I. INTRODUCTION

Following the conclusive demonstration that bleeding sap, or xylem exudate, contains cytokinins (Loeffler and van Overbeek, 1964; Kende, 1964,1965), a good deal of evidence has accumulated suggesting that cytokinins of root origin exert a regulatory control over the metabolism of the aerial parts of the plant (see Kende and Sitton, 1967; Letham, 1967). As part of a programme on the physiological significance of root cytokinins in grape vines, the writer has been studying cytokinin levels in their bleeding sap. Grape vines provide large quantities of sap (Skene, 1967) containing high levels of cytokinins (Skene, 1970).

The present paper describes some of the properties of cytokinins detected in the xylem transport system of grape vines, and discusses changes in cytokinin content in relation to experimental modifications to the root environment. In addition, a preliminary account is given of quantitative differences in the cytokinin levels of bleeding sap from Vitis vinifera and two grape vine rootstocks, Salt Creek and 1613. In past seasons it has been observed that these rootstocks modify the performance of V. vinifera scions grafted onto them; it is possible that the influence of rootstocks on scion development in fruit trees (Rogers and Beakhane, 1957) may in part be mediated by hormones produced by the rootstocks.

II. MATERIALS AND METHODS

For most of the experiments described, methods of growing plants and collection of bleeding sap followed the usual procedures (Skene, 1970). In brief, plants of Vitis vinifera were grown in aerated Hoagland's nutrient culture solution or perlite-sand mixtures. Bleeding sap was collected twice daily through polythene tubes attached to the bases of the stems after removal of the shoots.

In the rootstock studies, three types of fieldgrown vines were considered: (i) Sultana (Vitis vinifera, H5 clone) growing on its own roots; (ii) Sultana grafted onto the rootstock Salt Creek (V. Champini); (iii) Sultana grafted onto the rootstock 1613 (a hybrid derived from the species V. Longii, V. riparia, V. lubrusca and V. vinifera). These will be referred to as H5, H5/SC and H5/1613 respectively. Sap was collected from six vines of each type during the natural bleeding period in spring by inserting a rubber stopper and plastic tube into a hole bored in the trunk. In the case of the rootstocks, the hole was about 20 cm above the graft union. In order to establish any possible differences between the rootstocks and Sultana with the greatest possible precision, sap from each plant was processed and assayed separately.

Vacuum-extracted sap was collected from one-year-old canes of V. vinifera by the method of Bollard (1953). Canes were taken from dormant vines during winter and stored in plastic bags at 10°C for about ten months. Sap was collected either directly from these cold-stored canes or from similar stored canes which had subsequently been induced to root in a glasshouse and support growth of a newly emerged shoot.

Sap from all sources was immediately filtered, frozen and freeze-dried. The main method of cytokinin extraction and purification involved extraction of freeze-dried sap with MeOH. The resulting extracts were then purified by TLC on silica gel plates using a CHCl3-MeOH-H2O system.
dried sap in 80% (v/v) ethanol. After removal of the ethanol, cytokinin activity was estimated with soybean callus (Miller, 1965) either directly on serial dilutions of the supernatant, or after chromatography on Whatman No. 3 paper. Assay flasks contained four pieces of callus and 30 ml medium. Each fraction was assayed in triplicate. Chromatographic solvents included n-butanol/acetic acid/water (4:1:1, v/v), sec. butanol/acetic acid/water (70:2:28, v/v) and 0.03 M boric acid adjusted to pH 8.4 with sodium hydroxide. Sometimes additional purification of the extracts involved partitioning into water-saturated n-butanol from aqueous solutions at pH 7.0, especially before chromatography in the borate system.

Properties of the cytokinins in \textit{V. vinifera} sap were further investigated by precipitation from alkaline ethanolic solutions with barium acetate. Cytokinin activity in the supernatant, and activity recoverable from the barium precipitate with excess sodium sulphate at pH 2.0 was assayed after chromatography in sec. butanol/acetic acid/water. Solubility in n-butanol and chromatographic behaviour of the cytokinin precipitated by barium acetate was also determined after incubation overnight with calf intestine alkaline phosphatase (Calbiochem, B. grade, 1 mg/ml) at 32°C. The incubate, at pH 8.3, contained 0.01 M magnesium chloride.

Procedures for investigating the effects of root temperature and treatment with CCC ((2-chloroethyl) trimethylammonium chloride) on cytokinin levels of vine roots have been described elsewhere (Skene and Kerridge, 1967; Skene, 1970).

III. RESULTS AND DISCUSSION

Properties of the cytokinins in bleeding sap

Chromatogrammed extracts of bleeding sap from grape vines grown in nutrient culture solutions contained two regions of cytokinin activity (Fig. 1A), one of low mobility (Peak 1) and another of high mobility (Peak 2).

Mild acid hydrolysis of Peak 1 converted it to a substance having an Rf similar to that of Peak 2; Peak 2 passed into n-butanol from aqueous solutions, and was held by cationic exchangers, from which it could be recovered by elution with ammonium hydroxide. Treatment of 70% (v/v) ethanolic solutions of sap at pH 9.0 with barium acetate also separated the cytokinins of Peaks 1 and 2. The supernatant above the barium precipitate that formed during standing contained Peak 2 (Fig. 1C), its Rf coinciding with that of synthetic zeatin. Peak 1 could be recovered from an aqueous suspension of the barium precipitate by treatment with excess sodium sulphate at pH 2.0 (Fig. 1D). Further treatment with alkaline phosphatase of the activity recoverable from the barium precipitate converted it to a substance soluble in n-butanol with the chromatographic properties of Peak 2 (Fig. 1E). Chromatography of an n-butanol extract of Peak 2 in 0.03 M boric acid adjusted to pH 8.4 with sodium hydroxide resolved the original peak into two regions of activity (Fig. 1B), one of which co-chromatogrammed with zeatin; the other moved to an Rf to which zeatin nucleoside would be expected to migrate (Miller, 1965). Thus Peak 2 appears to consist of a free cytokinin and its nucleoside. The behaviour of Peak 1, especially after barium acetate treatment and hydrolysis with alkaline phosphatase suggests that it is a nucleotide of the Peak 2 cytokinin.

Zeatin or related compounds and their nucleosides and nucleotides have now been isolated from tissues of a wide range of plants (see Letham and Williams, 1969), and it is likely that zeatin is a constituent of the bleeding sap of sunflowers (Kende and Sitton, 1967). Nitsch (1968) has isolated a cytokinin from bleeding sap of grape vines that appears to be a nucleotide of zeatin, and he has suggested that this may be the water-soluble form in which cytokinins circulate. Considering the evidence for the widespread occurrence of zeatin and its derivatives, it is probable that the three cytokinins under consideration in vine sap are the nucleoside and nucleotide of zeatin or a closely related cytokinin, together with the free form of the same cytokinin.