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CHEMICAL ANALYSIS OF PEACH EXTRAFLORAL NECTARY EXUDATE*

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Abstract—Analyses of peach leaf nectary exudate confirmed the presence of seven carbohydrates. These included two amino sugars and inositol which have not been previously reported in nectary exudate of plants. Seventeen amino acids, and seven fatty acids were also detected. The major carbohydrate fraction (in decreasing order of concentration) consisted of fructose, glucose and sucrose.

INTRODUCTION

Extrafloral nectaries (EFN) are characteristic of certain species in at least 39 families of flowering plants [1]. These specialized structures are found on the leaf blade, leaf margin, petiole, rachis, stem, stipules, cotyledons and outer flower parts such as the calyx, corolla and fruit [1, 2]. Many varieties of peach, *Prunus persica* (L.) Batsch, have nectaries at the base of the leaf blade and on the petiole [3]. EFN exude a fluid, similar to floral nectar which contains carbohydrates and amino acids. Because of their location on the plant, EFN in contrast to floral nectaries, are not considered a factor in attracting insects to initiate pollination [1]. The true function of EFN has been debated with two factions attempting to determine if their function is physiological or if their presence is due to an evolved symbiotic relationship with ants. The ants reportedly protect the plant from marauding insects and herbivores, and in return, obtain nectar as food [1, 4, 5].

RESULTS AND DISCUSSION

Carbohydrates

Glucose, fructose and sucrose were found as well as small quantities of inositol, arabinose and two amino sugars, *N*-acetylglucosamine and *N*-acetylgalactosamine (Table 1). This is the first report of amino sugars and inositol in extrafloral exudate. Knapheisowana [6] found sugars in the exudate of EFN from five species of *Prunus* other than *P. persica*, but did not specify which sugars. Putman [7], using paper chromatography, identified glucose, fructose and sucrose in the exudate of peach EFN.

Glucose, fructose and sucrose are usually present in nectar, but the ratio of these three sugars varies from species to species. In peach EFN exudate, sugar concentrations decreased *ca* 50 mM with fructose > glucose > sucrose (Table 1). Wykes [8] found fructose ≃ glucose > sucrose in exudate from peach flower nectaries. Pickett and Clark [4] found glucose ≃ fructose > sucrose in exudate from EFN of a cactus, *Opuntia acanthocarpa* Engelm. et Bigelow var. *major* (Engelm. et Bigelow) Benson. On a molar basis, glucose > fructose > sucrose in stipular exudate of *Impatiens scabrida* DC. [9]. EFN exudate from two species of cotton, *Gossypium* spp., also contains glucose > fructose > sucrose [10]. An exception in this trend is reported by Elias and Gelband [2] who observed varying ratios of sugars in four EFN systems of trumpet creeper, *Campsis radicans* (L.) Seem. They found all three sugars present, but the exudates (depending on the EFN system), were either sucrose dominant or contained equal concentrations of all three sugars.

Carbohydrate concentrations in peach EFN exudate samples totaled 1.27 and 1.41 mol/litre (M). These values are within the range of carbohydrate concentrations from cotton EFN exudate; values of 0.9, 2.24 and 2.46 M were

Table 1. Carbohydrate composition of hydrolysed peach leaf nectary exudate

Carbohydrate	Concentration (mM)	
	Sample 1*	Sample 2†
Fructose	518.7	455.7
Glucose	466.8	424.1
Sucrose	409.7	372.4
Inositol	6.6	6.1
Arabinose	6.4	5.9
N-Acetylglucosamine	5.2	4.7
N-Acetylgalactosamine	0.4	0.4
Total	1413.9	1269.3

*50 μl sample of exudate collected on September 17, 1979.
†Total of 50 μl of exudate collected on September 26, 1979 and October 1, 1979.

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reported by Butler *et al.* [10]. Carbohydrate concentration in *Impatiens* stipular exudate was *ca* 1.45 M [8]. Carbohydrate concentrations in *Opuntia* EFN exudate was 10.0 ± 1.4 M [4].

Amino and fatty acids

Analyses revealed 17 amino acids (Table 2) in both peach EFN exudate samples. As with the carbohydrate samples, the sample collected later was less concentrated. Amino acids have been found to be a typical component of flower nectar, with 260 positive ninhydrin tests on nectar from 266 species [11].

Amino acid composition of peach EFN exudate is similar to that of EFN exudate from cotton [12] and *Opuntia* [4] with 14 amino acids common to all three species. Amino acid concentration in peach EFN exudate was 0.92 and 2.05 mM, slightly lower than in cotton EFN exudate which averaged 3.65 mM [12]. *Opuntia* EFN exudate is rich in amino acids with 169.8 mM and ranged from 55 to 1009 mM [4].

Our results support the conclusion of Baker and Baker [13], that serine, glycine, alanine, aspartic and glutamic acids are common constituents in nectar. These five amino acids were present in the greatest concentrations, compared to the concentrations of the 12 remaining amino acids, other than proline. Tryptophan, phenylalanine, methionine and proline, which were reported as uncommon in flower nectar [13], were found in peach EFN exudate.

The following fatty acids were present (mM) in exudate from peach EFN exudate: lauric, 0.64; myristic, 0.21; palmitic, 0.47; palmitoleic, 0.16; stearic, 0.14; oleic, 0.04; and linoleic, < 0.04. Linolenic and arachidonic acids were not detected. Total fatty acids equaled 1.69 mM. No previous reports were found that included the detection and measurement of fatty acids in EFN exudate.

Table 2. Amino acid composition of hydrolysed peach leaf nectary exudate

	Concentration (mmol/litre)	
Amino acid	Sample 1*	Sample 2†
Aspartic acid	0.85	0.14
Glutamic acid	0.24	0.11
Glycine	0.21	0.11
Proline	0.13	0.06
Serine	0.12	0.08
Alanine	0.12	0.14
Valine	0.08	0.05
Threonine	0.07	0.05
Cystine	0.00	Trace
Methionine	0.01	0.00
Isoleucine	0.03	0.02
Leucine	0.05	0.05
Tryosine	0.02	0.03
Phenylalanine	0.05	Trace
Histidine	0.01	0.01
Lysine	0.03	0.07
Arginine	0.02	0.02
Tryptophan	0.01	0.00
Total	2.05	0.92

*Sample was a total of 50 μl of exudate collected July 30, August 6, 9, 12, 13, 1979.

†Sample was a total of 44 μl of exudate collected October 1, 3, 8, 1979.

One must remember that, the aforementioned quantitative comparisons are useful only as a relative measure, since concentrations of chemicals in EFN exudate are most likely influenced by the same factors which cause variation in flower nectars [14]. These factors include environmental conditions, season, species variability, stage of growth and cultural practices.

EXPERIMENTAL

Collection of nectar. Nectary exudate was collected (July–Oct 1979) and stored for the analyses in 5 μl micropipettes. Exudate samples were collected between 0700 and 0930 hr from 25 two-year old 'Earli-Glo' peach trees at the University of Missouri South Farm in Columbia and were kept frozen until analysed. Due to variations in the amount of exudate produced, collections were made on several dates. Each tree was fertilized on May 24 with 450 g of 12-12-12 (N as NO_3 form) and 450 g of $CaCO_3$. Pesticides were not applied to these trees.

Carbohydrate analysis. Total carbohydrate composition was determined by using a modified GLC technique [15]. With this procedure, individual amino and neutral sugars were separated and identified. Two 50 μl samples were analysed independently. The first sample was collected on Sept. 17 and the other combined sample was collected on Sept. 26 and Oct. 1.

Amino and fatty acids. Amino acids were analysed by automated cation exchange chromatography using a Beckman® 121-M Amino Acid Analyzer following the methodology of ref. [16]. Nectar samples were hydrolysed with 6 N HCl for 22 hr at 110° prior to derivatization. Single analyses were performed on each sample with one reanalysed to check the precision of the method.

One sample, 36 μl, collected on Sept. 17, was analysed for fatty acids using the microdetermination method of ref. [17]. According to this procedure, a measured amount of heptadecanoic acid (C17:0), was used as the internal standard. The sample was then hydrolysed, acidified and the fatty acids were extracted into hexane. The fatty acids in the hexane extract were then extracted into 10 μl of TMTFTH and 1 μl of this extract was injected onto the GLC column. The small sample size did not permit precision and recovery analyses.

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