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HONEYDEW SUGARS ELIMINATED BY *STIGMACOCCUS* SP. NR. *ASPER* HEMPEL (HEMIPTERA: MARGARODIDAE) FEEDING ON LEGUMINOUS TREES IN BRAZIL.

ABSTRACT

HONEYDEW SUGARS ELIMINATED BY *STIGMACOCCUS* SP. NR. *ASPER* HEMPEL (HEMIPTERA: MARGARODIDAE) FEEDING ON LEGUMINOUS TREES IN BRAZIL.

The sooty mould coating the trunks of mature trees of *Schizolobium excelsum* in Brazil was found to be associated with honeydew being eliminated by an undescribed species of margarodid near *Stigmacoccus asper* Hempel. Analysis of the honeydew sugars by paper chromatography revealed a complex composition. The principal sugar was sucrose, but there were significant amounts of fructose, glucose and three components identified as di-, tri- and tetrasaccharides. The disaccharides were maltose, trehalose, trehalulose and a hexose-hexitol. The other, apparently novel, pair of oligosaccharides were composed of glucose(s) 1,4 linked to the glucose of sucrose. The sugar composition of the tree sap was also determined and found to be glucose and sucrose only. The findings, therefore, imply significant and novel metabolic transformations of sugars by the scale insect and/or its microbial symbionts.

Key words: Xylococcinae, sexual reproduction, stigmatrise, stigmatetraose, Amazonia.

INTRODUCTION

Black sooty moulds were noticed coating the trunks of mature trees of *Schizolobium excelsum* (Leguminosae). These trees are native to Amazonia, but have been planted in a small area of dense forest in the Botanic Garden of the Federal University of Santa Catarina, Florianopolis, Brazil. Closer investigation revealed frequent, hair-like, waxy, filamentous tubes protruding 5-8cm laterally from the bark, each bearing a clear honeydew droplet which was visited by flying insects. This striking phenomenon stimulated study of the scale insect involved and of the sugar composition of the tree sap and eliminated honeydew.

MATERIALS AND METHODS

Honeydew (10-20µl) was collected from each wax filament separately by capillarity into a fine glass tube. Approximately 50 specimens of the

honeydew-producing scale insects were collected by slicing beneath each insect to remove a thin layer of bark along with the specimen. This left the insect's severed stylets in the tree but insured that the clypeus and labium were undamaged. The insects were preserved in 80% ethanol before preparation as slide mounts, using the method in Williams & Watson (1988). Keys and descriptions in Morrison (1928) and Foldi (1995), and comparison with museum specimens of *S. asper* Hempel, were used to identify the slide-mounted insects on the basis of their morphology and collection data.

A few minutes after each scale insect had been excised from the tree bark, leaving the stylets *in situ*, small amounts of plant sap appeared and were absorbed into a piece of filter paper. The honeydew and the exuded plant sap were analysed by descending chromatography on Whatman 3MM paper in n-propanol:ethyl acetate:water (7:1:2). Sugars were visualised using aniline hydrogen phthalate reagent, and sucrose, fructose and glucose were recognized by reference compounds. Other sugars were isolated preparatively and their structures determined by a combination of FAB-MS analysis of permethylated derivatives and the standard methodology for polysaccharide linkage analysis by GC-MS (Biermann & McGinnis, 1989).

RESULTS

The honeydew-producing insects were identified as pre-adult females of an undescribed species of scale insect near *Stigmacoccus asper* Hempel (Margarodidae: Xylococcinae). Immature males were also collected, implying that reproduction in this species is sexual.

Paper chromatography showed that the principal honeydew sugar was sucrose (Table 1). Fructose was also clearly evident. There were lesser amounts of glucose and sugars with chromatographic mobilities indicative of di- and tri-saccharides. A tetrasaccharide, with the lowest chromatographic mobility, was the least abundant component.

The composition of the disaccharide group was complex, FAB-MS data showing an approximately 2:1 ratio of di-hexoses (m/z 477) to hexose-hexitol (m/z 493). The composition of the latter was not defined further. However, rechromatography and subdivision of the disaccharide region into 3 regions according to mobility enabled an interpretation of the complex GC-MS data for sugar linkage analysis as indicating the presence of trehalose, trehalulose and maltose.

The molecular masses of the permethylated trisaccharide and tetrasaccharide confirmed that each was composed only of hexose units.

Linkage analysis for the trisaccharide clearly showed glucopyranose linked either in the 1 position or in both 1 and 4 positions, and fructofuranose linked in the 2 position. In the tetrasaccharide, the amount of 1,4 linked glucose was doubled. Consequently, the deduced structures are as in Table 1 and the two are structurally related.

Table 1. Sugar composition of honeydew eliminated by *Stigmacoccus* sp. on *Schizolobium excelsum* at Florianopolis, Brazil, June 1998, listed in decreasing order of abundance of the six chromatographically separated groups of sugars.

| Sugar | Relative order of abundance | FAB-MS* | Monosaccharide composition by GC-MS |
|--------------------|-----------------------------|---------|---|
| SUCROSE | 1 | | G ₁ - ₂ F |
| FRUCTOSE | 2 | | F |
| GLUCOSE | 3 | 477 | G |
| DISACCHARIDE GROUP | 3 | 477 | G ₁ - ₄ G = MALTOSE |
| | | 477 | G ₁ - ₁ F = TREHALULOSE |
| | | 477 | G ₁ - ₁ G = TREHALOSE |
| | | 493 | HEXOSE-HEXITOL |
| TRISACCHARIDE | 3 | 681 | G ₁ - ₄ G ₁ - ₂ F "STIGMATRIOSE" |
| TETRA-SACCHARIDE | 4 | 885 | G ₁ - ₄ G ₁ - ₄ G ₁ - ₂ F "STIGMATETRAOSE" |

* measured mass of the sodiated molecular ion of permethylated compound(s).

Analysis of the liquid collected from the severed insect mouthparts showed that sucrose and glucose were the only sugars in the plant sap. There was no evidence of any of the other oligosaccharides which have been recognised in the honeydew eliminated by the scale insect.

DISCUSSION

Trehalose and trehalulose have already been described as components of scale insect honeydew (Fisher *et al.*, 1984; Bates *et al.*, 1990). However, the occurrence of a hexitol disaccharide, maltose and two oligosaccharides containing glucose connected by 1,4 links to the glucose of sucrose, appear to be novel findings. Predictably, maltose could arise from the trisaccharide by invertase cleavage of the sucrose bond. Since the oligosaccharides also appear to be novel sugars (Liptak *et al.*, 1991), the trivial names "Stigmatriose" and "Stigmatetraose" are proposed.

The significant difference between the carbohydrate composition of eliminated honeydew and the plant sap, implied by analysis of exudate from the severed insect mouthparts, is attributed to metabolism within the scale insect. At present, it is not possible to differentiate between activity by the insect's enzymes and those of any microbial symbionts, such as have been described for other "Homoptera" (Bates *et al.*, 1990; Tremblay, 1990). However, the present application of modern analytical techniques to very small amounts of natural material demonstrates the potential for novel compound discovery. It also emphasises the complex biotransformations within scale insects, which, in the present example, form an integral part of a food web involving a wide range of flying insects.

The undescribed species of scale insect will be described and named once adult specimens are obtained.

ACKNOWLEDGEMENTS

A. Bogo thanks CAPES (Brazil) for postgraduate studentship funding. The authors wish to thank Mr Richard Vane-Wright, Keeper of Entomology at the Natural History Museum, London, UK for permitting free access to the Museum's collection and library for identification of the scale insect, and Dr Imre Foldi of the Muséum National d'Histoire Naturelle, Paris, France, for helpful discussion of the undescribed species of scale insect.

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