composition in cotton undergoing changes in the availability of water is dynamic. Protein breakdown and FAA biosynthesis (Ingram and Bartels, 1996; Bray, 1997), lead to increases in specific FAAs such as proline and changes in broad FAA profiles (Streeter et al., 2001; Showler, 2002).

Proline, Glutamate, and Total FAAs. Free proline has been identified as reflecting water deficit stress in plants, including cotton, more consistently than other FAAs (Gzik, 1996; Showler, 2002). Suspension culture cells of drought-adapted tomato, *Lycopersicon esculentum* Mill., for example, exposed to polyethylene glycol had 300-fold more free proline because of a 10-fold increase in proline synthesis via the glutamate pathway (Rhodes et al., 1986). Osmotic stress in *A. thaliana* induced a rapid accumulation of free proline through *de novo* synthesis from glutamate (Oono et al., 2003). The response pattern of absolute proline concentration to re-hydration in cotton involved a rapid decline within minutes, extending through the first 4-5 d, and then an increase to the seventh day. That trend was shared by several other FAAs (arginine, glycine, phenylalanine, and tyrosine), but proline was the only FAA to show no proportional change at either the minutes or hours timelines. The early response of cotton to re-hydration may involve maintenance of proline at a minimal critical level. During recovery, free proline is oxidized to glutamate via proline dehydrogenase (Verbruggen et al., 1996). In the minutes and hours timelines, percentage of free glutamate increased after irrigation while absolute free proline levels declined. When drying occurred during the days timeline, absolute proline levels and percentage proline increased while glutamate and percentage glutamate levels declined. These results indicate the linkage between glutamate and proline accumulations across all time timelines.

Large pools of free proline and other FAAs in drought-stressed plants are indications of disrupted protein synthesis and/or increased proteolysis to component amino acids (Fernandes, 1991; Chiang and Dandekar, 1995). Plants adapted to dry conditions, like cotton, might also accumulate other osmoregulatory solutes (Golan-Goldhirsh et al., 1989; Bray, 1997; Bussis and Heineke, 1998). Osmoregulation in potatoes, *Solanum tuberosum* L., for example, occurred in two phases: during the first two days hexoses accumulated, and after seven days, accumulation of FAAs, especially proline, occurred (Bussis and Heineke, 1998). The response of cotton (Showler and Moran, 2003) and other plants (Streeter et al., 2001; Moran and Showler, 2005) to water deficit similarly involves increases in soluble sugars as well as FAAs. These trends explain why some FAAs lagged behind water potential, and why some FAA accumulations were not correlated with water potential.

Changes in total FAAs followed pattern 3 (Fig. 1C) during the minutes timeline, indicating that this pattern predominated among the individual FAAs. A decline in total FAAs continued during the hours timeline, but the days timeline showed how drying and increasing water potential were associated with an increase in total detectable FAAs. The mechanisms underlying the dynamics or changes in total FAAs are unclear, but could be related to osmoregulation, disruption of metabolic processes that utilize FAAs, increased lignification under drought conditions related to elevated peroxidase levels (Showler and Moran, 2003) and catabolism of organic molecules containing amino acids.

Application. Nutritive value as influenced by drought stress likely plays a role in host plant selection by herbivorous insects (Blaney and Simmonds, 1988; Moran and Showler, 2005; Reay-Jones et al., 2005) and has been associated with elevated populations of herbivorous insects (White, 1984; Mattson and Haack, 1990; McQuate and Connor, 1990). Beet armyworm, *Spodoptera exigua* (Hübner), eggs are more abundant on drought-stressed cotton plants than on well watered plants under field and laboratory conditions (Ruberson et al., 1994; Ruberson, 1996; Showler and Moran, 2003). Availability of amino acids in host plants is a critical factor in the population growth of many insect herbivores (McNeil and Southwood, 1978) possibly because FAAs are more available to insects for assimilation than protein-bound amino acids (Helms et al., 1971; Brodbeck and Strong, 1987). Despite higher oviposition rates of beet armyworms on drought-stressed cotton plants, larval survival was reduced, probably because water is the limiting factor for this pest (Showler and Moran, 2003; Moran and Showler, 2005).

Summed free essential amino acid content in cotton was correlated with water potential during all three time timelines. Re-hydration of cotton rapidly reduced the essential amino acid nutritive quality of leaf tissues for herbivores. In the minutes and hours timelines, increased proportional glutamate content may have occurred at the expense of greatly decreased proportional essential amino acid content. Renewed dehydration over seven days increased the absolute and proportional content of all essential amino acids excluding three (histidine, methionine, and threonine) above the levels observed before irrigation and in well watered plants while glutamate declined. Herbivores may thus benefit from rapid, repeated cycles of water deficit and irrigation in cotton, due to enhanced essential amino acid content during periods of dehydration.

Observations across the three timelines showed how concentrations of various FAAs change in cotton leaves in response to irrigation and subsequent drying (Showler, 2002). The shortest timeline (15-min intervals) revealed an initial decline and partial recovery in some FAA accumulations that was not observed in the two longer, more coarse timeline used (12-h and 1-d intervals). The 12-h timeline indicated shifts in percentages of some FAAs that were not observed in the other two timelines, and the daily timeline demonstrated how some FAAs increased with time and water potential. In the hours timeline, the line of best fit for 10 of the FAAs and the line of best fit for 13 FAAs and water potential in the days timeline were different, which contributes to the idea that dynamics and processes governing FAA changes are different at different timelines. The nature of the changes over time in absolute and percentage FAA concentrations varied too much among individual FAAs to be the result of simple water dilution or concentration effects over 360 min, 48 h, or 7 d. Nor can the changes be ascribed entirely to generalized connections between plant FAA biosynthesis pathways (Coruzzi and Last, 2000), illustrating the complexity of regulation of FAA accumulations.

ACKNOWLEDGMENTS

Thanks to Veronica Abrigo, and Mamoudou Setamou for technical assistance, and Waseem Akbar and Francis Reay-Jones for critical reviews. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

LITERATURE CITED

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