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Nonprotein Amino Acid Composition of Flatpea (*Lathyrus sylvestris* L.) as Affected by Ethephon Seed Treatments and Seedling Fertilization

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Abstract. Use of flatpea (*Lathyrus sylvestris* L.) as a forage is limited because of nonuniform seed germination and the potentially toxic effects of 2,4-diaminobutyric acid (A_2bu), a nonprotein amino acid found in seeds and vegetative tissues. The effects of ethephon (2-chloroethyl phosphonic acid) on seed germination, amino acid leachates of seeds, and amino acid composition (particularly A_2bu) of seedlings were investigated. Germination of flatpea seeds, imbibed for 16 h in 0, 100, 200, 400, 800, and 1600 mg/L ethephon, did not differ, but amino acid leachates tended to increase up to 200 mg/L ethephon and then decline at higher concentrations. The major amino acid constituents in leachates were A_2bu, 4-aminobutyric acid (Abu), and homoserine (Hse). Dry matter accumulation of seedlings grown from ethephon-treated seeds was reduced for second cuttings grown from ethephon-treated seeds and high nitrogen grown plants. During regrowth, free amino acid accumulation was most pronounced in leaves of plants supplied with high nitrogen. The most abundant free amino acids in flatpea tissues were the same as those in seed leachates, but concentration and relative abundance varied with nitrogen level, plant part, and ethephon treatment. Results suggest that ethephon seed treatments can have persistent effects on the growth and amino acid composition of flatpea seedlings grown under different nitrogen regimes.

Flatpea (*Lathyrus sylvestris* L.) is a deep-rooted, perennial legume that has potential as a forage species for marginal lands, such as those that occur in the Appalachian region of the United States. Slow and nonuniform seed germination and the occurence of 2,4-diaminobutyric acid (A_2bu), a potentially toxic nonprotein amino acid (Miller et al., 1948; O'Neal et al. 1968), have limited its use for feeding ruminant livestock (Grunder and Dickson 1948). A_2bu occurs at levels of 3% or more of the dry weight of flatpea seeds and vegetative tissues (Ressler 1964) and has been reported to promote the growth of specific rhizobia that nodulate flatpea roots (S. F. Wright, personal communication). Thus, leakage of such amino acids from the seed during imbibition and germination may have a significant effect on rhizobial establishment and seedling growth.

Seed germination can be hastened in some legumes and numerous weed species by treatment with ethylene or the ethylene-generating compound, ethephon (2-chloroethyl phosphonic acid), prior to germination (Egley 1984; Globerson 1977; Hargurdeep et al. 1985a,b; Taylorson 1979). Ethephon has also been reported to enhance precipitable protein levels, amino nitrogen, and de novo synthesis of nitrate reductase in potato roots and stems, but not leaves (Palmer 1985). Both nitrogen source and concentration can also influence amino acid composition (Haynes and Goh 1978). If flatpea is to be used as a forage crop, improved germination, seedling establishment, and cultural methods for controlling concentrations of potentially toxic nonprotein amino acids, such as A_2bu, (Przybylska and Rymowicz 1965; Ressler 1964) in the tissues are important. Hence, the purpose of the present study was to determine the influence of ethephon on flatpea seed germination, leakage of amino acids from imbibed-treated seeds, and the subsequent effects of ethephon seed treatments and nitrogen fertilization on the amino acid composition of vegetative tissues.

Table 1. Amino acid leakage from flatpea seeds after a 16-h imbibition in different concentrations of ethephon.

Amino acid	Ethephon concentration (mg/L)					
	0	100	200	400	800	1600
	Concentration (μg/50 seeds)					
Ser	0.4 b	0.4 b	0.5 a	0.4 b	0.4 b	0.2 c
Asp	0.8 b	0.9 b	1.1 a	0.8 b	0.8 b	0.2 c
Unk 1	0.7 cd	1.0 b	1.2 a	0.9 b	0.8 bc	0.5 d
Ala	1.1 b	1.3 b	1.7 a	1.2 b	1.1 b	1.2 b
Unk 2	1.6 b	1.9 b	2.5 a	1.8 b	1.9 b	0.2 c
Asn	2.2 ab	2.8 a	3.0 a	2.6 a	2.1 ab	1.1 b
Hse	160 c	260 b	320 a	260 b	260 b	220 b
Abu	520 b	560 b	790 a	550 b	500 b	510 b
A_2bu	6130 b	3600 b	10,370 a	5130 b	3750 b	2400 b

Values followed by the same letter within rows are not significantly different at the 0.05 level as determined by ANOVA. Separation of means was by LSD. Unknowns were calculated based on Asp equivalents.

Materials and Methods

Seed Imbibition Treatments and Growth of Seedlings

Groups of 50 medium-sized seeds (average weight, 2.1 g per 50 seeds) were selected and acid-scarified with concentrated H_2SO_4 for 20 min. The acid was decanted and the seeds rinsed three times with deionized-distilled water. Seeds were surface-sterilized with Clorox and water (1:5 vol/vol) for 10 min at 50°C and rinsed three times with 20 ml of sterile deionized-distilled water.

Ethephon solutions were prepared at concentrations of 0, 100, 200, 400, 800, and 1600 mg/L with deionized-distilled water. At the time of seed imbibition, ethephon solutions were adjusted to pH 6 with 0.1 N KOH, and 25 ml of each ethephon concentration were added to vials containing 50 seeds. There were four replications of each treatment. Vials were capped and incubated at 21°C with continuous agitation; and, after 16 h the imbibition solutions were decanted and frozen for subsequent amino acid analysis. Seeds were transferred to sterile Petri dishes containing two layers of Whatman no. 2 filter paper, previously moistened with deionized-distilled water. The dishes containing treated seeds were placed in an incubator at 25°C. Seeds were considered germinated when the tip of the radical was visible. Germination counts were performed daily.

Thirty germinated seeds from each of six treatments were divided equally (10/pot) among three 15 × 12 cm plastic pots. Pots were filled with a peat:perlite (1:1) mix. One pot from each treatment group was watered biweekly with 200 ml of one of three nutrient solutions containing different forms and concentrations of nitrogen (Clark 1982; Munns 1968; Steinberg 1953). The nitrogen forms and concentrations were (NO_3^-/NH_4^+ mM): Clark, 22.9/28; Munns, 1.0/2.0; Steinberg, 3.7/0.3. Differences in concentrations of other elements in these solutions were not considered important, compared to nitrogen, relative to their effects on amino acid levels. These nutrient solutions were selected because of the different forms and concentrations of nitrogen, the balance of other macro- and micronutrients, and their ability to support growth of legumes.

At approximately 5 weeks, shoots were cut just above the bottom set of leaves (first harvest), and the leaves and stems were then separated, weighed, frozen, ground in liquid nitrogen, and extracted in 100% ethanol. Following a 5-week regrowth period, the 10 plants in each pot were harvested in their entirety and separated into leaves, stems, and roots. Tissues were processed as above, except 80% (vol/vol) aqueous ethanol was used as an extraction solvent to improve extraction efficiency (Foster 1989).

Plant extracts were analyzed by high-performance liquid chromatography (HPLC) as outlined previously (Shen et al. 1989). Briefly, amino acid extracts were partially purified by passing them through a C-18 Sep-Pak (Waters Associates, Milford, MA, USA) eluted with 0.5 ml water and 1.0 ml methanol. Twenty microliters of eluent was reacted with 0.1 ml *o*-phthalaldehyde for 90 s and then injected onto an Altex (San Ramon, CA, USA) Ultrasphere-ODS reverse-phase analytical column (4.6 × 250 mm, 5 μm) and monitored using fluorescence detection (305–395 excitation and 430–470 emission). Amino acids were quantified and identified by comparing with known standards. Recovery was estimated at 70–80% based on penicillinamine used as the internal standard.

Results

Imbibition of seeds in differing concentrations of ethephon had no significant effect on germination rate or percentage (unpublished observations). After 8 days approximately 50% of the seeds germinated in all treatments.

Table 1 summarizes the concentrations of several amino acids in leachates following 16-h seed treatments at the specified concentrations of ethephon. The most abundant amino acid detected in the leachates was A_2bu followed by 4-aminobutyric (Abu), homoserine (Hse), and asparagine (Asn). Smaller

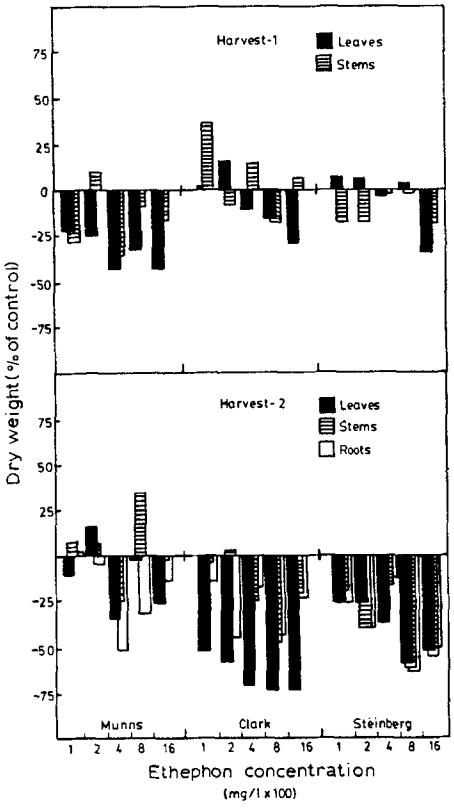

Fig. 1. The influence of ethephon seed-imbibition treatments and nitrogen source on subsequent seedling growth. Values are the combined dry weights of 10 plants in each treatment expressed as a percent of control. Control values in g were Harvest-1: Munns-leaf 0.65, stem 0.16; Clark-leaf 0.58, stem 0.15; Steinberg-leaf 0.49, stem 0.15; Harvest-2: Munns-leaf 0.81, stem 0.35, root 0.54; Clark-leaf 1.85, stem 0.37, root 0.56; Steinberg-leaf 1.15, stem 0.53, root 0.66.

amounts of alanine (Ala), two unknowns (Unk 1 and 2), aspartic acid (Asp) and serine (Ser) were also detected. Except for Asn, amino acid leakage was significantly higher at 200 mg/L ethephon compared to other treatments. Amino acid leakage tended to increase up to 200 mg/L ethephon and then declined at higher rates. Several amino acids (Ser, Asp, Unk 2, Hse) were significantly lower in the 1600 mg/L treatments compared to controls.

Ethephon seed treatments generally reduced dry matter accumulation in all tissues, but the effect was most pronounced in tissues of the second harvest (Fig. 1). In the first harvest, the greatest reduction in dry weight was observed in plants grown in Munns'(3 mM N) nutrient solution. In the second harvest, the greatest reduction in dry weight was in plants grown in Clark's (50.9 mM N) followed by Steinberg's (4.0 mM N) and Munns' media. There was a trend toward greater growth inhibition as the

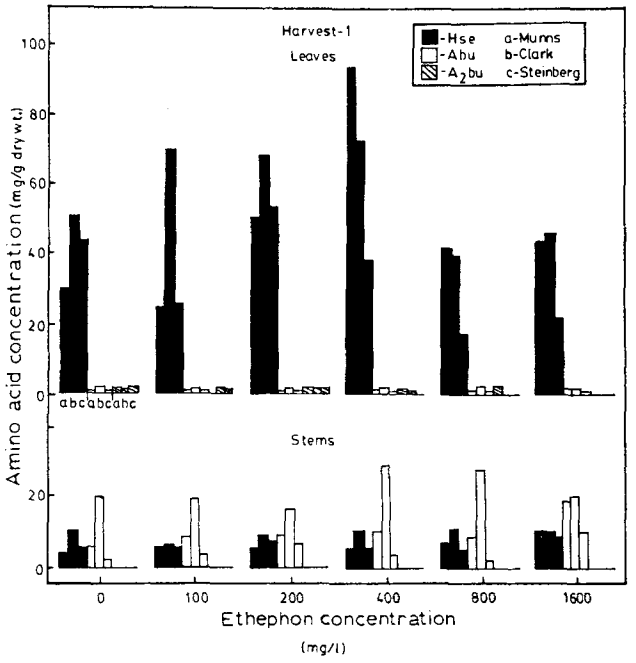

Fig. 2. The influence of ethephon seed-imbibition treatments and nitrogen source on Hse, Abu, and A_2bu concentrations in plant tissues (Harvest-1) derived from treated seeds.

concentration of ethephon increased. This was particularly evident in plants of the second harvest fertilized with Clark's and Steinberg's solution.

The free amino acids detected in the tissues of flatpea plants grown from seeds imbibed with ethephon included: Asp, glutamic acid (Glu), Asn, Hse, Ala, Abu, and A_2bu. As with the seed leachates, the most abundant amino acids detected in all tissues were Hse, Abu, and A_2bu (Fig. 2).

In the first harvest, leaves had higher concentrations of Hse and A_2bu than the stems in most treatments (Fig. 2). A_2bu was not detected in stems. The concentration of Abu was higher in the stems than the leaves for all treatments. The relative concentration of Abu to Hse was higher in the stems than in the leaves. Plants treated with high nitrogen or NH_4^+ fertilizers (Munns and Clark solutions) frequently exhibited higher concentrations of amino acids than those treated with only NO_3^- at low levels (Steinberg solution). This was particularly true of Hse in the leaves and Abu in the stems. Ethephon, up to 400 mg/L, tended to increase levels of Hse in the leaves, whereas higher concentrations caused a general decline in Hse.

In the second harvest, leaves had the highest concentrations of Hse and A_2bu followed by stems and roots (Fig. 3). Stems and roots both exhibited higher concentrations of Abu than the leaves. The

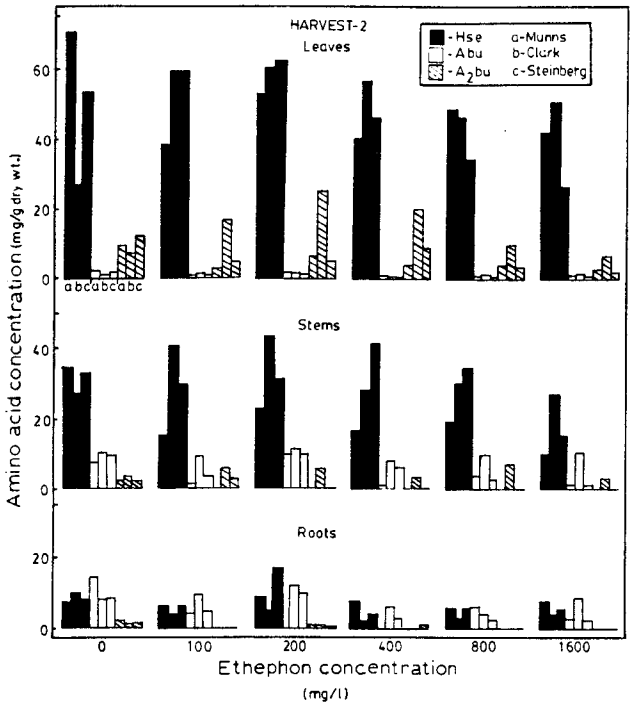

Fig. 3. The influence of ethephon seed-imbibition treatments and nitrogen source on Hse, Abu, and A_2bu concentrations in plant tissues (Harvest-2) derived from treated seeds.

relative proportion of Abu to Hse in the stems was much less than that observed in the stems of the first harvest for all treatments. High nitrogen (Clark solution) resulted in elevated levels of A_2bu in the leaves and Hse, Abu, and A_2bu in the stems of most ethephon treatments. In the roots, Hse concentrations were less in the high nitrogen treatments. Total free amino acids averaged over dosages of 0, 100, and 200 mg/L ethephon resulted in increases in leaves, stems, and roots of 25, 30, and 90%, respectively, compared to averages over the higher dosages (400, 800, and 1600 mg ethephon/L).

Discussion

Although acid scarification improves the germination rate and percentage in flatpea seeds, after 12 days only 50% of the controls germinated, indicating factors other than hard seed coats affected germination. Embryo dormancy appears to be one of the major factors contributing to seed dormancy in legumes (Globerson 1977). Seed treatments, using ethylene or ethylene-generating compounds, KNO_3 or combinations of the two, have met with some success in increasing germination rates and percentages in a number of different plant species (Egley 1984; Globerson 1977; Hargurdeep et al. 1985a,b; Taylorson 1979). The results of the present study indicate that the ethylene-generating chemical, ethephon, does not affect the germination percentage or rate in flatpea under the conditions employed. Consideration of treatment temperature and embryo maturity has proven important in other studies (Globerson 1977).

Ethylene is known to increase membrane permeability in plant cells (Suttle and Kende 1980) and to bind to membranes of bean cotyledon protein bodies allowing the entrance of proteolytic enzymes (Evans et al. 1982). Such a mechanism may explain the leakage of amino acids from flatpea seeds at the lowest ethephon concentrations but does not explain reduced leakage at elevated levels. Recent studies indicate that A_2bu stimulates the growth of specific rhizobia that colonize the roots of flatpea (S. F. Wright, personal communication). Thus, leakage of A_2bu, which was the most abundant amino acid in flatpea leachates, may be important in the establishment of nodulation.

Growth of flatpea seedlings derived from ethephon-imbibed seeds was inhibited. The effect was concentration-dependent, occurred in all tissues, and was more pronounced in the second cutting. Similar effects with ethephon have been reported for purslane (*Portulaca oleracea* L.), wild oat (*Avena fatua*), and *Lathyrus sativus* (Choudhuri 1972; Egley 1984; Hargurdeep et al. 1985a).

Both nitrogen concentration and source can influence protein and amino acid composition in plants (Haynes and Goh 1978). Thus, the high concentrations of amino acids in the leaves and stems of flatpea plants grown in high nitrogen or NH_4^+ probably reflects the greater availability of nitrogen for amino acid synthesis.

Ethephon enhances levels of precipitable protein, amino nitrogen, and de novo synthesis of nitrate reductase in potato roots and stems but not leaves (Palmer 1985). Thus, it is conceivable that nitrate reductase is stimulated in flatpea at ethephon concentrations of up to 400 mg/L resulting in elevated levels of amino acids. At higher ethephon concentrations, nitrate reductase may be inhibited resulting in lower levels of amino acids. Such affects on amino acid synthesis may be important in view of the numerous environmental stresses that can influence natural ethylene production in plants (Hale and Orcutt 1987).

In the present study, there appears to be no advantage to pretreating flatpea seeds with ethephon for increasing germination. Such treatments were detrimental to seedling growth and resulted in the accumulation of nonprotein amino acids, including the potentially toxic amino acid A_2bu. Also, it is

apparent that the type and amount of nitrogen-containing fertilizer applied to plants can substantially affect the levels of such compounds and potentially affect the quality of forage.

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