

Influence of Benzyladenine, Leaf Darkening, and Ringing on Movement of ^{14}C -labeled Assimilates Into Expanded Leaves of *Vitis vinifera* L.

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Abstract. Leaves of *Vitis vinifera* L., nearly fully expanded, imported only trace amounts of ^{14}C following assimilation of $^{14}\text{CO}_2$ by a lower leaf on the same shoot, but benzyladenine (BA) application at 4.4×10^{-3} M caused a marked increase in the movement of ^{14}C into these leaves. Older leaves near the shoot base were less responsive; BA treatment alone had little effect on import of labeled assimilates from adjacent leaves but when the BA-treated leaves were darkened there was an increased import of labeled materials. When these 2 treatments were combined and applied to leaves on shoots with ringed bases, relatively high levels of radioactivity were detected in the BA-treated leaves but under these conditions darkening, without the application of BA, also resulted in an increased import of ^{14}C . Accumulation of imported ^{14}C was found to be restricted to the area of the leaf blade treated with BA. Separation of labeled compounds in ethanol extracts of treated leaves showed a lower percentage of radioactivity present in the sugar fraction from BA-treated leaves and an increased percentage present in the amino acid fraction.

Exogenous applications of cytokinins to aerial parts of plants are known to cause a wide range of growth effects (6,9). These compounds have been shown to be strong mobilizing agents causing movement of amino acids into cytokinin-treated areas of detached leaves (12) and retention of amino acids applied to treated tissues (11). Other compounds have also been shown to be attracted to sites of cytokinin application. Working with detached leaves Muller and Leopold (13) demonstrated the mobilization of ^{32}P and Gunning and Barkley (4) showed movement of ^{14}C -labeled photosynthates and ^{32}P to zones treated with cytokinin. Black and Osborne (1) found an increased auxin movement through petiole segments following benzyladenine treatment.

Chvojka *et al.* (2) found that cytokinin application caused buds to swell on young apple trees and resulted in the production of shoots from these buds; an increased incorporation of ^{32}P in apical meristems was also demonstrated. Application of benzyladenine to an attached bean leaf hastened senescence of other leaves on the stem (8) and a cytokinin applied to extending shoots of a grapevine caused an increased acropetal movement of ^{14}C -labeled photosynthates (15).

The experiments reported here were designed to examine the effect of benzyladenine application on the movement of ^{14}C -labeled assimilates into expanded leaves of *Vitis vinifera* L.

Materials and Methods

The grapevines used were mature vines of the variety 'Muscat of Alexandria', growing in a vineyard at the University of California, Davis. Leaves on non-fruiting shoots were used.

In all experiments benzyladenine (BA) was applied at a concentration of 4.4×10^{-3} M in the presence of Tween 20 wetter (0.1% v/v). The solution was applied with a soft paint brush to both upper and lower surfaces of a pair of adjacent leaves on opposite sides of the stem. Forty-eight hr after BA application 1 or 2 leaves on the shoot were allowed to photosynthesize in an atmosphere containing $^{14}\text{CO}_2$, each leaf being contained singly in a polyethylene bag, sealed around the petiole with adhesive tape. Labeled carbon dioxide was generated in a polyethylene reservoir, containing a known amount of air, by the addition of lactic acid to ^{14}C -labeled barium carbonate of specific radioactivity 129 μC per mg. Each leaf was supplied with 25 μC of $^{14}\text{CO}_2$ contained in 10 ml of air, transferred from the reservoir and injected into the polyethylene bag by a hypodermic syringe. After 30 min the polyethylene bag was removed and the shoots were collected after 24 hr. The shoots to be autoradiographed were segmented, mounted on paper and oven dried. When ^{14}C distribution within a leaf was to be examined (Fig. 3) the leaf was freeze-dried.

Autoradiographs were prepared according to Yamaguchi and Crafts (17), with an exposure period of 5 days.

In the experiments to be described, agreement between replicates used for autoradiography was generally good.

In the first experiment, replicated 4 times, an adjacent pair of approximately fully-expanded leaves was treated with BA or water containing wetter (control) and a single leaf lower down the shoot was fed with $^{14}\text{CO}_2$ (Fig. 1). This leaf was known to be exporting photosynthates in both upward and downward directions (5). In subsequent experiments, carried out later in the season when shoot growth had stopped, the leaves used were near the base of the shoot. These leaves were at least 12 weeks old, from the time of full expansion. $^{14}\text{CO}_2$ was supplied to a pair of leaves directly above the treated ones; these were known to be exporting photosynthates mainly basipetally to the parent vine (Fig. 2).

A preliminary experiment with the older leaves showed that these imported only trace amounts of ^{14}C following treatment of adjacent leaves on the shoot with $^{14}\text{CO}_2$. BA application as the only treatment had little effect on the amount of ^{14}C imported. In a subsequent experiment other treatments were introduced; a pair of adjacent leaves on shoots spaced widely over several vines was treated as follows: (i) water alone (control), (ii) water + leaf darkened, (iii) water + leaf darkened + shoot base ringed, (iv) BA alone, (v) BA + leaf darkened, (vi) BA + leaf darkened + shoot base ringed. In treatments (i) to (iii) the water contained wetter. Leaf darkening was achieved by enclosing the leaf in a shade constructed from aluminum foil. The BA was applied 48 hr, and the darkening and ringing treatments 24 hr, before $^{14}\text{CO}_2$ was supplied to the 2 leaves above the treated leaves.

Two shoots per treatment were prepared for autoradiography and from the other 4 replicates the 2 painted leaves were removed, dried, and ground in a Wiley micromill to pass a 40-mesh sieve, and 20 mg powder samples were weighed on planchets and counted in a proportional gas flow counter.

Treatments (iii) and (vi), replicated 5 times, were repeated 2 weeks later. The 2 painted leaves were removed from each shoot 24 hr after $^{14}\text{CO}_2$ treatment of the 2 leaves above, and cut into small pieces before extraction in boiling 80% ethanol. Leaves from untreated vines were used as controls. The methods of extraction and separation of each extract into amino acid, organic acid and sugar fractions were those described by Kliewer (7). Samples were taken from each fraction and counted in a Beckman LS100 liquid scintillation system.

Results and Discussion

A grape leaf is dependent upon a supply of photosynthates from older leaves during its early stages of growth but when it has achieved approximately half its final area it starts exporting assimilates to the growing shoot tip (5) and rapidly becomes independent of a supply of photosynthates from the mature foliage. It is clear from the autoradiograph shown in Fig. 1a that leaves nearing the point of full expansion import only trace amounts of ^{14}C following photo-assimilation of $^{14}\text{CO}_2$ by a lower leaf on the same shoot; upward movement of assimilates from this leaf was predominantly to the shoot apex and young leaves. Treatment with BA resulted in a marked increase in the amount of ^{14}C imported in a 24-hr period (Fig. 1b). This effect was not so readily achieved by the treatment of older leaves with BA. When applied to mature leaves near the base of the shoot, BA alone had no effect on import by the treated leaves of labeled materials exported by adjacent leaves on the shoot (table I). However, when the 2 BA-treated leaves were darkened they imported a significant amount of the isotope, but the darkening treatment applied without BA had little effect (table I). The direction of assimilate movement from the lower leaves on the shoot was downward to the parent vine (Fig. 2) but when movement out of the shoot *via* the phloem was prevented by ringing and the 2 BA-treated leaves darkened, import of assimilates by these leaves was greatly increased. Under these conditions an increased movement of ^{14}C into darkened leaves oc-

Table 1. *Effect of Benzyladenine, Leaf Darkening and Ringing on Import of ^{14}C -Photosynthate Into Mature Grape Leaves From Leaves Immediately Above Them*

Data transformed to logarithms for analysis of variance. Figures in parentheses are back-transformed means (cpm above background, per 20 mg powder)

Water + leaves darkened	Water + leaves darkened + shoot ringed	BA	BA + leaves darkened	BA + leaves darkened + shoot ringed	L. S. D. between treat- ment means
0.8733 (7)	2.7172 (521)	0.8451 (7)	1.9045 (80)	2.9263 (844)	0.8990

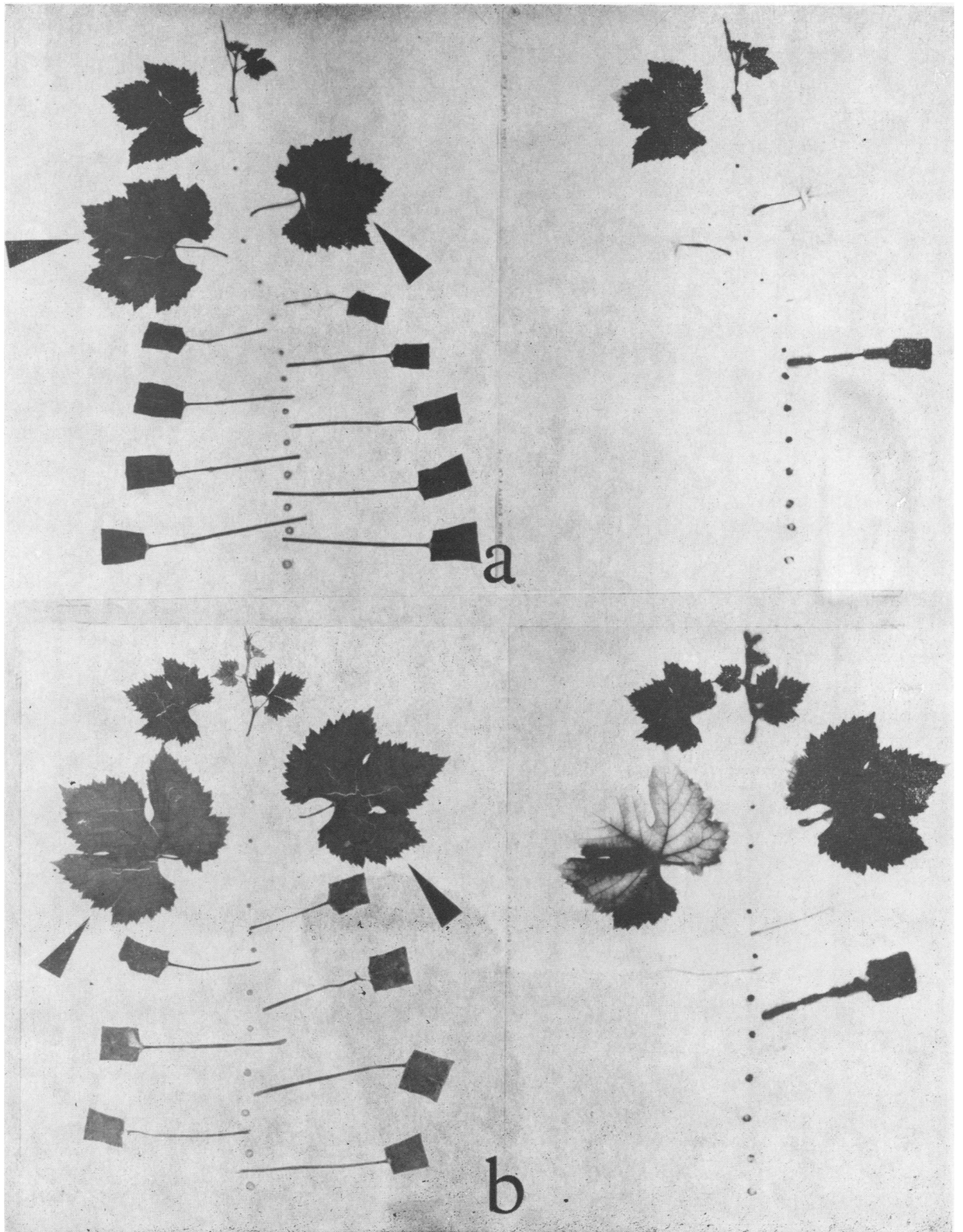


FIG. 1. Effect of BA on movement of ^{14}C -labeled photosynthate into nearly expanded grape leaves. Plant specimen on left of each pair and corresponding autoradiograph on the right. Two nearly fully-expanded leaves (arrowed) treated (a) with water containing wetter or (b) with 4.4×10^{-3} M BA. The third leaf below the lower arrowed leaf was fed with $25 \mu\text{C } ^{14}\text{CO}_2$.

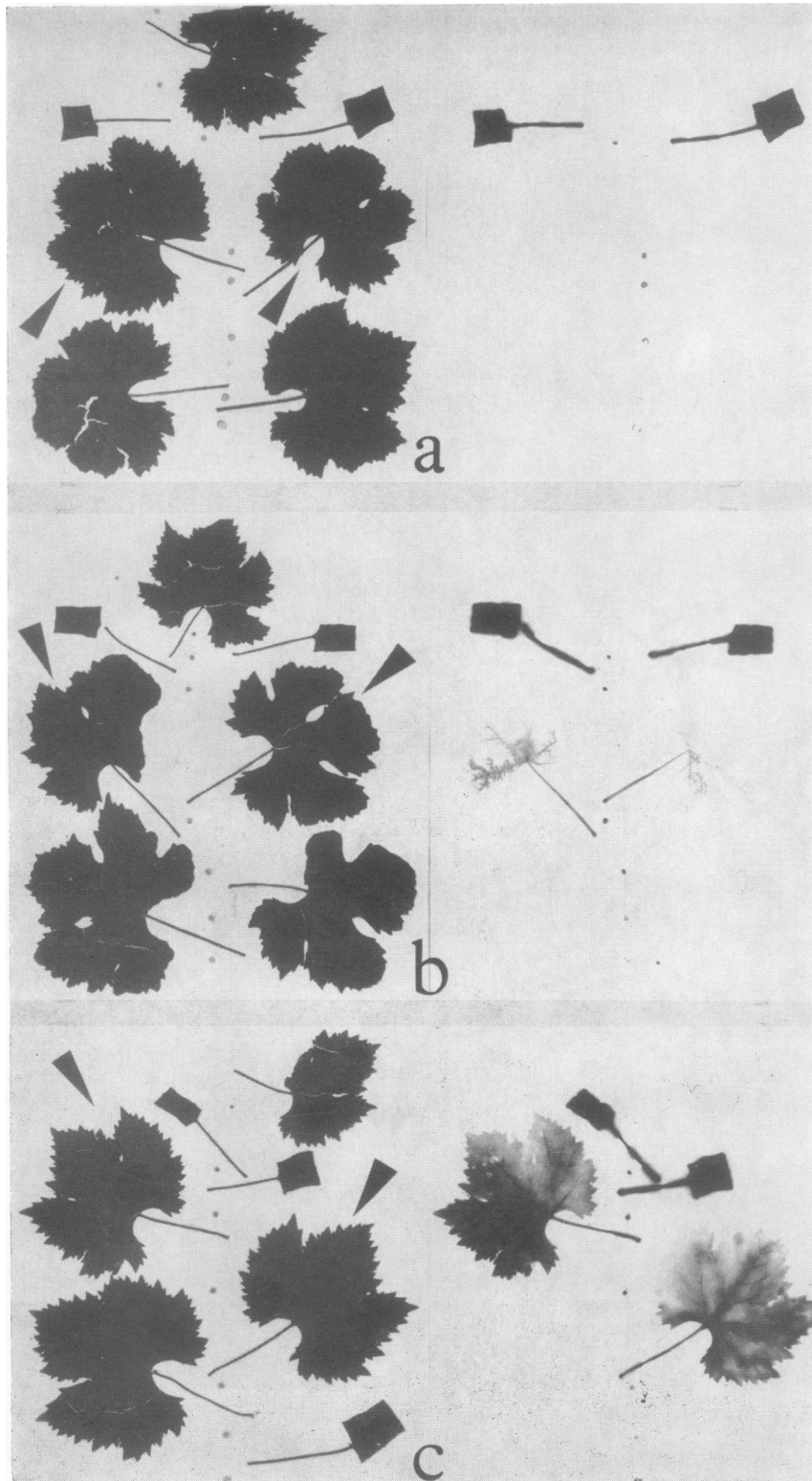


FIG. 2. Effect of BA, darkening and ringing on movement of ^{14}C -labeled photosynthate into mature grape leaves. Two adjacent lower leaves (arrowed) treated (a) and (b) with water containing wetter, (c) with 4.4×10^{-3} M BA. Arrowed leaves in (b) and (c) darkened and shoot bases ringed. The two leaves immediately above the arrowed ones were each fed with $25 \mu\text{c } ^{14}\text{CO}_2$.

curred even when the BA treatment was omitted (Fig. 2 and table I). Crafts and Yamaguchi (3) demonstrated a similar effect by shading a primary leaf on a bean plant, while at the same time removing the shoot apex. Translocation *via* the phloem into the darkened leaf was induced, as indicated by the movement of labeled amitrole. Therefore, provided a darkened leaf is not competing with a capacious sink, darkening may induce the import of phloem translocates.

Results of an experiment not described here, gave no indication that the ringing treatment influenced translocation *via* the phloem into unshaded BA-treated mature leaves.

Leaf darkening increased the effectiveness of BA application particularly in non-ringed shoots. This may have resulted from an improved penetration of the shaded leaf by the BA. Alternatively, the reduction in the level of available food materials within the leaf, as a result of shading, may have increased the capacity of the leaf as a sink for assimilates produced in other leaves.

In order to separate the effect of the BA application from that of the darkening and ringing treatments a lateral half of a mature leaf on a ringed shoot was treated with BA and the other half with water containing wetter; the whole leaf was darkened. The distribution of the imported ^{14}C , shown in Fig. 3, indicates that accumulation occurred only in the part of the leaf blade treated with the BA.

An examination of the distribution of imported ^{14}C in the amino acid, organic acid and sugar fractions of ethanol extracts of leaves from the 2 treatments, with and without applied BA, which imported the most isotope (Fig. 2b and c) showed that in both cases over 90% of the total radioactivity was present in the sugar fraction, but a significantly lower value was obtained from the leaves treated with BA (table II). The BA treatment did not alter the small percentage of radioactivity in the organic acid fraction but increased that present in the amino acid fraction. Levels of individual amino acids were not determined but it is known that cytokinins are effective in maintaining protein levels in detached leaves (14) and may also stimulate protein synthesis (10). Recently, Weaver *et al.* (16) have shown that treatment of a fruit cluster of 'Black Corinth' grape with cytokinin results in more radioactivity in the sugar fraction but less in the amino acid fraction after a leaf above the cluster is treated with $^{14}\text{CO}_2$.

Mothes (11) suggests that kinetin has a rejuvenating effect on aging leaves and insofar as it causes import and accumulation of phloem translocates benzyladenine may be regarded as having this effect on expanded leaves of the grapevine. However, leaf age is an important factor; leaves that are not fully expanded respond more readily than do older ones.

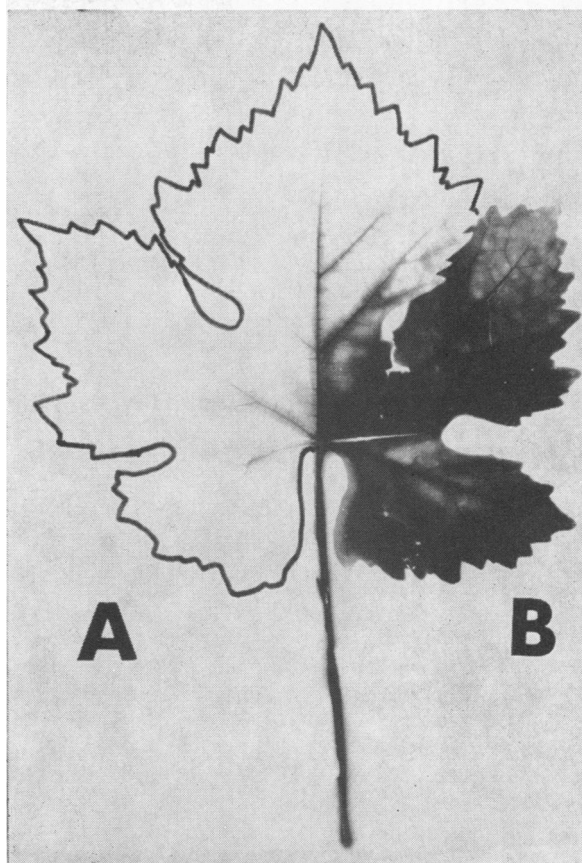


FIG. 3. Autoradiograph showing the distribution of imported ^{14}C within a mature darkened grape leaf following application of BA to half of the leaf. Lateral half (A) treated with water containing wetter, half (B) with 4.4×10^{-3} M BA. The shoot base was ringed and the 2 leaves immediately above the treated one were each fed with $25 \mu\text{c } ^{14}\text{CO}_2$.

Table II. Effect of Benzyladenine on Distribution of ^{14}C -Activity in Ethanol Extracts of Darkened Mature Leaves on Ringed Shoots of 'Muscat of Alexandria' Grapevines

Leaves immediately above the treated and extracted ones were fed $^{14}\text{CO}_2$. The extract was passed through a cation exchange column (Dowex 50 W-X8) and the amino acid fraction was eluted with 10% NH_4OH . Organic acids were separated from the effluent by passage through a Dowex 1-X8 anion exchange column and eluted with a 1.5 N solution of $(\text{NH}_4)_2\text{CO}_3$.

Leaf darkened, shoot ringed	Amino acid fraction	Organic acid fraction	Sugar fraction
$\% ^{14}\text{C}$ in Ethanol Extract			
Without BA	4.31	1.61	94.08
With BA	6.14	1.82	92.04
Significance of difference	P<0.05	N.S.	P<0.05

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