EFFECT OF ABSCISIC ACID ON GROWTH CORRELATION IN *VITIS VINIFERA* L.*

By M. G. Mullins† and Daphne J. Osborne‡

The inflorescences of woody grape vine cuttings usually atrophy if bud burst precedes emergence of adventitious roots (Mullins 1967). Roots are a site of cytokinin synthesis (Kende 1964; Itai and Vaadia 1965), and the ascending sap of vines is rich in endogenous cytokinin (Loeffler and Van Overbeek 1964; Nitsch and Nitsch 1965; Skene and Kerridge 1967). The inflorescences of vine cuttings are retained and continue to grow if roots are initiated before bud burst, and the inflorescences of non-rooted cuttings are retained when treated with synthetic cytokinins (Mullins 1967).

In non-rooted cuttings the demands of expanding leaves for available hormones or metabolites appear to take precedence over those of the inflorescences because inflorescences are retained if leaves are excised from the expanding bud. Furthermore, the inflorescences of non-rooted vine cuttings respond to gibberellic acid if leaves are removed, but inflorescences do not elongate in the presence of leaves unless gibberellic acid and a cytokinin are applied in combination (Mullins 1968).

Work with both intact plants and detached plant organs indicates that cytokinins have a regulatory function in nucleic acid and protein synthesis, possibly by incorporation into soluble RNA (Fox 1966; Fox and Chen 1967). Some growth-promoting effects of cytokinins can be counteracted by abscisic acid (ABA), and ABA has been shown to depress RNA and protein synthesis (Crispeels and Varner 1966; Osborne 1967; Esashi and Leopold 1968).

This paper reports an effect of foliar applications of ABA on inflorescence growth in non-rooted vine cuttings.

Materials and Methods

Dormant canes of the cultivar Cabernet Sauvignon were stored under refrigeration (4°C) until required, and single-node cuttings were propagated by methods described earlier (Mullins 1967). Plants were treated when they bore three unfolded leaves. For experiments on the incorporation of amino acid into protein, ABA (250 mg/l) was applied to one-half of the leaf up to the main vein with a camel-hair brush, and water supplied to the other half. After 1 day, and again after 5 days, disks were cut from the treated and control halves of leaves and incubated for 2 hr at 25°C on the surface of 0·8 ml of [^{14}C]leucine (specific activity 10 mCi/mM) at 1 μCi/ml in the presence of 40,000 units of penicillin per millilitre. The disks were killed by boiling in 80% ethanol, and protein was extracted by the method of Osborne (1962). Determinations of total uptake

* Manuscript received September 8, 1969.

† Division of Horticultural Research, CSIRO, Private Bag No. 1, Glen Osmond, S.A. 5064.

‡ Agricultural Research Council, Unit of Experimental Agronomy, Department of Agricultural Science, University of Oxford, England.

of ^{14}C per sample showed no differences between samples. An aliquot of the protein hydrolysate was assayed for radioactivity with a Beckman Lowbeta gas-flow end-window counter. Assay of total protein in the extracts was by the microKjeldahl method.

Results

When vine inflorescences emerge the flowers are enclosed by bracts. As growth and development proceeds the bracts fold back and the flowers protrude. Later, the rachis becomes elongated and the flowers separate. Effects of foliar applications of ABA on inflorescence growth in vine cuttings were made in three series of experiments; one at Oxford, England, and the others at Adelaide, South Australia. Similar responses were found in all experiments.

In control plants protrusion of flowers from the bracts was observed in up to 10% of inflorescences, but most inflorescences failed to expand. All inflorescences subsequently turned brown and died, and none survived longer than 3 weeks from the beginning of experiments. In treated plants there was an increase in the number of

Fig. 1.—Effect of foliar application of abscisic acid (250 mg/l) on inflorescence growth. *A*, treated; *B*, control. Plants photographed 3 weeks after the hormone was first applied.

inflorescences which showed continued growth and development (as evidenced by flower protrusion), and the inflorescences which did not expand remained viable and retained their green coloration and turgidity.

The scale of experiments was necessarily small because of the scarcity of ABA and because relatively large amounts of hormone were required to produce effects on inflorescence growth. Less than 5% of plants produced expanded inflorescences in response to single applications of ABA at a concentration of 250 mg/l (Fig. 1).

A greater proportion of plants responded when ABA was applied for 3 consecutive days, and stimulation of inflorescence growth occurred with greatest frequency (see following tabulation) when plants were defoliated so that one leaf remained:

ABA Concn. (mg/l)	No. of Inflorescences with Flowers Emerging	
	Intact Plants	Partly Defoliated Plants
0	4	5
50	6	16
100	10	14
250	10	17

No effects of ABA concentration were observed and three consecutive applications of either 50, 100, or 250 mg/l all produced similar results. Measurements of leaf and shoot growth were made at the end of experiments when plants were destructively sampled (after 3 weeks) but not during the growing period. There were no significant differences between treated plants and controls in the numbers of leaves produced, in total leaf area, or shoot elongation. Applications of ABA to leaves did not induce leaf abscission. In one experiment (involving 250 treated plants) 30 inflorescences survived to anthesis, and bunches of grapes were produced by eight plants.

TABLE 1

INCORPORATION OF [^{14}C]LEUCINE INTO PROTEIN. DISKS CUT FROM ABA-TREATED AND CONTROL HALVES OF VINE LEAVES 1 AND 5 DAYS AFTER TREATMENT

Control leaves treated with water. ABA applied at a concentration of 250 mg/l. Results for each of two replicates given

	Protein Content (mg)	Total Radioactivity Incorporated (counts/min)	Specific Activity (counts/min/ mg protein)
Day 1			
Control leaves	2·40, 2·33	2570, 3270	1070, 1403
ABA-treated leaves	2·66, 2·68	2020, 1610	759, 601
Day 5			
Control leaves	2·23, 2·14	2700, 2440	1210, 1184
ABA-treated leaves	2·44, 2·39	2360, 2040	968, 854

Whilst effects of ABA on inflorescence growth were variable its effects on protein synthesis were unequivocal. It is seen from Table 1 that the application of ABA to the vine leaf results in a marked depression in the incorporation of amino acid into protein by the first day, but some recovery of synthesis occurs by the fifth day.

Discussion

Effects of ABA on plant growth are often transient (reviewed by Addicott and Lyon 1969), and it has been shown that ABA is readily metabolized in plant tissues (Milborrow 1968). Inactivation of ABA by the tissues concerned could well explain why the retardation of protein synthesis diminishes with time from application, and

why differences in leaf and shoot growth were not detected. Here, it is likely that plants had recovered from effects of ABA before they were sampled at the end of the experiment. Nevertheless, three successive foliar applications of ABA produced marked but variable differences in inflorescence growth between treated and control plants. There was continued development in up to 35% of inflorescences in treated plants, and 8 out of 250 treated plants subsequently bore fruit. None of the inflorescences of control plants survived to anthesis.

These results suggest that compensatory growth of inflorescences resulted from the reduced protein synthetic rate of leaves following applications of ABA, an inhibitor of protein synthesis. Inflorescence growth in non-rooted vine cuttings is normally limited by cytokinin supply in the stem (Mullins 1968). If the presence of cytokinins in cells is essential to maintain protein synthesis and growth processes, then the effect of applying ABA to leaves, and thereby reducing their levels of protein synthesis, could in turn reduce the demand for cytokinin by the leaves and permit diversion of metabolites and cytokinins for inflorescence growth.

In these experiments it is suggested that foliar applications of ABA produced a cytokinin-sparing effect since inflorescences were retained by many plants; an effect hitherto found *only* when inflorescences were treated with exogenous cytokinin, when leaves were excised from expanding buds, or when plants were propagated so that adventitious root emergence preceded bud burst (Mullins 1967, 1968).

The results presented here indicate the possibility of modifying the potency of metabolic sinks within a plant in two ways. First, by use of growth regulators which stimulate nucleic acid and protein synthesis at the point of application; and second by temporarily reducing such processes in one region, by use of inhibitors such as ABA, and thereby permitting an enhanced competitive growth of another plant part.

Acknowledgments

This work was supported by a CSIRO Post-Doctoral Studentship to M. G. Mullins. We thank M. M. Rives, Directeur, Station de Recherches de Viticulture, INRA, Pont-de-la-Maye (Gironde), France, for gifts of vine cuttings, and the Shell Research Centre, Sittingbourne, Kent, England, for abscisic acid.

References

Addicott, F. T., and Lyon, J. L. (1969).—Physiology of abscisic acid and related substances. *A. Rev. Pl. Physiol.* **20**, 139–64.

Chrispeels, M. J., and Varner, J. E. (1967).—Hormonal control of enzyme synthesis: On the mode of action of gibberellic acid and abscisin in aleurone layers of barley. *Pl. Physiol., Lancaster* **42**, 1008–16.

Esashi, Y., and Leopold, A. C. (1968).—Regulation of tuber development in *Begonia evansiana* by cytokinin. In "Biochemistry and Physiology of Plant Growth Substances". pp. 923–41. (Runge Press: Ottawa.)

Fox, J. E. (1966).—Incorporation of a kinin, N.6-benzyladenine into soluble RNA. *Pl. Physiol., Lancaster* **41**, 75–82.

Fox, J. E., and Chen, C. (1967).—Characterization of labelled ribonucleic acids from tissue grown on C14-containing cytokinins. *J. biol. Chem.* **242**, 4490–4.

Itai, C., and Vaadia, Y. (1965).—Kinetin activity in root exudate of water stressed sunflower plants. *Physiologia Pl.* **18**, 941–4.

KENDE, H. (1965).—Kinetin-like factors in root exudate of sunflowers. *Proc. natn. Acad. Sci. U.S.A.* **53**, 1302–7.

LOEFFLER, J. E., and VANOVERBEEK, J. (1964).—Kinetin activity in coconut milk. In "Régulateurs Naturels de la Croissance Végétale". pp. 77–82. (Cent. Natn. Rech. Scient.: Paris.) Colloques Int., Gif-sur-Yvette, July 1963.

MILBORROW, B. V. (1968).—Identification and measurement of (+)-abscisic acid in plants. In "Biochemistry and Physiology of Plant Growth Substances". pp. 1531–45. (Runge Press: Ottawa.)

MULLINS, M. G. (1967).—Morphogenetic effects of roots and of some synthetic cytokinins in *Vitis vinifera* L. *J. exp. Bot.* **18**, 206–14.

MULLINS, M. G. (1968).—Regulation of inflorescence growth in cuttings of the grape vine (*Vitis vinifera* L.) *J. exp. Bot.* **19**, 532–43.

NITSCH, J. P., and NITSCH, C. (1965).—Présence de phytokinines et autres substances de croissance dans la sève d'*Acer saccharum* et de *Vitis vinifera*. *Bull. Soc. bot. Fr.* **112**, 11–18.

OSBORNE, D. J. (1962).—Effect of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. *Pl. Physiol., Lancaster* **37**, 595.

OSBORNE, D. J. (1967).—In "Aspects of the Biology of Ageing". pp. 305–21. (Academic Press, Inc.: New York.)

SKENE, K. G. M., and KERRIDGE, G. H. (1967).—Effect of root temperature on cytokinin activity of root exudate of *Vitis vinifera* L. *Pl. Physiol., Lancaster* **42**, 1131–9.