Effect of Root Temperature on Cytokinin Activity in Root Exudate of Vitis vinifera L.

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Summary. Root exudates of plants of *Vitis vinifera* L. cv. Thompson Seedless, grown in nutrient cultures with root temperatures maintained at either 20° or 30° and with shoots at a common air temperature, were assayed for cytokinin activity. After chromatography of freeze-dried sap on paper with *n*-butanol/acetic acid/water (4:1:1), activity was detected with a soybean callus assay. For both root temperatures, major activity appeared between R_F 0.6 and R_F 0.8, at about the same concentration in each case. The major difference between the 2 samples was the presence of activity at R_F 0.1 to 0.2 in the 20° sample and its absence in the 30° sample.

The higher root temperature resulted in increased shoot and root elongation, increased dry matter accumulation by both shoots and roots, and also altered the morphological appearance of the roots.

Interest in the general significance to the rest of the plant of hormones originating in roots has been stimulated by the demonstration of cytokinin activity in the root exudate of various species (2, 3, 4, 7). There is evidence that these cytokinins indeed do originate within the roots (3, 10), and that their levels can be experimentally modified by treatments which affect the growth and metabolic processes of the rest of the plant (1). Furthermore, experiments of the type described by Mullins (6), in which synthetic cytokinins have successfully substituted for roots in maintaining growth of young inflorescences in *Vitis vinifera* cuttings, provide additional, though indirect, evidence of the importance of roots as a source of cytokinins.

When plants of *Vitis vinifera* are grown at different root temperatures, there is a marked effect on shoot growth, on fruit set, and on growth and appearance of the roots (11). In view of these results, it seemed of interest to determine the effect of root temperature on cytokinin production by the root.

Materials and Methods

Hardwood cuttings of *Vitis vinifera* L. cv. Thompson Seedless (syn. Sultana) were rooted in perlite. After roots were established the plants were transferred to aerated nutrient cultures of Hoagland's solution contained in 30 liter plastic drums, which in turn were immersed in tanks of water maintained at either 20° or 30° (11). Each drum contained 5 plants; a total of 15 plants was subjected to each root temperature. All but 1 shoot was removed from each plant, and throughout the experiment laterals were removed from the primary shoot as they appeared. The tops of the plants in each treatment were exposed to the conditions prevailing in a glasshouse with vigorous air circulation. Air temperature was controlled between the limits of 20° (night) and 35° (day). The difference in air temperature 15 cm above the surface of the 2 tanks at no stage was greater than 1°. The 2 experiments reported were carried out between September and December 1966, and during this time the plants received no supplementary lighting.

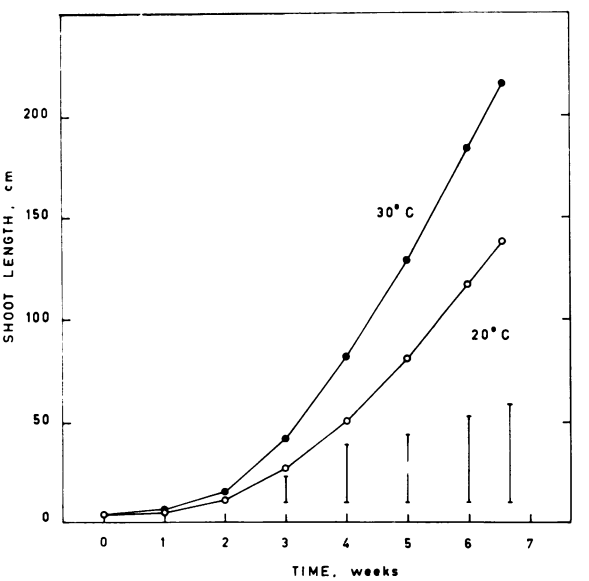

FIG. 1. Growth of primary shoots of Thompson Seedless vines with roots held at either 20° or 30°. Vertical bars represent LSD (P = 0.01) on each occasion.

Shoot length was measured at weekly intervals. After 4 weeks in the first experiment and 7 weeks in the second, the primary shoot was cut back 2 cm from its base and a polythene tube was attached. Root exudate was collected twice daily; at each collection period the sap from all the plants of each treatment was bulked and stored at −15°. Only sap collected during the first 36 hours was considered for cytokinin bioassay. Finally, dry weights of shoots and roots were determined.

Treatment of root exudate and its bioassay for cytokinin activity were based on procedures described by Kende (3), using cultures of soybean callus (*Glycine max* cv. Acme) (5). Freeze-dried sap was applied to Whatman No. 3 chromatography paper and developed in *n*-butanol/acetic acid/water (4:1:1) until the solvent front had advanced 25 cm. After drying, chromatograms were cut into 10 equal transverse sections, plus 1 control section from below the start line, and eluted with 80 % (v/v) ethanol. The fractions were reduced to dryness and transferred to 125 ml Erlenmeyer flasks containing 30 ml of nutrient medium. Each fraction was assayed in duplicate with 4 pieces of callus in each flask. Calluses were cultured at 27° in the dark. Responses were assessed by measuring the fresh weight of each piece of callus.

Results

The effects of root temperature on shoot elongation during the second experiment are shown in figure 1. Differences in final shoot length, shoot dry weight and root dry weight were all highly significant. In addition, the roots at 30° were longer and thinner in diameter than those at 20°. The appearance of the roots at each temperature is shown in figure 2. The same qualitative effects on root growth were observed in the first experiment (fig 3), although differences in growth of the tops were less marked (mean final shoot length at 20° and 30° was 38 cm and 48 cm respectively).

The results of the cytokinin bioassays are shown in figures 4 and 5. Two, and possibly 3, regions of cytokinin activity are evident. In all cases the major peak of activity occurs between fractions 6 to 9 (R_F 0.5–0.9), whereas activity in fraction 2 (R_F 0.1–0.2) of the sap from roots grown at 20° is absent from the 30° samples. In the second experiment there also appears to be activity in fraction 4 of the 20° sample.

It is doubtful whether root temperature has caused any marked differences in the quantitative levels of cytokinin activity appearing in fractions 6 to 9, particularly when one considers the overall data for both experiments expressed in terms of kinetin equivalents (table I). However, the noteworthy effect of root temperature is that it seems to have influenced the qualitative pattern of cytokinins detected (figs 4, 5).

Discussion

The main peak of cytokinin activity (fractions 6–9) in the chromatogrammed exudate of roots grown at either 20° or 30° corresponds in R_F to factor II detected in sunflower sap by Kende (3). There seems to be little effect of root temperature on the appearance of this cytokinin. Although in the second experiment the total amount of activity detected in the sap over the 36 hour bleeding period was greater at 30° than at 20°, the difference became less evident when activity was expressed on a concentration basis in terms of either kinetin equivalents per liter of sap or per kg dry weight of roots (table I). If one assumes that the dry weight of the roots is an approximate measure of the potential number of sites for cytokinin production, there is little difference in the levels of cytokinin produced in fractions 6 to 9 at each root temperature.

The major effect of root temperature is on the appearance of cytokinin activity in fractions of lower R_F, particularly in fraction 2. Whether this latter fraction bears the same relationship to fractions 6 to 9 as Kende's (3) factor I bears to his factor II is not known, although it would be attractive to think that root temperature is influencing the conversion of a "bound" cytokinin to its uncomplexed form. However, it should be noted that the factor I of Kende did not promote cell division after the chromatographic treatment reported above (3).

Table I. *Effect of Root Temperature on Cytokinin Levels in Root Exudate of Thompson Seedless Vines****

Expt	Fraction* no.	Activity in total sap		Kinetin equivalents Activity per liter sap		Activity per kg dry wt roots	
		20°	30°	20°	30°	20°	30°
		μg	μg	μg	μg	μg	μg
1	2	0.04	0	0.39	0	3.6	0
	4	0	0	0	0	0	0
	6–9	0.10	0.12	1.04	0.90	9.6	11.0
2	2	0.04	0	0.22	0	1.1	0
	4	0.03	0	0.18	0	0.9	0
	6–9	0.21	0.61	1.18	2.77	6.1	9.3

* Fraction 2 = R_F 0.1 to 0.2; fraction 4 = R_F 0.3 to 0.4; fractions 6 to 9 = R_F 0.5 to 0.9.
** A factor of 30 has been omitted from the calculations; actual activity levels are 30 times higher than indicated.

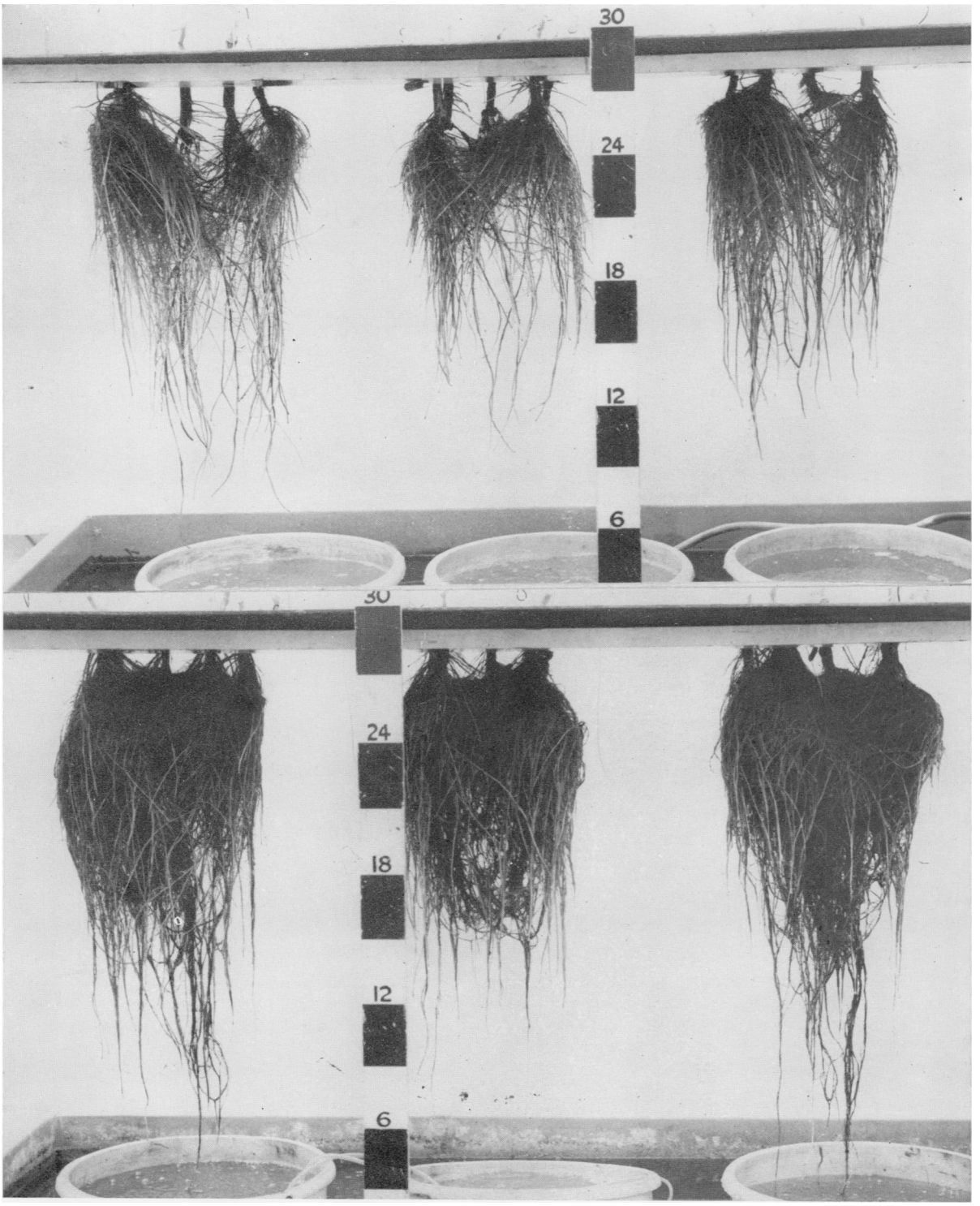

FIG. 2. Effect of root temperature on the growth of Thompson Seedless roots. Seven weeks growth. Upper, 20°. Lower, 30°.

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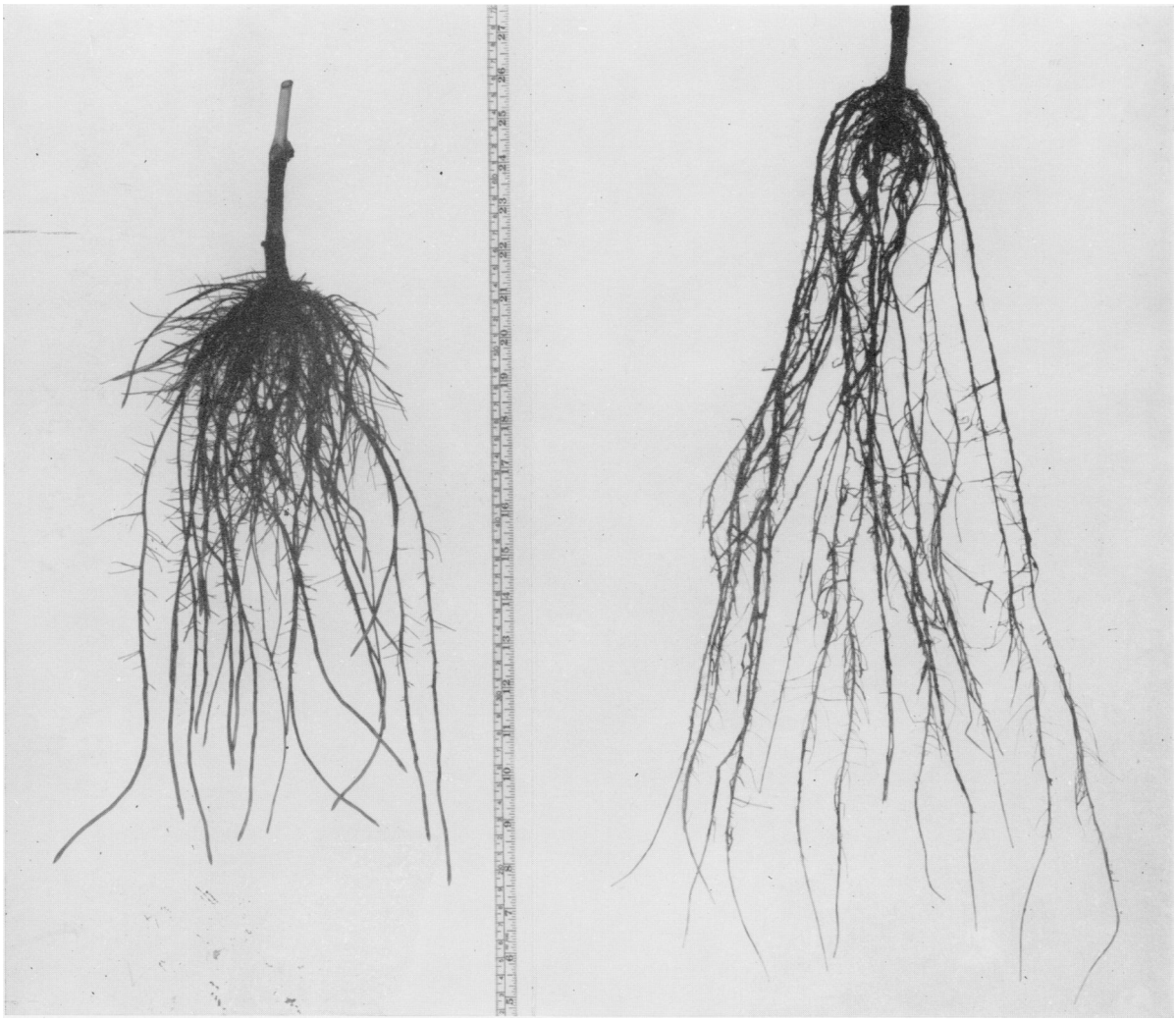

FIG. 3. Appearance of Thompson Seedless roots after 4 weeks growth at root temperatures of 20° (left) and 30° (right).

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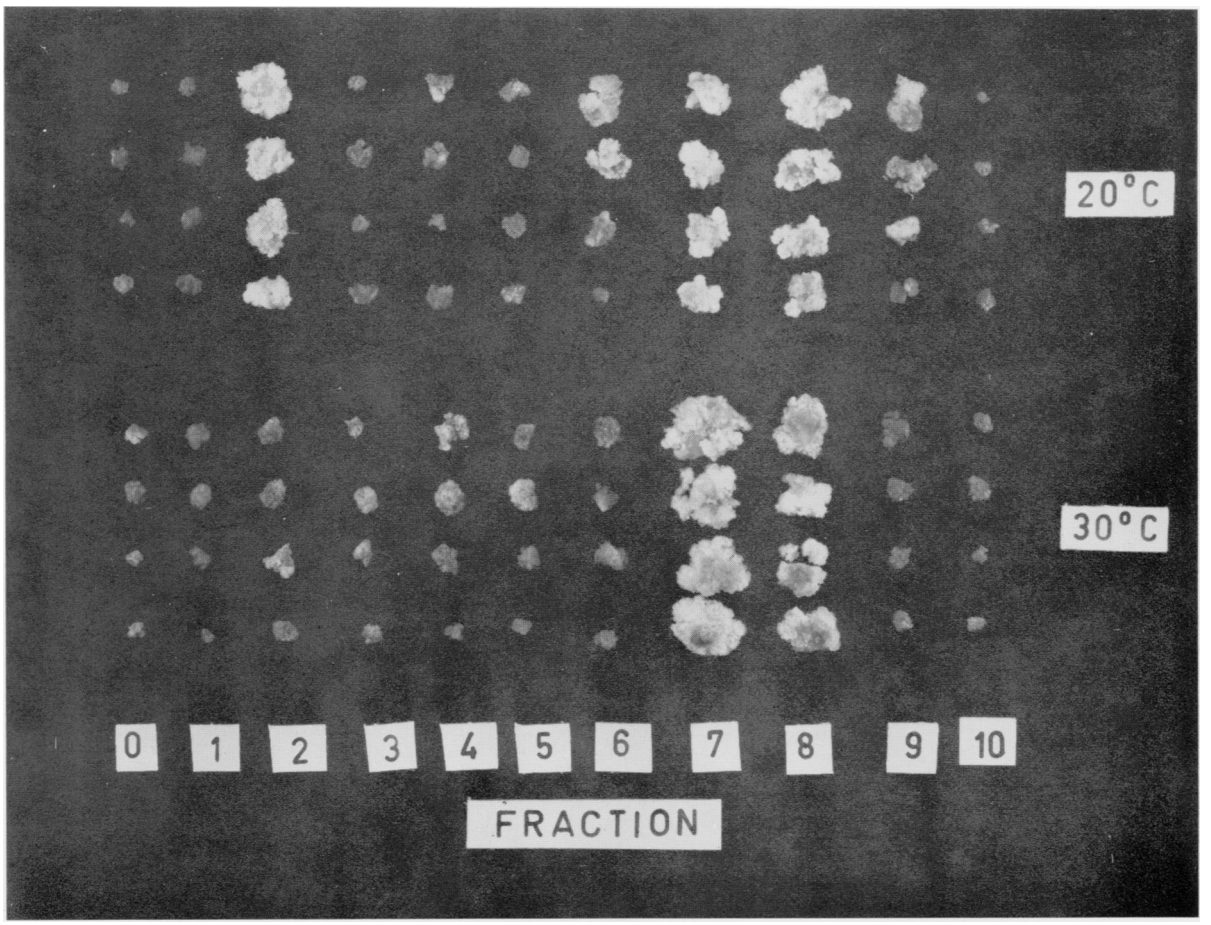

FIG. 4. Response of soybean callus to chromatogrammed root exudate from Thompson Seedless vines grown at root temperatures of either 20° or 30° (expt 1). Fraction 0 = control; fractions 1 through 10 correspond to R_F 0.0 to 0.1 through R_F 0.9 to 1.0. Each fraction was exposed to the equivalent of 46 ml sap in the case of the 20° sample, and 67 ml in the case of the 30° sample. Calluses were grown for 5 weeks at 27°.

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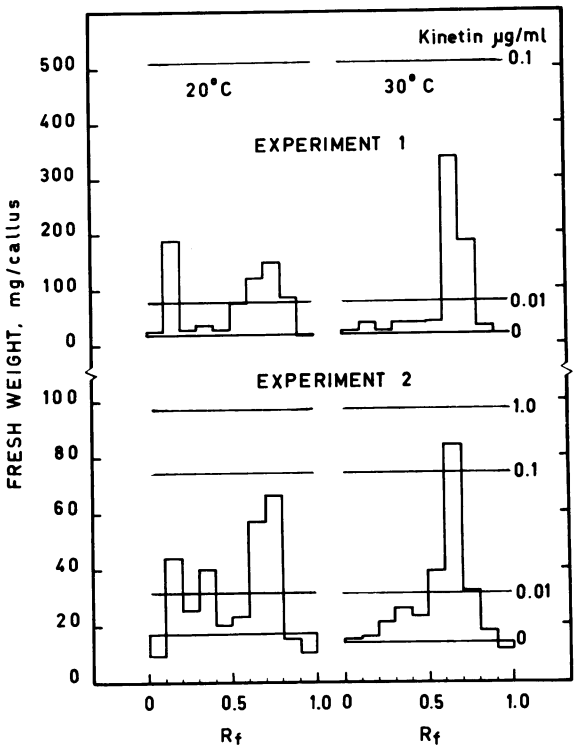

FIG. 5. Response of soybean callus to chromatogrammed root exudate from Thompson Seedless vines grown at root temperatures of either 20° or 30°. Each fraction was exposed to the equivalent of 46 ml sap (expt 1) or 90 ml sap (expt 2) in the case of the 20° samples, and 67 ml (expt 1) or 110 ml (expt 2) in the case of the 30° samples. Calluses were grown at 27° for 5 weeks (expt 1) or 3 weeks (expt 2).

Irrespective of the nature of the cytokinin detected in fraction 2, the possibility does exist that differential destruction or interconversions of the cytokinins occurred at the 2 root temperatures after the plants were decapitated and whilst the roots were exuding sap. Although this is not regarded as being very likely, with the data available one cannot overlook the possibility. Nor can one be sure whether the same pattern of cytokinin movement would take place in the intact plant.

It is not suggested that the morphological differences recorded for each root temperature necessarily can be explained in terms of the observed cytokinin patterns in the root exudate. Root exudate of grape vines also contains gibberellin-like substances (8), which may be implicated for instance in the effects of root temperature on shoot elongation. However, in view of the effects of synthetic cytokinins on fruit set in *Vitis* (9), the stimulatory effects of a 30° root temperature on fruit set of Sultana grapes reported by Woodham and Alexander (11) may well be explained on the basis of an altered cytokinin production by the roots. The main interest of the results, we feel, is the modification by temperature of the type of cytokinins appearing in the root exudate. Deciding whether this is an effect on cytokinin production or interconversion, or is even due to utilization by the roots for their own growth, awaits further experimentation.

Acknowledgments

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