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FREE AMINO ACIDS IN THE LEAF TISSUE OF *EUCALYPTUS BLAKELYI*

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Key Word Index—*Eucalyptus*; Myrtaceae; amino acids; nitrogen; insect feeding.

Abstract—The free amino acid composition and concentration in young and mature leaves of *Eucalyptus blakelyi* were determined. These data are compared with published data on the total nitrogen in similar tissue.

INTRODUCTION

Variation in total nitrogen of the host plant, induced by leaf age or environmental conditions, has been reported to influence phytophagous insect population dynamics [1, 2]. The performance of two species of aphids has been associated with host plant age and changes in soluble *N* and individual amino acids [3]. Sap-sucking psyllids (Insecta: Homoptera), of the genus *Glycaspis* favour young foliage [4, 5]. In order to investigate further the hypothesis that this preference is a function of variation in free amino acid composition and concentration with leaf age, it is desirable to quantify the amino acids in leaf tissue of the host tree species of *Glycaspis*, *Eucalyptus blakelyi* Maiden. In this communication are presented the results of an investigation into the free amino acid content of young and mature leaves of the host.

RESULTS AND DISCUSSION

The results presented in Table 1 using an amino acid analyser indicate that young leaves contain an overall concentration of free amino acids which is *ca* 17% greater than that exhibited by mature leaves. There is a lack of precision in these data because base line irregularities and overlapping peaks produced some difficulty in calculating exactly the concentration of amino acids from tryptophan onwards.

The main differences between young and mature leaves occurs in glutamic acid, asparagine and, especially, isoleucine whose concentrations in mature leaves are over twice those in young leaves. Compensating for this in terms of total amino acids, however, in young leaves, are threonine, serine and, especially, alanine, proline and methionine whose concentrations are over twice that exhibited by mature leaves. The six dominant amino acids in this study were proline, arginine, alanine, glutamine, phenylalanine and methionine, the first three also being dominants in lemon leaves [6]. None of these 6, however, were among the dominants in *Ricinus communis* [7], where glutamic acid, aspartic acid and threonine accounted for most of the amino acid content.

The concentration of total nitrogen in *Eucalyptus blakelyi* leaves falls between 1.33 and 1.85% on a dry weight basis [8]. Using the conversion of N to protein constant of 6.25, this range represents 8.31–11.56% protein. The total free amino acid concentration in Table 1 is above that observed during the noon peak in diurnal variation of soluble protein in *Picea glauca* [12], 1.4% fr. wt, or lemon [6], 3% dry wt. A comparison of this range in total protein with the data in Table 1 showing total free amino acids reveals that the latter represents 33–50% of the total leaf protein. This is considerably higher than the value of 20% reported for lemon leaves [6].

It has been suggested that the analysis of leaf material should provide a guide to the levels of soluble nitrogen in the phloem [9]. A similar assumption is made in the extrapolation of these results to amino acid in the phloem.

The observation that there is a difference in the concentration of various amino acids between young and mature leaves is in agreement with the hypotheses that differences in growth rate of insects on these food sources may be a function of nutritional variability. The high

Table 1. Free amino acid content of *Eucalyptus blakelyi* leaves expressed on a dry wt basis

	Young leaves		Mature leaves		
	µg/g	As % of total	µg/g	As % of total	Young/ Mature
Aspartic acid	924	2.1	—	—	—
Threonine	1368	3.1	631	1.7	2.2
Serine	1363	3.1	564	1.5	2.4
Asparagine	940	2.1	1797	4.8	0.5
Glutamic acid	1645	3.7	3408	9.0	0.5
Glutamine	3935	8.9	4299	11.4	0.9
Proline	5145	11.7	2382	6.3	2.2
Glycine	662	1.5	748	2.0	0.9
Alanine	5690	12.9	1665	4.4	3.4
Valine	2297	5.2	2098	5.6	1.1
Leucine	1312	3.0	831	2.2	1.6
Isoleucine	1556	3.5	6524	17.3	0.2
Tyrosine	2401	5.4	2261	6.0	1.1
Phenylalanine	3250	7.4	3107	8.2	1.1
Methionine	3488	7.9	696	1.8	5.0
Tryptophan	488	1.1	1355	3.6	0.4
Lycine	455	1.0	752	2.0	0.6
Histidine	1360	3.1	361	1.0	3.8
Arginine	4094	9.3	1573	4.2	2.6
Ornithine	1771	4.0	2735	7.2	0.7
Total	44.1 mg/g		37.8 mg/g		

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proportion of total leaf amino acid nitrogen which was found to be present as free form suggests, further, that variation in concentration of these substances, as reported to occur diurnally in *Picea glauca* [12], may also influence population dynamics in insects which consume the entire leaf and acquire all protein present.

In relation to sap-sucking insects it should be noted also, however, that a significant proportion of the total amino acid content available to insects from the phloem comprises protein. In *Ricinus communis*, for example, about one third is in the form of protein [7]. Analyses of a number of species suggest that much of this is P-protein [7, 10, 11]. Information on the variability of this substance and its significance for insect nutrition is not available.

EXPERIMENTAL

Young leaves are those still expanding. Mature leaves are fully expanded and darker green, at least 2–3 months old. As soluble nitrogen varies diurnally [12], samples were collected at dawn from the north quadrant of a mature tree, 2.5 m above the ground, where the xylem pressure potential was consistently −2 to −3 bars. One sample comprised 10 or more leaves, depending on size. Two samples of each age class were taken. Leaves were collected at random but those with evidence of other organismic activity were discarded. Leaves were excised from shoots and stems and petioles discarded. Leaf areas were determined in the field by means of a Lambda L1300 Leaf Area Meter (Lambda Instruments Corp., Nebraska) for 4 subsamples of *ca* 3 g each which were sealed in polythene bags. One was immediately packed in dry ice and all were returned to the laboratory. Fresh and dry wts (72 hr at 105°) were taken for the 3 non-analytical samples, thus allowing estimation of these parameters for the analytical sample.

The analytical sample was stored at −20° until processing. Then the sample was treated with *ca* 250 ml liquid N_2 and ground for about 2 min with 4 g of acid washed sand. To ground material 10 ml of H_2O were added followed by 10 ml 0.1 mM norleucine to check amino acid recovery rate (mean 4.0% but variable) to this, 20 ml MeOH were added and the suspension washed into a 250 ml separating funnel with 10 ml 50% MeOH. To this an extraction was performed by adding 50 ml of $CHCl_3$, the soln was then gently swirled, allowed to stand and the $CHCl_3$ layer discarded. This lipid extraction was repeated at least once.

To this soln trichloroacetic acid (TCA) was added to produce a 5% w/v soln, this being allowed to stand for 60 mins. The soln was then centrifuged at 8000 K for 20 mins and the supernatant carefully decanted into a measuring cylinder and the vol. recorded. Here it may be saved. From this a sub-sample of 10 ml was pipetted to rotovap down to 2–3 ml. This was then made up to 10 ml with pH 2.2 buffer (0.05 M Na citrate). In order to overcome a serious problem of high phenolic content the sample was then passed through a 5 cm column of Dowex 50 W × 8 (200–400 mesh), which was washed with 15–20 ml H_2O and then subjected to an eluting medium of 15 ml 2.0 M NH_4NO_3. The first 10 ml of the sample through the column was collected and evapd to dryness in a rotovap in a H_2O bath at 30–35°. This was then made up to 10 ml using Na citrate buffer. The column was restored with HCl. This soln was then subjected to a Beckman 120C amino acid analyser and calculations performed according to the manual (A-1M-3).

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