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Factors affecting the qualitative and quantitative levels of cytokinins in xylem sap of grapevines

by

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Faktoren, welche die qualitative und quantitative Zusammensetzung der Cytokinine im Blutungssaft von Reben beeinflussen

Zusammenfassung. — Im Gewächshaus und in Phytotronkammern wurde der Einfluß von Sorte, Unterlage und Düngung auf die Cytokininkonzentration im Blutungssaft von Reben, die bei Wurzeltemperaturen von 12 und 25 °C gehalten wurden, untersucht. Im Gewächshaus bewegte sich die Lufttemperatur zwischen 15 °C bei Nacht und 30 °C am Tag. In der rotierenden Phytotronkammer betrug die Temperatur tagsüber (6.00 — 18.00) 27 °C, in der stationären Kammer 17 °C; die Nachttemperatur betrug in beiden Kammern 12 °C.

Nach der Extraktion des gefriergetrockneten Blutungssaftes wurde die Cytokininaktivität mit Hilfe des Gurkenkotyledonentestes und auf gaschromatographischem Wege nachgewiesen. Im Blutungssaft von Reben der Sorte Pinot noir lag bei beiden Wurzeltemperaturen (12 und 25 °C) eine niedrigere Cytokininkonzentration vor als bei Cabernet Sauvignon. Bei einer Wurzeltemperatur von 25 °C war die Cytokininaktivität des Blutungssaftes von Cabernet Sauvignon und Pinot noir meist signifikant höher als bei einer Wurzeltemperatur von 12 °C; die Cytokiningehalte von Reben, die bei Lufttemperaturen von 17 oder 27 °C gehalten wurden, unterschieden sich dagegen bei derselben Wurzeltemperatur nicht signifikant. Die Höhe der Wurzeltemperatur äußerte sich bei papierchromatographischer Auftrennung des Blutungssaftes in einer unterschiedlichen Cytokininzusammensetzung: Bei 25 °C war die Aktivität im R_f-Bereich 0,2—0,7 verstärkt; bei 12 °C überwog dagegen die R_f-Fraktion 0,8. Die qualitative Analyse mittels GLC ergab für die bei 12 °C aufgewachsenen Reben nur einen einzigen Cytokinin-Peak, während bei 25 °C vier Peaks der Cytokininaktivität vorlagen.

Wurden Topfreben von Cabernet Sauvignon mit N, P und K gedüngt, so war bei Austrieb die Cytokininkonzentration im Blutungssaft sowohl bei einer Wurzeltemperatur von 12 wie von 25 °C leicht erhöht. Im Freiland dagegen zeigten ausgewachsene Reben der Sorte Thompson Seedless, die mit 224 und 448 kg N/ha gedüngt worden waren, zur Zeit des Austriebes in ihrem Blutungssaft eine signifikant höhere Cytokininkonzentration als Reben, die nicht oder mit 672 kg N/ha gedüngt worden waren.

Introduction

The carbohydrate flux from shoots to roots and the auxin supply produced in the above-ground portion of the plant is considered to be essential for the ultimate growth and development of the root system (26). The presence of a well-developed root system is vital for the development of inflorescence in grapevines (14). The findings of Mullins (14), Alexander (1), and Alexander and Woodham (2) suggest that roots are the source of one or more growth regulators that are translocated to the above-ground parts and stimulate stem growth and inflorescence development. Mullins (14) indicated that the effect of roots on inflorescence retention is enhanced by applying the synthetic cytokinin, 6-benzylamino purine (BAP) to the base of

unrooted cuttings. He determined that the movement of the BAP in the xylem sap of vines was acropetal. Early investigations indicated that roots are an important source of cytokinin to the above-ground portion of plants and the root tip is the primary site of cytokinin synthesis (13, 24). The correlation of the effects of roots and synthetic cytokinin on inflorescence retention and fruit set suggests a role for the endogenous cytokinin in the ascending sap of vines (14).

Soil environment (moisture, temperature, aeration, mineral nutrients) are believed to have an effect on synthesis and translocation of cytokinins. Low root temperature dramatically reduced plant growth and the amount of exported cytokinin, whereas the export of inhibitors was increased (3). Hence, low root temperature seems to affect the growth habit of the above-ground organs by altering the balance of growth regulators that are exported to the shoots.

The primary concern of this study was to determine the effect of root temperature and its interaction with N, P, K fertilization, rootstock, and air temperature on the level and kinds of cytokinins in the xylem sap of Cabernet Sauvignon and Pinot noir vines.

Materials and methods

Plant material and cultural system

2-year-old dormant *Vitis vinifera* L. vines growing in 20-l containers in a mixture of sand : soil : peat (2 : 2 : 1, v/v) were used in each of the greenhouse and phytotron experiments. The vines were pruned to two 10-node canes just prior to initiation of temperature and fertilizer treatments. Four experiments were conducted with vines growing in either a temperature controlled greenhouse or phytotron rooms using water baths held at 12 and 25 °C for controlling root temperature (29). Specific details of individual experiments will be described under "Results and discussion".

Xylem sap collection

Just prior to the collection of the xylem sap, potted vines were positioned horizontally to aid the flow of sap into test tubes. The canes were cut back to 2 cm from base and surgical rubber tubing was fitted tightly over the cut end of the cane. The other end of the tubing was connected to a stoppered test tube through a glass tube. The test tubes were covered with aluminium foil to control the possible destruction of kinetin-like substances by solar radiation (18). Ethanol was added to the test tubes to control growth of microorganisms.

Xylem exudate was also collected from Thompson Seedless vines grown in the University Vineyard at Davis with different levels of nitrogen (0, 112, 224, 448 and 672 kg N/ha). Immediately after collecting the bleeding sap, the samples were frozen for cytokinin assay.

Cytokinin extraction

The frozen samples were thawed and ethanol was removed at 40 °C *in vacuo*. 10-ml samples were freeze-dried to determine the concentration of cytokinin in each sample. The freeze-dried samples were redissolved in 80 % ethanol, extracted overnight at room temperature, and centrifuged for 10 min in a clinical centrifuge. The supernatant was decanted into small test tubes and reduced to 1 ml for paper chromatography or to dryness for cucumber cotyledon bio-assay.

Cytokinin assay

Paper chromatography: 1 ml of each of the concentrated samples was streaked on a 20 × 25 cm Whatman 3MM paper and developed in an ascending system of n-butanol : acetic acid : water (4 : 1 : 1, v/v) solvent until the solvent front had advanced 20 cm. The chromatograms were dried overnight at room temperature and then cut into ten equal strips (2 × 20 cm) representing various R_f regions. Each strip was cut into smaller pieces and placed in a small glass jar and eluted with 5 ml of 80 % ethanol for gas chromatography or with 5 ml of 2 mM potassium phosphate (KH_2PO_4) buffer for bio-assay.

Gas chromatography: The paper strips were soaked in 5 ml of 80 % ethanol for about 2 h, and then agitated gently on a shaker for 2—3 h. The solutions were decanted into 10-ml screw-cap culture tubes, dried in a stream of air at room temperature, and placed in a desiccator for future assay.

GC was carried out on a Varian Aerograph instrument (Varian-1400) with flame ionization detectors. Standard conditions for cytokinin analysis were: 3 % SE_{52} (on 80—100 mesh Gas Chrom Q) columns (2.8 m × 3 mm), temperature programmed 220—300 °C at 4 °/min. The flow rate of the carrier gas (N_2) was 30 ml/min. This procedure was a modification of Horgan *et al.* (11) and Ohkawa (16). Trimethyl-

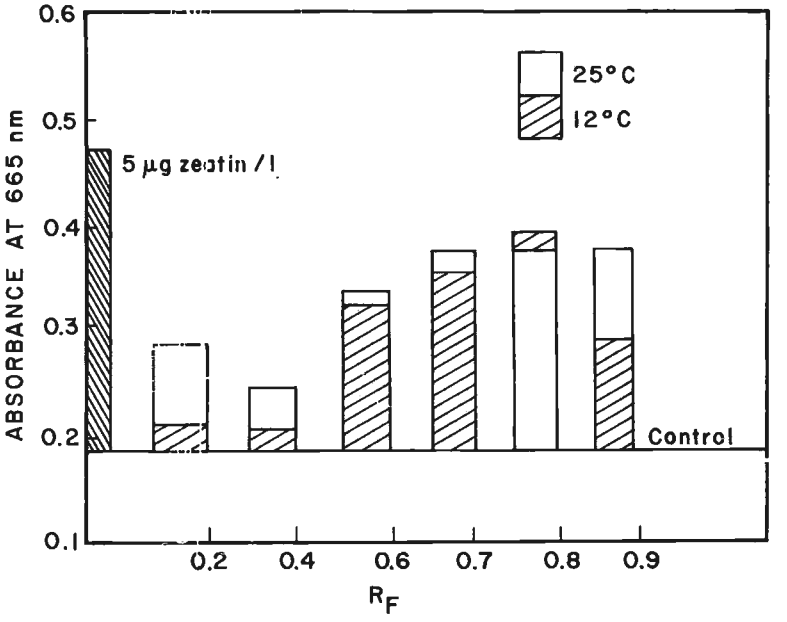

Fig. 1: Cucumber cotyledon (*Cucumis sativus* L. cv. Chicago Pickling) chlorophyll bioassay of xylem sap chromatogrammed in n-butanol : acetic acid : water (4 : 1 : 1) for cytokinin-like substances obtained from Cabernet Sauvignon vines grown at 12 and 25 °C root temperatures in a greenhouse.

Gurkenkotyledonentest (*Cucumis sativus* L. cv. Chicago Pickling) zum Nachweis cytokininartiger Substanzen im Blutungssaft von Cabernet Sauvignon. Chromatographische Auftrennung des Blutungssaftes mittels n-Butanol : Essigsäure : Wasser (4 : 1 : 1). Haltung der Reben im Gewächshaus bei Wurzeltemperaturen von 12 und 25 °C.

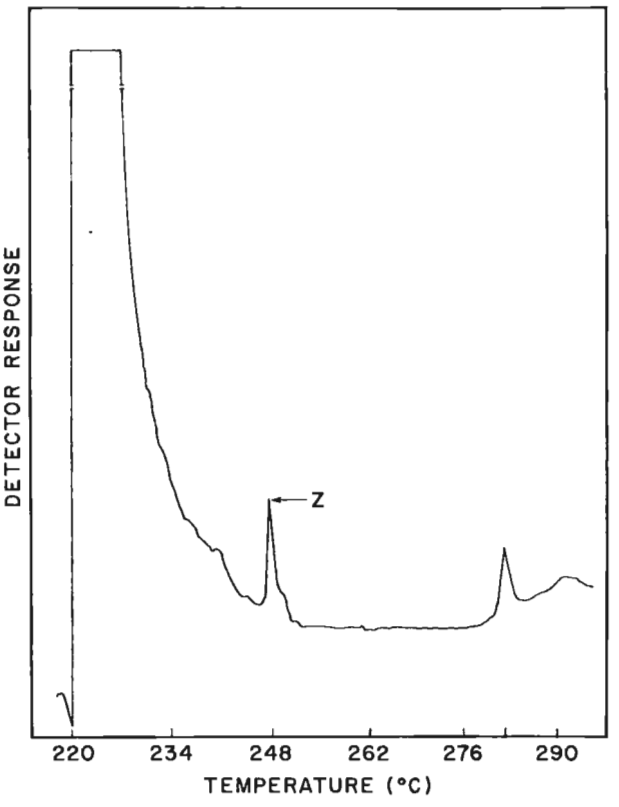

Fig. 2: Total ionization current traces of partially purified extract from xylem sap of Cabernet Sauvignon grapevines grown at 12 °C root temperature. The one elution temperature peak corresponds with authentic zeatin peak (Z).

Gaschromatogramm eines teilweise gereinigten Blutungssaftextraktes von Cabernet Sauvignon. Wurzeltemperatur von 12 °C. Peak Z = Zeatin.

silyl-derivatives were prepared by adding to the dried extracts 100 μl of N,O-bis(trimethylsilyl)trifluoroacetamide : acetonitrile (1 : 3) followed immediately by 100 μl of pyridine. The mixture was incubated at 90 °C for 45 min in small screw-cap culture tubes. 4-μl samples of the derivatives were used for GC. Peak crests for authentic standards[1]) of 6-(γ,γ-dimethylallylamino)-purine (2iP), zeatin, 6-(γ,γ-dimethylallylamino)-purine adenosine (2iPA), and zeatin riboside (at 233, 248, 274 and 292 °C, respectively) were determined. The types of cytokinin in the combined R_f zones of the chromatographed samples were identified by comparison with these standards.

Bio-assay: The cytokinin activity in the sap was estimated using FLETCHER's (7) cucumber[2]) cotyledon greening assay.

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[1]) The standards 2iP and 2iPA were obtained from SIGMA Chemical Company, zeatin and zeatin riboside from CALBIOCHEM.

[2]) *Cucumis sativus* L. variety Chicago Pickling seeds were obtained from Peto Seed Co., Woodland, California.

Table 1

Effect of root temperature and cultivar on the concentration of cytokinin (μg zeatin equivalent/l) in xylem sap of grapevines at budbreak

Einfluß von Wurzeltemperatur und Rebsorte auf die Cytokininkonzentration (μg Zeatinäquivalente/l) im Blutungssaft von Reben zur Zeit des Knospenaustriebes

Cultivars	Root temperature (°C) 12	25
Pinot noir	2.5[b1)]	4.3[a*]
Cabernet Sauvignon	4.7[b*]	7.1[a*]

[1]) Means with a common letter within the row were not significantly different at 5 % level.

* Differences between means in the same column were significant at the 5 % level.

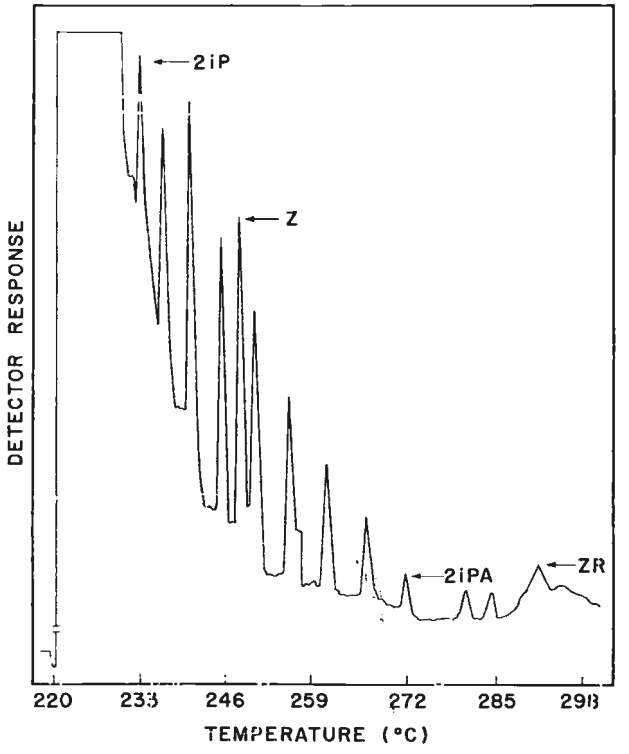

Fig. 3: Total ionization current traces of partially purified extract from xylem sap of Cabernet Sauvignon grapevines grown at 25 °C root temperature. The four elution temperature peaks correspond with standard peaks (2iP = dimethylallylamino-purine, Z = zeatin, 2iPA = dimethylallylamino-purine adenosine, and ZR = zeatin riboside).

Gaschromatogramm eines teilweise gereinigten Blutungssaftextraktes von Cabernet Sauvignon. Wurzeltemperatur von 25 °C. Peaks: 2iP = Dimethylallylaminopurin, Z = Zeatin, 2iPA = Dimethylallylaminopurin-Adenosin, ZR = Zeatinribosid.

Results and discussion

The effects of the root temperature, rootstock and fertilization treatments on bud-break, shoot growth, and composition of Cabernet Sauvignon vines have been published elsewhere (30).

Greenhouse experiments

Two greenhouse experiments were conducted, each with vines grown at 12 and 25 °C root temperatures. In the first experiment the levels of cytokinin in the xylem sap of Pinot noir and Cabernet Sauvignon were compared (Table 1). In the second experiment levels of cytokinin in the xylem sap of Cabernet Sauvignon on its own roots and grafted to A × R # 1 (Ganzin No. 1), with half of the vines receiving 2.4 g of N and 1.2 g of P and K each, and the other half receiving no supplemental nutrients, were determined. In both experiments there were four single vine replicates per treatment. The greenhouse ambient temperature for both experiments ranged between 27 to 30 °C in the daytime and 15 to 17 °C at night.

The data in Figs. 1 to 3 indicate that the kind of cytokinins in the xylem exudate was greatly affected by root temperature treatment, in agreement with the findings of Skene and Kerridge (21). However, in this study, unlike the findings of these authors, the cucumber cotyledon bio-assay of various paper chromatograph fractions did not show significant differences in cytokinin activity in xylem exudates between vines grown at 12 and 25 °C (Fig. 1). Many of the R_f fractions from the paper chromatograms had so little cytokinin that it was not possible to qualitatively determine the cytokinin from the individual fraction by GLC. However, when the entire chromatographed sample was eluted from paper and subjected to GLC analysis cytokinin-like compounds were detected. There was a treatment-dependent difference in the kinds of cytokinin present in the combined R_f fractions. At 12 °C root temperature the main peaks coincided with the elution peak of the zeatin standard (Fig. 2); whereas, at 25 °C there were additional peaks (Fig. 3).

Material coeluting with zeatin, zeatin riboside, 6-(γ,γ-dimethylallylamino)-purine (2iP), and 6-(γ,γ-dimethylallylamino)-purine adenosine (2iPA) was detected in the combined R_f fractions at 25 °C root temperature (Fig. 3), whereas at 12 °C the main peak coincided only with zeatin (Fig. 2). At both root temperatures (12 and 25 °C), there was an unidentified common peak at about 284 °C. The difference in cytokinin content of xylem sap between vines grown at 25 and 12 °C root temperature may be explained by the possible reduction in water flow at the lower root temperature (12) and/or the possible modification by temperature of the types of cytokinin appearing in the xylem exudate, as suggested by Skene and Kerridge (21).

Itai and Vaadia (12) found that cytokinin biosynthesis in roots of sunflower plants ceased when the water tension in leaves increased. They suggested that the alteration of cytokinin effects was due to either the accumulation of inhibitors during stress or transformation of the cytokinin molecule. Several investigators (22, 27, 28) have also suggested that the accumulation of inhibitors, such a abscisic acid (ABA), in extracts could interfere in bio-assays masking cytokinin activity.

Atkin *et al.* (3) reported that increasing root temperature to 28—30 °C increased the rate of cytokinin export. In the present investigation the increase in export of cytokinin at 25 °C compared to 12 °C may be related to the development of more roots at the high root temperature than at the low temperature (21), and consequently increased the number of sites for cytokinin synthesis, such as root apices at the higher root temperature (24).

Table 2

Effect of root temperature, rootstock and fertilization with N, P and K on concentration of cytokinin (μg zeatin equivalent/l) in xylem sap of Cabernet Sauvignon grapevines at fruit maturity

Einfluß von Wurzeltemperatur, Unterlage und N,P,K-Düngung auf die Cytokininkonzentration (μg Zeatinäquivalente/l) im Blutungssaft von Cabernet Sauvignon zur Zeit der Beerenreife

Rootstock	Fertilizer treatment	Root temperature (°C) 12	25
A × R	F	1.8[a1)]	2.2[a]
	NF	1.7[a]	1.5[a]
Own-rooted	F	1.4[a]	1.7[a]
	NF	1.1[a]	1.5[a]

[1]) Means with a common letter within the row were not significantly different at 5 % level.
F = Fertilized with 20 ml of 12 : 6 : 6 % N, P, K/20-l pot.
NF = Not fertilized.

Table 3

Effect of root and air temperature on the concentration of cytokinin (μg zeatin equivalent/l) in xylem sap of Cabernet Sauvignon grapevines grown in phytotron rooms at fruit maturity

Einfluß von Wurzel- und Lufttemperatur auf die Cytokininkonzentration (μg Zeatinäquivalente/l) im Blutungssaft von Cabernet Sauvignon zur Zeit der Beerenreife · Die Reben wurden in Phytotronkammern gehalten

Air temperature (°C)	Root temperature (°C) 12	25
17	2.3[b1)]	4.9[a]
27	2.3[b]	4.2[a*]

[1]) Means with a common letter in the same column were not significantly different at the 5 % level.
* Differences between means within the row were significant at the 5 % level.

The concentration of cytokinin in xylem exudate of Cabernet Sauvignon vines was significantly greater than in exudate of Pinot noir vines at budbreak at both 12 and 25 °C root temperatures (Table 1). Cabernet Sauvignon is a more vigorous cultivar than Pinot noir (29) and may have had greater root development than Pinot noir. However, more work is necessary before this can be definitely established as the reason for difference in level of cytokinin between the two cultivars.

Cabernet Sauvignon vines on A × R rootstock and own-rooted vines that received 2.4, 1.2 and 1.2 g of N, P, K respectively per 20-l pot had slightly higher

concentration of cytokinin in the xylem sap during budbreak than vines that received no fertilizer treatment at both root temperatures; however, the difference was not significant at 5 % level (Table 2). The findings of Sattelmacher and Marschner (19), who studied the relation between nitrogen nutrition, cytokinin activity and tuberization in *Solanum tuberosum,* are in general agreement with the result of this investigation. They reported a fertilization- dependent increase in cytokinin activity in the xylem exudate while there was a considerable decrease in the activity of cytokinins extracted from roots and shoots of fertilized potato plants. How nitrogen fertilization increased the activity of cytokinin only in the xylem sap is not known.

Phytotron experiments

Two experiments were conducted using a rotating and a stationary phytotron. These phytotron rooms have been well described by Zscheile *et al.* (1965). Both rooms were equiped with Partlow temperature control systems that allow the programming of the rate of temperature increase or decrease and both rooms were maintained at ± 1 °C of the indicated temperature. Natural sunlight was the source of illumination in both the roating and stationary rooms, which averaged about 75,000 lx and 43,000 lx, respectively.

In the first experiment the interaction of two air temperatures with two root temperatures on the level of cytokinin in xylem sap of Cabernet Sauvignon was investigated. The daytime (6 a. m. to 6 p. m.) air temperature in the rotating and stationary rooms were 27 and 17 °C, respectively. Night time (8 p. m. to 4 a. m.) air temperature was the same for both rooms (12 °C), with a 2-h period allowed for temperature changes from day to night and vice versa. The root temperatures were 12 and 25 °C. Each of the four treatments had four single vine replicates. Xylem sap from each vine was collected at fruit maturity and the level of cytokinin determined as described under "Materials and methods".

The concentration of cytokinin in xylem sap of Cabernet Sauvignon vines grown in the phytotron rooms at 25 °C root temperature was approximately twice that of plants at 12 °C at both high and low air temperatures (Table 3). Differences in air temperatures did not have a significant effect on the level of cytokinin in the sap (Table 3).

In the second phytotron experiment, conducted in the rotating phytotron, changes in the level of cytokinin in Cabernet Sauvignon xylem sap at four different physiological stages of development and at both 12 and 25 °C root temperature were determined (Table 4). The daytime and nighttime air temperatures were 27 and 12 °C, respectively.

At 12 and 25 °C root temperatures the level of cytokinin in the xylem sap increased slightly between budbreak and bloom, declined at veraison, and increased again during fruit ripening (Table 4). The concentration of cytokinin in xylem exudate of vines at both root temperatures followed the same trend from budbreak to veraison. However, the highest concentration of cytokinin was detected in the exudate of vines grown at the higher root temperature, the differences being significant at budbreak and midway between veraison and fruit maturity (Table 4).

Two peaks of cytokinin activity over the growing season were observed in the xylem sap from Cabernet Sauvignon vines (Table 4). While the initial peak in the level of cytokinin coincided with the period of inflorescence development, the reduction in the cytokinin level coincided with the cessation of shoot growth (29). This

Table 4

Effect of root temperature on changes in the concentration of cytokinin (μg zeatin equivalents/l in xylem sap of Cabernet Sauvignon grapevines at different stages of vine development · Vines were grown in a rotating phytotron room

Einfluß der Wurzeltemperatur auf die Veränderung der Cytokininkonzentration (μg Zeatinäquivalente/l) im Blutungssaft von Cabernet Sauvignon während verschiedener Phasen der Beerenentwicklung · Die Reben wurden in einer rotierenden Phytotronkammer gehalten

Root temperature (°C)	Stages of development			
	A[1])	B	C	D
12	$2.4^{b1\,2)}$	3.2^{a1}	1.6^{a2}	2.0^{b2}
25	3.5^{a2}	3.6^{a2}	2.2^{a3}	5.2^{a1}

[1]) A, B, C and D designate stages of vine growth at budbreak, bloom, veraison, and midway between veraison and fruit maturity, respectively.

[2]) Means with a common letter in the same column and with the same number within the row were not significantly different at the 5 % level.

seems to suggest that in intact plants some hormonal influence from the growing shoot apices passes to the roots, where cytokinin production is regulated (20). Cytokinins are known to play a primary role in cell division; however, berry enlargement and fruit set may be primarily controlled by other hormones, such as auxin and gibberellin (6, 23).

Several investigators have followed seasonal changes in the levels of cytokinin in the xylem exudate and in tissues of various plant species. Hewett and Wareing (9) found the maximum cytokinin activity in the leaves of *Populus robusta* in mid summer, prior and subsequent to the cessation of shoot elongation. Chacko *et al.* (5) reported maximum cytokinin activity in Bangalore Blue grape berries at anthesis and at 7 and 14 d after anthesis. Beever and Woolhouse (4) stated a five-fold increase of cytokinin in florally-induced plants *(Perilla frutescens)* during flower and fruit formation.

The data in Table 4 indicate a reduction in the cytokinin level of xylem sap about 30 d after anthesis, in agreement with the results obtained by Chacko *et al.* (5), who observed a reduction in the concentration of cytokinin in berries during the lag period of berry growth (4 weeks after anthesis). During the berry ripening period the level of cytokinin is believed to be directly associated with the second period of rapid root growth, which usually occurs after berries have matured (10).

Field nitrogen fertilization experiment

10-year-old own-rooted Thompson Seedless (syn. Sultana) vines grown in the University vineyard at Davis were fertilized with 0, 112, 224, 448 and 672 kg N/ha, applied as ammonium nitrate in December. Each treatment was replicated six times with three vines per replicate. At budbreak in mid March, xylem sap was collected from recently cut spurs located in the head of the vine over a period of 5—6 h (10 a. m. to 4 p. m.) and immediately frozen for future cytokinin assay. Samples of each treatment and replicate were assayed separately.

Table 5

Effect of nitrogen-fertilization on the concentration of cytokinin (μg zeatin equivalent/l) in xylem exudate of field-grown Thompson Seedless vines at budbreak

Einfluß der Stickstoffdüngung auf die Cytokininkonzentration (μg Zeatinäquivalente/l) im Blutungssaft von Freilandpflanzen der Sorte Thompson Seedless zur Zeit des Knospenaustriebes

I. Analysis of variance

Source of variation	df	MS	F
Among N-level	4	6.925	11.17**
Within N-level	5	0.62	

** Significant at 1 % level.

II. Duncan multiple-range test

Treatments (kg N/ha)	0	672	112	224	448
Mean	3.24^{c}	3.50^{c}	5.10^{bc}	5.80^{b}	7.90^{a}

Means with a common letter are not significantly different at 5 % level.

Thompson Seedless vines fertilized with 448 kg N/ha had significantly higher concentration of cytokinin in xylem sap than the other treatments (Table 5). The level of cytokinin in xylem exudate of vines fertilized with 112 and 224 kg N/ha was also higher than in unfertilized vines. The concentration of cytokinin in xylem sap of vines that received 672 kg N/ha did not differ significantly from control vines (0-nitrogen) or vines receiving 112 kg N/ha. Fertilization with very high dosage of nitrogen (672 kg N/ha) apparently either decreased synthesis and/or export of cytokinin from roots to aerial portion of the vines.

Summary

The effects of cultivar, rootstock, and fertilization on the concentration of cytokinin in xylem exudate of vines grown at 12 °C and 25 °C root temperatures were studied in greenhouse and phytotron rooms. The air temperature in the greenhouse ranged between 15 °C at night to 30 °C during the day. The daytime (6 a. m. to 6 p. m.) air temperature in the rotating and stationary rooms was 27 °C and 17 °C, respectively; the night time temperature was 12 °C in both rooms.

After extraction of freeze-dried xylem exudate, cytokinin activity was detected by the cucumber cotyledon bioassay and GLC. The level of cytokinin in xylem exudate from Pinot noir vines was less than in Cabernet Sauvignon vines at both 12 and 25 °C root temperatures. Cytokinin activity in xylem sap of Cabernet Sauvignon and Pinot noir vines grown at 25 °C root temperature was usually significantly greater than at 12 °C root temperature, whereas cytokinin levels in vines grown at 17 and 27 °C air temperature at the same root temperature did not differ significantly. The major difference in cytokinins between the two root temperatures

was a greater activity at R_f 0.2 to 0.7 on paper chromatograms of 25 °C samples. Xylem exudate from vines grown at 12 °C root temperature had higher acitivity at R_f 0.8. Qualitative analysis (GLC) showed a single cytokinin peak for vines grown at 12 °C, whereas at 25 °C there were four peaks showing cytokinin activity.

Fertilization of potted Cabernet Sauvignon vines with N, P and K slightly increased the concentration of cytokinin in xylem exudate during budbreak at both 12 and 25 °C root temperatures. Under field conditions, however, mature Thompson Seedless vines that were fertilized with 224 and 448 kg N/ha had significantly higher concentration of cytokinin in xylem exudate at budbreak than vines not fertilized and vines fertilized with 672 kg N/ha.

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