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Free Amino Acid Profiles in Reproductive and Rind Portions of Cotton Fruiting Bodies

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ABSTRACT

Different portions of cotton, *Gossypium hirsutum* L., squares and bolls have been reported to influence adult boll weevil, *Anthonomus grandis grandis* Boheman, longevity and fecundity to various extents. The reproductive portion (in squares: anthers, stamens, style, and ovary; in bolls: ovaries and developing seeds and lint) and rinds (developing calyx and petals of squares; the outer casing, or husk, of bolls) of match-head (2–3 mm diameter), medium (3.1–5.4 mm diameter), and large (5.5–8 mm diameter) squares, and post-bloom (1–2 d after petal senescence), young (5–10 d old), and mature (3–5 wk old) bolls were each analyzed for presence and concentrations of seventeen free amino acids (FAAs). Many differences in free amino acid levels were detected and they were related to previously published findings on boll weevil longevity and fecundity. Free methionine appears to have an important role in adult boll weevil survival and reproduction, and cystine levels were associated with degrees of egg formation.

Additional Index Words: *Anthonomus grandis grandis*, boll weevil, bolls, *Gossypium hirsutum*, nutrition, squares

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Concentrations of free amino acids (FAAs) can change in plants in response to various stresses, including water deficit (Bussis and Heineke, 1998; Showler et al., 2007; Showler and Castro, 2009), soil salinity (Joshi and Naik, 1980; Reay-Jones et al., 2005), pathogen infection (Showler et al., 1990; Singh et al., 1993; Moran and Showler, 2007), nematode infestation (Showler et al., 1991), and competition with weeds (Showler, 2002). Levels of FAAs also differ between plant species (Showler, 2001; Reay-Jones et al., 2007), varieties (Reay-Jones et al., 2005, 2007), and accessions (Moran and Showler, 2007).

Plant stress-induced accumulations of FAAs have been associated with elevated populations of herbivorous insects (White, 1984; Blaney and Simmonds, 1988; Moran and Showler, 2005; Reay-Jones et al., 2005, 2007; Showler and Castro, 2009). In cotton, boll weevils, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) feed primarly on cotton, *Gossypium* spp. (Malvaceae), fruiting bodies (Rummel and Summy, 1997). Adults can feed on cotton fruit from the pinhead square (1–2 mm diameter) to boll stages, but large squares (5.5–8 mm diameter) are preferred (Showler, 2005) and accelerate reproduction (Showler, 2004). Adult boll weevils fed certain portions of cotton fruiting bodies at particular stages of growth had different mean longevities and rates of egg production (Showler, 2008), suggesting different nutritional quality of those plant tissues. Amino acid composition of food sources influences adult boll weevil survival and egg production (Vanderzant, 1963) which suggests that, among other important constituents of boll weevil nutrition, different availabilities of free amino acids, more readily absorbed through insect digestive tracts than other forms of N (Brodbeck and Strong, 1987), can influence health and reproductive potential. The purposes of this study were to determine the concentrations of FAAs in different parts of cotton fruiting bodies at selected stages of growth and to relate those concentrations to published data on boll weevil longevity and fecundity.

MATERIALS AND METHODS

This study was carried out in a laboratory at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, Hidalgo County, Texas, and used only *Gossypium hirsutum* L. (variety NK 2837) as a

source for fruiting bodies.

Field-collected match-head (2–3 mm diameter), medium (3.1–5.4 mm diameter), and large squares (5.5 –8 mm diameter), and post-bloom (1–2 d after petal senescence), young (5–10 d old), and mature bolls (3–5 wk old) were dissected with a scalpel and forceps to separate the outer rinds (developing calyx and petals of squares; the outer casing, or husk, of bolls) of each fruiting body size or age from the interior reproductive portion (in squares: anthers, stamens, style, and ovary; in bolls: ovaries and developing seeds and lint).

FAA Analyses. Rinds and reproductive portions of each size of square or age of boll (excluding mature bolls which were too hard for sample extractions) were collected such that each sample to be analyzed represented tissue from a single plant. For example, several match-head square rinds were required from the same plant to constitute one sample. The 10 replicates of each treatment were therefore representative of 10 individual plants rather than individual fruiting bodies. Each 1-g tissue sample was homogenized with 10 ml of 0.1 N HCl using a Virtishear homogenizer (Virtis, Gardiner, NY, USA). Five g of homogenate from each sample was placed in separate 10-ml tubes and centrifuged at 10,000 rpm for 30 min. The extract samples were stored at -80ºC. One ml of supernatant from each sample was filtered through a 0.5-ml filter fitted to a 5-ml plastic syringe. Samples were placed in the autosampler of an Agilent 1100 Series (Agilent Technologies, Atlanta, GA, USA) 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 ml triethylamine (TEA) + sufficient acetic acid to bring the pH to 7.2 and solvent B [1.36 g sodium acetate trihydrate + 100 ml purified HPLC grade water (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 m column (Agilent Technologies). Absorbances at 262 and 338 nm were monitored on a variable wavelength detector for 48 min per sample. The autosampler measured and mixed 6 ml sodium borate buffer (0.4 N, pH 10.2 in water), 1 ml 9-fluorenylmethylchloroformate (FMOC), and 1 ml ophthalaldehyde (OPA) derivitizing agents, and 2 ml of sample, then injected 2 ml for chromatographic separation of FAAs. Identification and quantification of 17 derivitized FAAs (alanine, arginine, aspartic acid, cystine, glutamic acid, 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. Results are presented as pmoles of FAA per ml of extract.

Statistical Analyses. One-way ANOVA, analyzed as a completely randomized arrangement of treatments, was used to detect treatment differences in FAA concentrations (Analytical Software, 1998). Differences in tissue categories (rind versus reproductive portion), and squares versus bolls were analyzed using a 2 × 2 factorial design for the two boll weevil bioassays and for each FAA (Analytical Software, 1998). Means were separated with Tukey's HSD (Analytical Software, 1998).

RESULTS

Seventeen FAAs were detected in this study, and treatment differences were evident for each with df = 9,90 and $P \geq 0.0001$ unless otherwise indicated: alanine ($F = 17.37$), arginine ($F = 15.49$), aspartic acid ($F = 13.52$), cystine ($F = 264.58$), glutamic acid ($F = 8.18$), glycine ($F = 18.32$), histidine ($F = 17.78$), isoleucine ($F = 19.10$), leucine ($F = 50.37$), lysine ($F = 3.96$, $P = 0.0003$), methionine ($F = 6.31$), phenylalanine ($F = 33.49$), proline ($F = 115.23$), serine ($F = 8.41$), threonine ($F = 9.52$), tyrosine ($F = 2.51$, $P = 0.013$), and valine ($F = 7.18$). Other FAAs may have been present but were not detectable using this method.

Free alanine was 1.9-, 2.4-, and 1.6-fold greater in the reproductive portion of match-head squares than in the reproductive portion of medium and large squares, and post-bloom bolls, respectively (Fig. 1A). Medium square rinds had 1.5-fold more free alanine than rinds of match-head squares and ≥ 2-fold more than rinds of large squares and bolls of either age group. Free alanine was relatively sparse in the rind and reproductive portion of large squares, and while levels remained low in rinds of bolls, free alanine increased in the reproductive portion of bolls as they aged (Fig. 1A). Factorial analysis determined that free alanine was 1.2-fold more abundant ($F = 5.67$, $df = 1, 96$, $P = 0.0193$) in reproductive tissue than in rind tissue, but no difference was detected between squares and bolls.

Levels of free arginine were consistent in the reproductive portion and rind of squares and bolls, excluding post-bloom boll rinds with ≥ 2.2-fold more than in any other treatment (Fig. 1B). While relatively low compared with free arginine levels in post-bloom boll rinds, the average level in the rind of young bolls was 7.7- and 4.2-fold greater than in rinds of medium and large squares, respectively (Fig. 1B). Factorial analysis showed that free arginine levels were 1.8-fold greater ($F = 17.00$, $df = 1, 96$, $P = 0.0001$) in rind tissue than in reproductive tissue, and 2.8-fold greater ($F = 43.63$, $df = 1, 96$, $P < 0.0001$) in bolls than in

squares.

Lowest levels of free aspartic acid were observed in match-head squares, but increased 94-198% in the reproductive portion of other fruiting body categories (Fig. 1C). In the rinds of match-head and medium squares, aspartic acid levels remained relatively low but increased 4.1- and 3.2-fold in the rinds of large squares and post-bloom bolls, respectively. Young boll rinds had only 38% and 48% of the aspartic acid found in large square and post-bloom boll rinds, respectively (Fig. 1C). Factorial analysis did not detect a difference between reproductive and rind tissues, but bolls had 1.4-fold more (F = 8.58, df = 1, 96, P = 0.0042) free aspartic acid than squares.

Free cystine was relatively low (0–47.5 pmoles/ml extract) in rinds of each fruiting body category, and in the reproductive portion of match-head squares and both boll ages (Fig. 1D). The reproductive portion of medium and large squares, however, had ≥ 25.4- and 18.1-fold more cystine than any other fruiting body component analyzed (Fig. 1D). Free cystine was 38.9-fold more abundant in reproductive tissue than in rind tissue (F = 40.39, df = 1, 96, P < 0.0001) as determined by factorial analysis, and 51.9-fold more abundant in squares than in bolls (F = 41.44, df = 1, 96, P < 0.0001).

Free glutamic acid in the reproductive portion of medium squares was 1.5-fold more abundant than in the reproductive portion of large squares, but no other differences within the reproductive portion of other fruiting categories were detected (Fig. 1E). The level of free glutamic acid in the rinds of match-head squares was 2.4-fold greater than in the rind of young bolls (Fig. 1E). Factorial analysis showed that free glutamic acid in reproductive tissue was 1.4-fold more concentrated than in rind tissue (F = 24.12, df = 1, 96, P < 0.0001) and 1.3-fold more in squares than in bolls (F = 19.46, df = 1, 96, P < 0.0001).

Free glycine in the reproductive portion of match-head squares declined 34–55% to levels observed in the reproductive portion of the other four fruiting body categories (Fig. 1F). Free glycine in the rind of large squares was 47–52% lower than in match-head squares, and post-bloom and young bolls (Fig. 1F). The reproductive portion of match-head squares had 1.9-fold more free glycine than the corresponding rind (Fig. 1F). Factorial analysis revealed that free glycine was 1.4-fold more concentrated in reproductive tissue than in rind tissue (F = 23.77, df = 1, 96, P < 0.0001).

Levels of free histidine in the rind of match-head and medium squares, and post-bloom bolls were 1.4-, 2.1, and 1.8-fold greater, respectively, than in the corresponding reproductive portion (Fig. 1G). Levels dropped 56% and 35% from the reproductive portion and rind of match-head squares, respectively, to medium squares (Fig. 1G). A further decline from the rind of medium squares to large squares brought free histidine to the same relatively low level found in the reproductive portion of large squares (Fig. 1G). Differences in free histidine concentrations between the reproductive portion and rind of large squares and young bolls were not detected (Fig. 1G). Factorial analysis determined that rind tissue had 1.4-fold more free histidine than reproductive tissue (F = 14.00, df = 1,96, P = 0.0003), but differences were not detected between squares and bolls.

Free isoleucine in the reproductive portion of match-head and medium squares gradually declined from ≈ 123 pmoles/ml to zero in young bolls but statistical differences were not detected (Fig. 1H). In contrast, free isoleucine in the rind of match-head and medium squares was <10 pmoles/ml, but became 57- and 75-fold more abundant in the rind of large squares and post-bloom bolls, respectively (Fig. 1H). Free isoleucine in the rind of large squares and post-bloom bolls was ≥ 3.3-fold and ≥ 4.3-fold more concentrated, respectively, than in the reproductive portion of other sizes or ages of fruiting bodies examined in this study. Free isoleucine was 3.6-fold more abundant in rind tissue than reproductive tissue (F = 19.62, df = 1, 96, P < 0.0001), but factorial analysis did not detect a difference in concentrations of free isoleucine between squares and bolls.

Rinds of cotton fruiting bodies contained 0–8.9 pmoles of free leucine per ml of extract and no differences were detected between them (Fig. 1I). The reproductive portion of match-head squares had 290.8 ± 27.2 pmoles of free leucine per ml of extract and declined 49.4%, 57.8%, 72.3%, and 80% in the reproductive portion of medium and large squares, and post-bloom and young bolls, respectively (Fig. 1I). Free leucine was more abundant in the reproductive portion than in the corresponding rind of each fruiting stage, excluding young bolls (Fig. 1I). Factorial analysis showed that free leucine was 17.2-fold more abundant in reproductive tissue than in rind tissue (F = 126.69, df = 1, 96, P < 0.0001), and it was 2.5-fold more concentrated in squares than in bolls (F = 29.48, df = 1, 96, P < 0.0001).

The 24.6-fold greater concentration of free lysine in the reproductive portion of match-head squares than in the rind of large squares was the only observed treatment difference (Fig. 1J). Factorial analysis determined that free lysine in reproductive tissue was 1.6-fold more abundant than in rind tissue (F = 6.94, df = 1, 96, P = 0.0098).

The reproductive portion of match-head squares had 117.8 ± 8.4 pmoles of free methionine per ml of extract which gradually declined to zero in bolls (Fig. 1K). Free methionine was absent in the rind of match-

head squares and bolls. Although only 12.8 ± 12.8 pmoles of free methionine per ml of extract was detected in the rind of medium squares, this increased 12 -fold in the rind of large squares, which was 4.3-fold greater than in the reproductive portion (Fig. 1K). The reproductive portion of match-head squares, on the other hand, had more free methionine than the corresponding rind (Fig. 1K). Free methionine was determined by factorial analysis to be 11-fold more abundant in squares than in bolls ($F = 18.79$, $df = 1, 96$, $P < 0.0001$) but a difference between reproductive and rind tissue was not detected.

Free phenylalanine levels were 7- and 35-fold higher in the rind of match-head and medium squares, respectively, than in each corresponding reproductive portion (Fig. 1L). Levels of free phenylalanine in the rind of match-head and medium squares declined ³92% and ³88%, respectively, to levels in large square and boll rinds (Fig. 1L). Differences in levels of free phenylalanine in the reproductive portion of all fruiting body categories, and the rind of large squares and bolls of any age, were not detected (Fig. 1L). Free phenylalanine was 5.9-fold more concentrated in rind tissue than in reproductive tissue as determined by factorial analysis ($F = 27.33$, $df = 1, 96$, $P < 0.0001$), and it was 5.7-fold more abundant in squares than in bolls ($F = 26.63$, $df = 1, 96$, $P < 0.0001$).

Free proline levels in the rind did not differ between the various fruiting body categories, ranging from 104.4 to 224.1 pmoles/ml of extract (Fig. 1M). The reproductive portion of match-head squares had 8.8-fold more free proline than the rind of match-head squares, and free proline in the reproductive portion of medium and large squares was 2.9-fold more concentrated than in the reproductive portion of match-head squares. Free proline in the reproductive portion of bolls declined to levels comparable to those found in rind (Fig. 1M). Factorial analysis determined that free proline was 7.2-fold more abundant in reproductive tissue than in rind tissue ($F = 89.34$, $df = 1, 96$, $P < 0.0001$), and 7.5-fold more concentrated in squares than in bolls ($F = 91.55$, $df = 1, 96$, $P < 0.0001$).

Differences in concentration of free serine between the reproductive portion of each fruiting body category and its corresponding rind were not detected (Fig. 1N). Free serine in the reproductive portion of match-head squares was 1.5- to 1.6-fold greater than in the reproductive portion of any other fruiting body category, and free serine in the rind of match-head squares was 1.7- and 1.5-fold greater than in the rind of large squares and young bolls, respectively (Fig. 1N). Factorial analysis indicated that free serine was 1.2-fold more concentrated in reproductive tissue than in rind tissue ($F = 12.88$, $df = 1, 96$, $P = 0.0005$).

No differences in free threonine levels were found between the reproductive portion of each square category and young bolls. Free threonine was 1.5- and 1.6 -fold greater in the reproductive portion of match-head squares and young bolls, respectively, than in post-bloom bolls (Fig. 1O). In rinds, free threonine decreased from match-head to medium and large squares by 47% and 43%, respectively, but increased in post-bloom bolls to the match-head rind level and declined 12% from post-bloom to young bolls (Fig. 1O). The reproductive portion of match-head squares had 1.7- and 1.6-fold more threonine than in the corresponding rind of large squares and young bolls, respectively, but this FAA was most concentrated in the reproductive portion of young bolls (Fig. 1O). Free threonine was 1.2-fold more abundant in reproductive tissue ($F = 12.38$, $df = 1, 96$, $P = 0.0007$) than in rind tissue, and no difference was detected between squares and bolls according to factorial analysis.

Free tyrosine in the reproductive portion of fruiting bodies gradually declined from 73.9 pmoles per ml of extract to zero in young bolls but statistical differences were not detected (Fig. 1P). The rind of medium squares contained no tyrosine but increased to 368.1 pmoles/ml of extract in the rind of large squares, and free tyrosine in the rind of large squares was 19.7- and 47.2-fold more abundant than in the reproductive portion of post-bloom bolls and young bolls, respectively, and no free tyrosine was detected in the reproductive portion of young bolls (Fig. 1P). Squares had 5.2-fold more free tyrosine than bolls as determined by factorial analysis ($F = 5.40$, $df = 1, 96$, $P = 0.0223$), but a difference between reproductive and rind tissues was not found.

Quantities of free valine in the reproductive portion of match-head squares declined 70% and 63% in post-bloom and young bolls, respectively (Fig. 1Q). Free valine in the rind was 82% and 76% lower in medium squares and young bolls, respectively, than in match-head squares (Fig. 1Q). Differences between free valine levels in the reproductive portion of each fruiting category and free valine levels in the corresponding rind were not found (Fig. 1Q). Factorial analysis detected 1.7-fold more free valine in reproductive tissue than in rind tissue ($F = 11.92$, $df = 1, 96$, $P = 0.0008$), and 1.8-fold more in squares than in bolls ($F = 14.09$, $df = 1, 96$, $P = 0.0003$).

Of the 10 FAAs that were more abundant in reproductive tissue than in rind tissue, four were essential amino acids (leucine, lysine, threonine, and valine), and all four FAAs that were more concentrated in rind tissue were essential (arginine, histidine, isoleucine, and phenylalanine). Of the eight FAAs that were more abundant in squares than bolls, five were essential (leucine, methionine, phenylalanine, tyrosine, and valine), and of the two FAAs that were more abundant

in bolls, only free arginine was essential. Free cystine, glutamic acid, leucine, proline, and valine (leucine and valine are essential) were most abundant in both reproductive tissue and squares, free arginine (essential) was most concentrated in both rind tissue and bolls, and phenylalanine (essential) was more abundant in both rind tissue and squares. Some free essential amino acids, however, were absent from certain treatment tissues (Table 1).

Total free essential amino acids in the reproductive portion and rind of match-head squares declined by 42% and 50%, respectively, in medium squares and, in the reproductive portion, total free essential amino acids remained at that relatively low level in large squares and each boll category (Fig. 1R). The same was true of rinds, excluding that of post-bloom bolls which had 3.2-fold more total free essential amino acids than the corresponding reproductive portion (Fig. 1R). Factorial analysis determined that total free essential amino acids were 1.4-fold more abundant in rinds than in reproductive tissue ($F = 4.67$, $df = 1, 96$, $P = 0.0331$), and 1.4-fold more abundant in bolls than in squares ($F = 7.29$, $df = 1, 96$, $P = 0.0082$).

Total FAAs in the reproductive portion of medium squares were 1.6- and 1.5-fold more concentrated than in the reproductive portion of post-bloom and young bolls, respectively (Fig. 1S). The rind of match-head and large squares had 1.5-fold more total FAAs than the rind of medium squares (Fig. 1S). Although the rind of post-bloom bolls had 1.7- to 2.4-fold more total FAAs than the rind of squares, the rind of young bolls was similar to the rind of squares in terms of total FAAs (Fig. 1S). Factorial analysis showed that bolls had 1.1-fold more total FAAs than squares ($F = 10.04$, $df = 1, 96$, $P = 0.0021$).

Total free essential amino acids were more prevalent in rinds than in reproductive tissue, and bolls had more total free essential amino acids and total FAAs than squares. FAAs that were more abundant in reproductive tissue than rinds were alanine, cystine, glutamic acid, glycine, leucine, lysine, proline, serine, threonine, and valine, which constituted 59% of the detectable FAAs in this study, and 44% of the free essential amino acids. FAAs more abundant in squares than bolls were cystine, glutamic acid, leucine, methionine, phenylalanine, proline, tyrosine, and valine, constituting 47% of the detectable FAAs and 44% of the free essential amino acids.

DISCUSSION

The ten amino acids recognized as being essential to the growth and development of most insects, including boll weevils, are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Vanderzant, 1958, 1963; Chen, 1985; Kaur & Srinastava, 1994), nine of which were detectable in this study (the HPLC method used does not detect tryptophan although it likely occurred). Lea et al. (1956) determined that omission of arginine, isoleucine, leucine, lysine, phenylalanine, threonine,

Table 1. Free essential amino acids that were absent in reproductive or rind tissues of cotton fruiting bodies ($n = 10$).

Treatment[a]	Absent free essential amino acids
Reproductive portion[b]	
Match-head squares	all free essential amino acids present[d]
Medium squares	phenylalanine
Large squares	phenylalanine
Post-bloom bolls	methionine, phenylalanine
Young bolls	isoleucine, methionine
Rind[c]	
Match-head squares	isoleucine, methionine
Medium squares	arginine, isoleucine, leucine
Large squares	leucine, lysine
Post-bloom bolls	methionine
Young bolls	leucine, methionine, phenylalanine

[a] Match-head square, 2–3-mm-diameter;

tryptophan, or valine in the diet of yellow fever mosquitoes, *Aedes aegypti* (L.), halted egg development, and omission of cystine, glutamic acid, histidine, or methionine reduced fecundity. Similarly, Chang (2004) found that in addition to essential amino acids, alanine, glutamic acid, and serine were essential for Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), fecundity, and that deletion of aspartic acid, cystine, glycine, and tyrosine reduced egg formation. Olive fruit fly, *Bactrocera oleae* (Gmelin), egg production decreased from lack of alanine, arginine, cystine, glutamic acid, glycine, histidine, leucine, methionine, or proline (Zografou et al., 1998). While amino acids are important for growth and reproduction, different insect species have different amino acid requirements despite some commonalities (Dadd, 1973). Such differences are probably because of variation in the nutrient reserves of the insects (House, 1962) and the essential amino acids must usually be supplemented by a number of 'nonessential' amino acids for optimal growth (Dimond et al., 1956; Vanderzant, 1958)

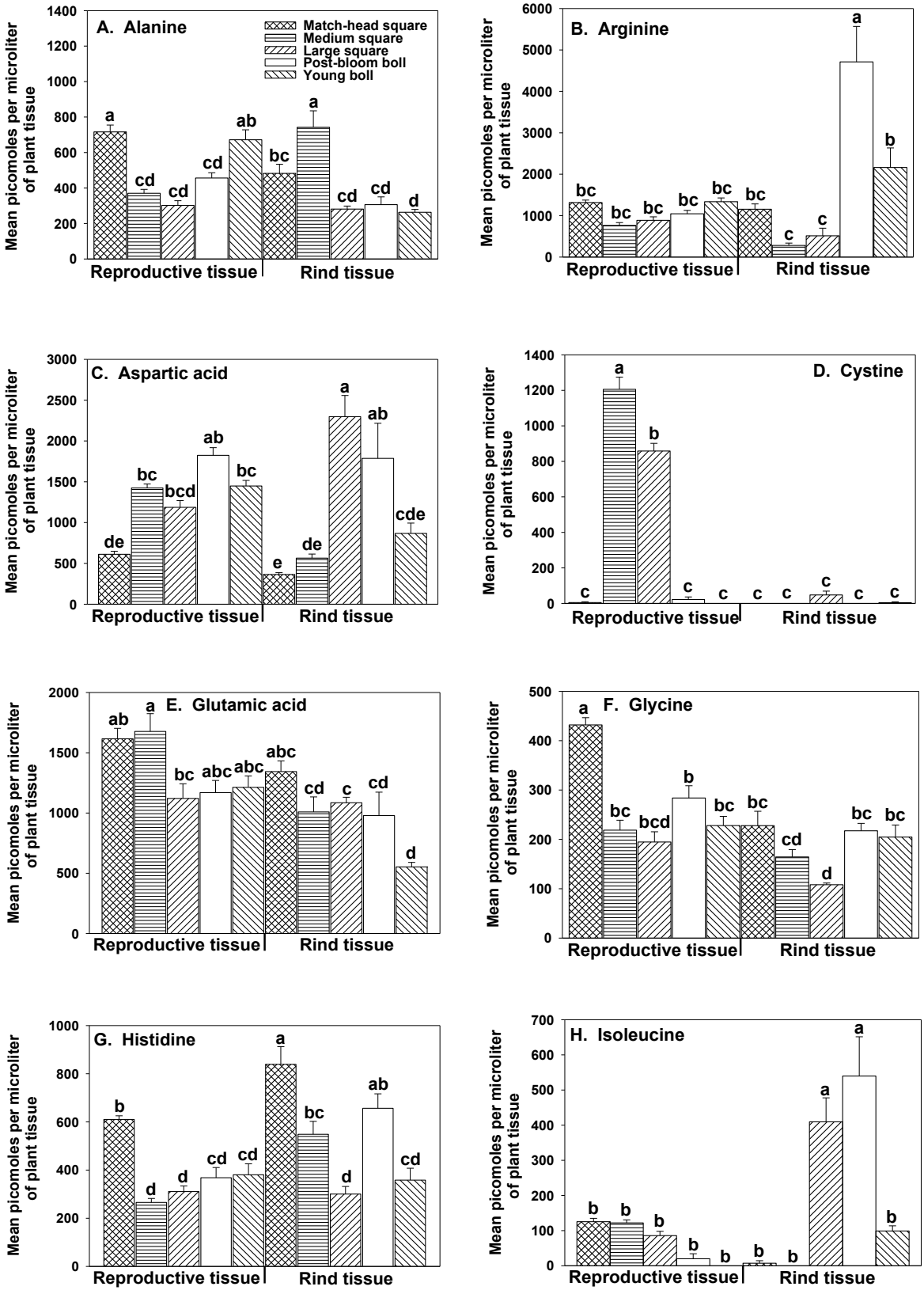
Boll weevil feeding on pinhead (1–2 mm diameter) and match-head squares under field conditions is negligible compared with the amount of feeding that occurs on medium and large squares (Showler, 2005). Hence, it is unlikely that the free amino acids available in those square sizes contribute substantially to boll weevil nutrition. Adult boll weevils were previously assumed to penetrate the rinds of cotton squares to feed on the anthers inside (Burks & Earle, 1965), but recent work demonstrated that in many instances the rind is not completely penetrated, carpel damage was not observed 65% of the time (Showler & Cantú, 2007), and adult female boll weevils exclusively fed rinds of cotton fruiting bodies became gravid, especially regarding rinds of large squares and post-bloom bolls (Showler, 2007). Despite the ability to become gravid by feeding on the rind of squares, fecundity was 2.2-fold greater when boll weevils fed on the reproductive portion of large squares than on the corresponding rind (Showler, 2008). Use of both the rind and reproductive portion of squares as food allows boll weevils access to free essential amino acids that are low in one tissue but not the other, such as free leucine in the inner reproductive portion of all square size categories and free phenylalanine in match-head and medium square rinds.

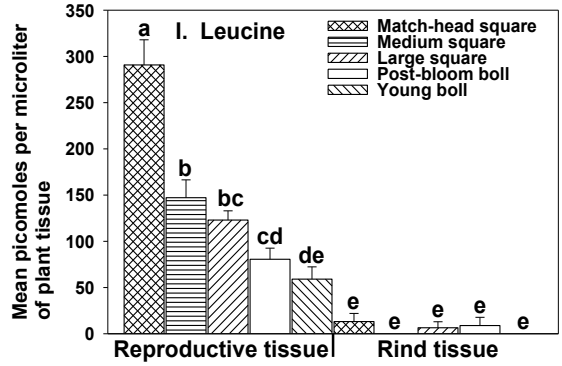
Although square rinds are relatively thin and can be penetrated (Showler & Cantú, 2007), rinds of bolls are considerably thicker (1.4 – 2.2-fold) and less apt to be penetrated by boll weevil mouthparts (Showler, 2007). Hence, when cotton fruit reaches the boll stage, boll weevils derive most of their nutrition from the rind in the absence of squares. In field conditions, however, numbers of squares are often available, albeit limited, even after cut-out when bolls predominate (Guinn, 1986; Cothren, 1999). While post-bloom bolls have relatively thin rinds (~1.4-fold that of large squares), the rinds contain substantially more free essential amino acids than the internal reproductive portion. Adult boll weevil longevity and fecundity, however, are not enhanced by feeding on the rinds over the reproductive portion (Showler, 2008), suggesting that total amounts of free essential amino acids might be less important than accumulations of specific FAAs. Once young bolls form, the rind thickness increases to 2.2-fold that of large squares (Showler, 2007), making it more unlikely that adult boll weevils have access to the nutrients in the reproductive portion. Injury to carpels within developing bolls frequently results (Showler, 2006) from adult feeding on squares (Showler and Cantú, 2008), but not necessarily bolls.

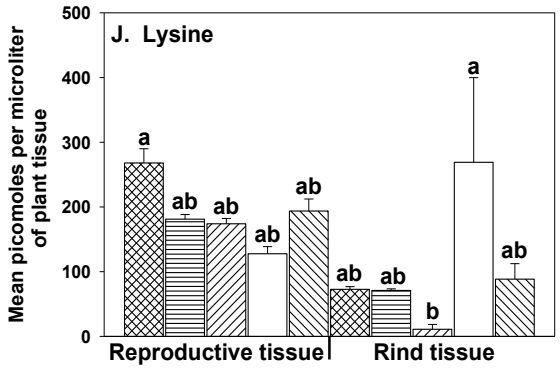
Tryptophan, an essential amino acid not identified from cotton using chemical methods, is present in cotton fruiting bodies as determined by microbiological assay (Parrott et al., 1969), and in boll weevils (Mitlin et al., 1966, 1968). However, the tryptophan content in the rind or the reproductive portion of various sizes and ages of cotton fruit has not been determined.

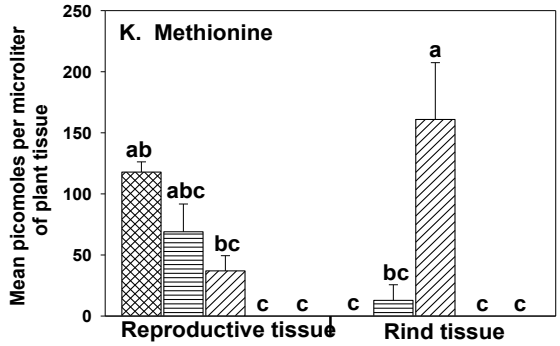
Tyrosine is synthesized from phenylalanine, hence phenylalanine can replace tyrosine, but not vice-versa (absence of phenylanaline in the absence of tyrosine stops growth) (Vanderzant, 1973). This study shows that while squares had some of both in the form of FAAs, levels were relatively low in boll rinds. Boll weevils that fed exclusively on post-bloom and young boll rinds nevertheless lived on average 68 and 32 d, respectively (Showler, 2008). When absent, lysine is the only amino acid reported to inhibit boll weevil growth (Vanderzant, 1973), but it was present in all treatments excluding the rind of large squares which still supported boll weevils for an average of 57 d (Showler, 2008). Deficiencies of certain FAAs might be compensated by the same amino acids in bound form (e.g., in peptides or proteins).

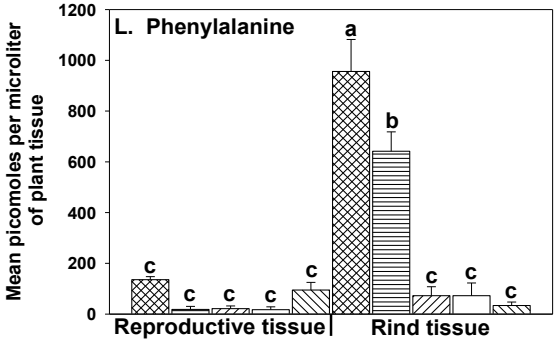
It is, however, likely that longevity was affected by the availability of individual FAAs and completeness of the set of essential amino acids in the diet. Total free essential amino acids were >1,000 pmole/ml of extract in each treatment, but longevity was lowest (<35 d) with the reproductive portion of post-bloom and young bolls, and with the rind of young bolls (Showler, 2008). Individual free essential amino acids such as histidine, threonine, and valine were available in every treatment and thus were probably not governing longevity. The other free essential amino acids were absent or detected at low levels in some fruiting body tissues that nevertheless kept adult boll weevils alive for >100 d (Showler, 2008) (e.g., free phenyla-

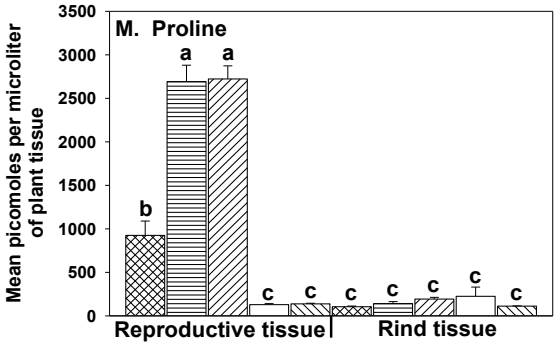

A. Alanine
Match-head square
Medium square
Large square
Post-bloom boll
Young boll
B. Arginine
C. Aspartic acid
D. Cystine
E. Glutamic acid
F. Glycine
G. Histidine
H. Isoleucine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue

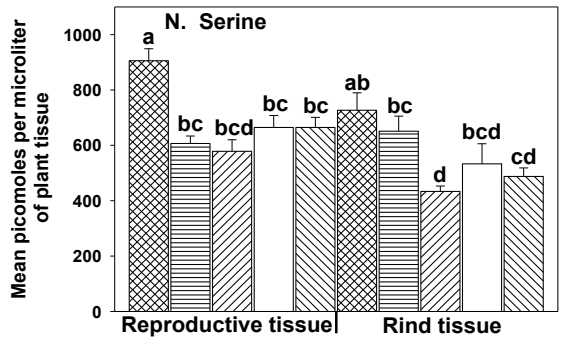

I. Leucine
Match-head square
Medium square
Large square
Post-bloom boll
Young boll
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue

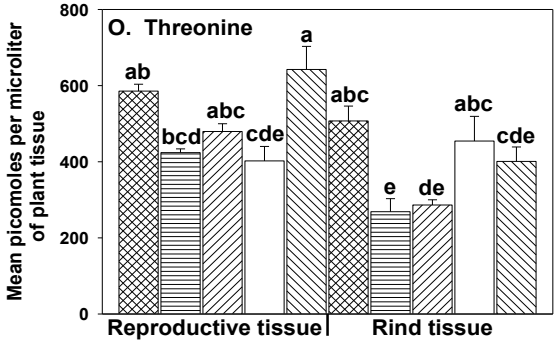

J. Lysine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue

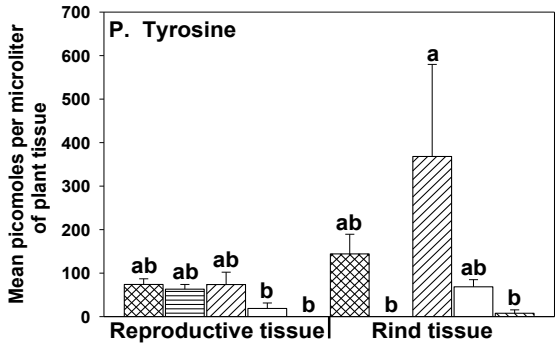

K. Methionine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue


L. Phenylalanine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue


M. Proline
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue


N. Serine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue


O. Threonine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue


P. Tyrosine
Mean picomoles per microliter of plant tissue
Reproductive tissue
Rind tissue

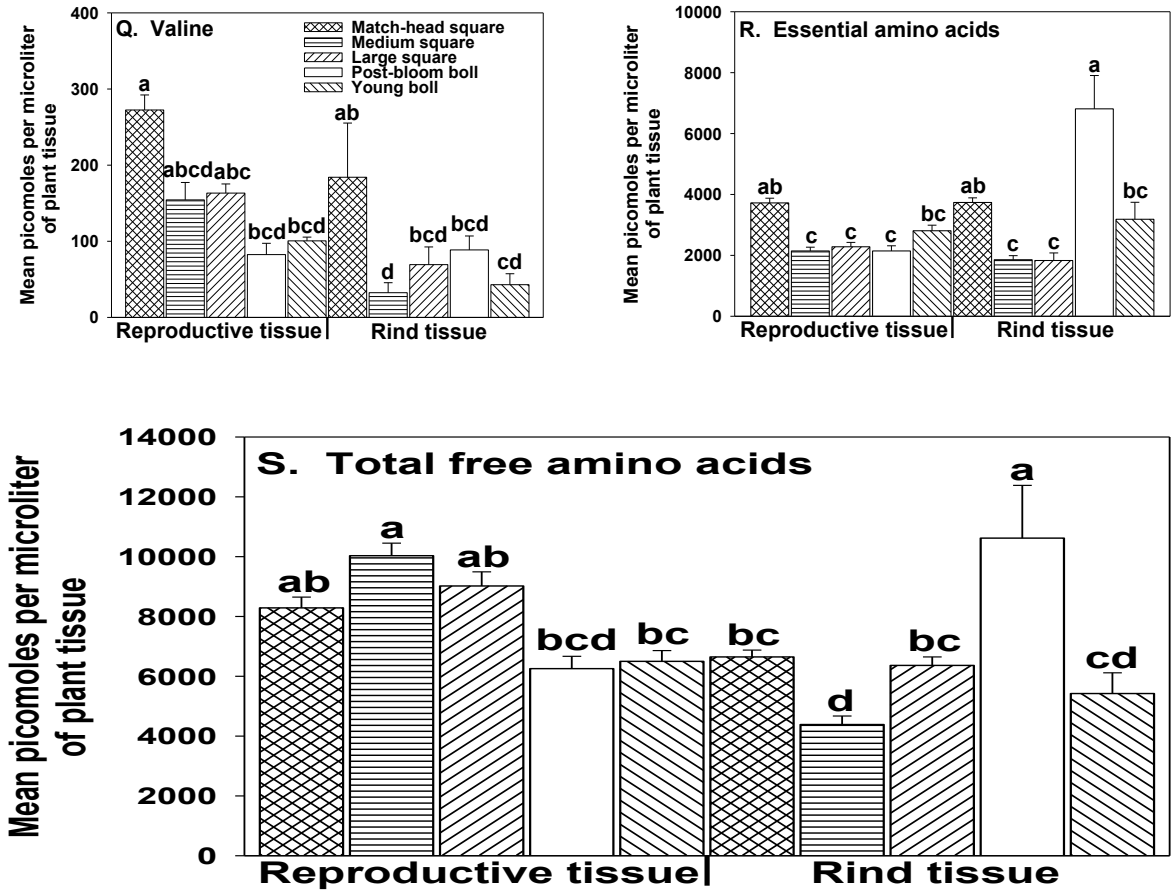


Fig. 1A–S. Mean (±SE) picomoles of 17 free amino acids per ml of the reproductive portions (in squares: anthers, stamens, style, and ovary; in bolls: ovaries and developing seeds and lint) or rinds (developing calyx and petals of squares; the outer casing, or husk, of bolls) of match-head (2–3 mm diameter), medium (3.1–5.4 mm diameter), and large (5.5–8 mm diameter) squares, and post-bloom (1–2 d after petal senescence), young (5–10 d old), and mature (3–5 wk old) bolls.

lanine in the reproductive portion of medium and large squares, and free lysine in the rind of medium squares) which indicates that those essential amino acids are available by ingestion of the bound form or synthesized by the weevil. Nutrients in addition to essential amino acids are also needed for optimal boll weevil health, including minerals (Vanderzant, 1965), biotin (Dadd, 1970), and lipids (Vanderzant & Richardson, 1964), and sugars (Vanderzant & Davich, 1961). While it is unwarranted to ascribe longevity to dietary free amino acids alone, lack of free methionine was consistently associated with average longevity of ≤64 d in contrast to average longevities of ³108 d in treatments that included free methionine.

The influence of nutrition on boll weevil reproduction, particularly with regard to amino acids, has been poorly understood, but fat-deficient diets are inferior to those with fat (Vanderzant & Richardson, 1964). Reduced dietary N reduces boll weevil egg production (Hilliard & Keeley, 1984) and when any essential amino acid is deleted from artificial boll weevil diet, no eggs are produced but eggs are laid when adults are supplied with the 10 essential amino acids plus glutamic acid and glycine as the only sources of N (Vanderzant, 1963). Cystine-, aspartic acid-, and glutamic acid-deficient diets are each known to suppress fecundity of *Drosophila melanogaster* Meigen (Sang & King, 1961). Because aspartic and glutamic acids were available in every treatment in this study, it is unlikely that their levels explain observed differences

in boll weevil fecundity.

Cystine is important to egg development in many insects (Dimond et al., 1956; Lea et al., 1956; Sang & King, 1961), and it is the major sulfur-containing amino acid of insect egg shells (Kawasaki et al., 1971; Inokuchi, 1972; Sumioka & Yoshitake, 1974). Methionine is metabolized to cystine for chorion formation (Ogawa & Tojo, 1981; Seo et al., 1998) and the two intermediates in the conversion, cystathionine and lanthionine, are incorporated into egg shell protein (Inokuchi, 1972; Shinbo, 1978). Free cystine was most abundant in the reproductive portion of medium and large squares which corresponded with enhanced boll weevil fecundity. Although free methionine was present in the reproductive portion of match-head squares, fecundity of boll weevils fed the reproductive portion of match-head squares was relatively low (Showler, 2008). Unlike in *D. melanogaster*, free methionine in boll weevils might not be converted to free cystine efficiently, or free methionine is used instead for other metabolic or structural functions in boll weevils. While both free methionine and free cystine were detected in the reproductive portion of squares, neither was found in leaf tissue (Showler, 2001, 2002b; Showler & Moran, 2003) which, as a source of food, does not result in egg development (Showler & Abrigo, 2007). The changing levels of dietary cystine and methionine in the progression of fruiting body development appear to influence boll weevil fecundity.

Individual dietary FAAs are not solely responsible for degrees of weevil health and egg production, which is true for other insects (Vanderzant, 1958; Dadd, 1973; Chang, 2004), and the magnitude of increase in certain FAAs may not proportionally match the magnitude of changes in longevity and fecundity. The roles of methionine and cystine in egg production, however, are likely significant to the longevity and reproductive capacity of boll weevils when large squares become available under field conditions (Showler et al., 2005) as a result of heightened fecundity (Showler, 2004). It is conceivable that cotton bred for reduced levels of cystine, methionine, or both, in cotton fruiting body tissues can retard egg production and possibly curtail it.

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