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The active control of calcium allocation in avocado trees

Progress report

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ABSTRACT

IAA export and ^{45}Ca uptake by excised avocado flower, fruitlet and shoots from the vegetative flush, were determined in conditions which limited transpiration flow to a minimum. Treatments using auxin transport inhibitors were included to ascertain if IAA played a role in the allocation of Ca. IAA levels were determined using radioimmunoassay. IAA export and ^{45}Ca uptake were strongest for the vegetative flush and weaker for the reproductive structures, for the first five weeks after flowering. The competition for and control of Ca partitioning is discussed.

Abbreviations:

IAA = lndole-3-acelic acid; TIBA = 2,3,5-triiodobenzoio acid; RIA = radioimmunoassay; DPM = disintegrations per minute

INTRODUCTION

The South African avocado industry is heavily dependent on exports of refrigerated fruits by air and sea to the northern hemisphere. Export by sea involves road and sea journeys of over 10 000 km at a temperature of about 5,5°C, and an interval of about four weeks from harvest to disembarkation in Europe. Fruit quality problems are magnified by this extended postharvest period (Cutting *et al*, 1988)

Bower (1985) found that avocado fruits with a low Ca level had a greater potential for physiological disorders and poor postharvest quality. Low levels of Ca in avocado fruits has been associated with, rapid softening after harvest (Wills & Tirmazi, 1982), and with susceptibility to chilling injury (Chaplin & Scott, 1980). The avocado appears to have a very limited ability to take up Ca from orchard sprays (Veldman, 1983) or dipping solutions (Tingwa & Young, 1974). Eaks (1985) observed that the benefits of vacuum infusion of Ca were negated by the adverse effect on external quality, when ripened after storage.

In general it is agreed that Ca-related disorders arise from its internal distribution problems and its allocation between mature and growing regions of the plant (Bangerth, 1979; Marschner, 1983). It is usually accepted that transport of Ca occurs mainly in the xylem vessels and in Ca exchange sites along xylem walls (Hanson, 1984). Plants have

to be continuously supplied with Ca, as little or no redistribution occurs to new growth zones after accumulation in one site (Poovaiahn, 1985). Competition between sinks is intensified when the calcium content in xylem sap is low and transpiration great (Clarkson, 1984). Other studies have, however, shown this relationship not to be so simple. For example, the influx of Ca in leaves declines after maturity, even though a constant transpiration rate is maintained (Koontz & Foote, 1966). It would therefore appear that plants have additional or alternative mechanisms of regulating their Ca distribution, besides its strong relation to water supply and movement in the xylem.

There is considerable evidence that auxin (IAA) transport plays an important role in Ca allocation to developing tissues (Banuelos *et al*, 1987). The application of auxin transport inhibitors such as TIBA led to Ca-related deficiency disorders in tomatoes and apples (Bangerth, 1973; 1976). Whitney *et al* (1986) found that avocado fruits on trees with reduced vegetative vigour accumulated more Ca. In the present paper the relationship between vegetative and reproductive flushing, auxin export, sink strength for Ca and its allocation during flowering and early fruit growth of Fuerte avocados, was investigated.

MATERIALS AND METHODS

General

Flower and vegetative flushes of Fuerte avocados were removed from the authors' orchard during August and September 1988. All experiments were conducted in replications of three or four, with ten plant parts (flowers or vegetative flushes) per replication. All results are presented as the means of the replicates. All of the results were subjected to analysis of variance, and treatment comparisons were done using the Student-Newmans Kreuls Comparison Test, the Duncan's new Multiple Range Test and the Tukey Comparison Test. The comparison which gave the strictest test is that presented in the results.

TIBA in lanolin with Tween 20 was used to inhibit auxin transport. It was applied to the base of the newly emerging vegetative flush, or the expanding flower panicle 48 hours prior to removal from the tree. The different plant parts (with or without TIBA depending on treatment) were placed on 0,5 ml 1,5 per cent agar in a specially modified low absorption (Nunc 466982) polyethylene tube (Figure 1). The excised plant parts were then placed in a container with 100 per cent RH, together with small containers of KOH crystals (to remove $C0_2$) and potassium permanganate (to remove ethylene) and left for 48 hours in a 12-hour light, 12-hour dark cycle.

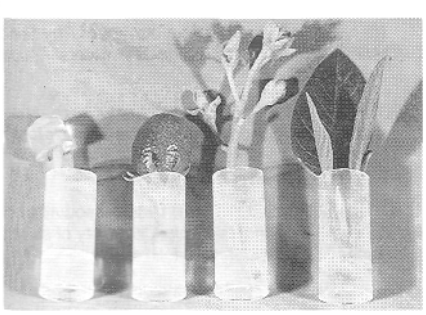

Fig 1 The method used for determining IAA export and ^{45}Ca uptake. The agar blocks in the base of the modified tube are assayed for IAA. The top agar block, fruitlet, flowers or vegetative shoot were ashed for Ca determination (magnification x3).

Determination of rate of basipetal auxin transport

Flower peduncles 25 mm long with the flowers removed, were individually placed on an agar block. Agar blocks with IAA concentrations from 0 to 1,25 jug were then placed on top of the peduncle and placed in the respective containers. After 48 hours, the bottom agar block was assayed for IAA content, using a standard radioimmunoassay (RIA) procedure (Cutting *et al*, 1986). The antiserum characteristics were essentially the same as those reported by Weiler (1981). The experiment was repeated using TIBA as above.

Determination of Ca uptake

^{45}CaCI (*ca* 90 000 DPM) was incorporated in agar blocks, which were used as the bottom agar blocks and the experiment conducted as above, but using the following treatment differences. Treatments with 1,25 µg IAA in the top agar block as well as flower, vegetative and young fruit material both with and without TIBA were used. The experiment was repeated twice. IAA was determined in the agar using RIA. The method of Banuelos *et al* (1987) was used for the determination of radioactive ^{45}Ca. The flowers and vegetative flushes were ashed for 60 minutes at 550°C and dissolved in 2 m 2M HCL A 0,1 m sample was then added to 5 ml liquid scintillant and the radioactivity counted. Radioactivity determinations were done in triplicate.

Comparison of auxin export to vegetative and reproductive structures

Portions of flower panicles containing six to eight flowers (approximately one-tenth of the total flower panicle) and single vegetative flushes from the centre of the flower's panicle (Figure 2) were placed on agar blocks and left for 48 hours as above. The experiment was repeated five times from just prior to flower-opening, to when the fruit had reached ca 30 mm. All the experiments included TIBA treatments. The diffused IAA content in the agar was determined, using RIA.

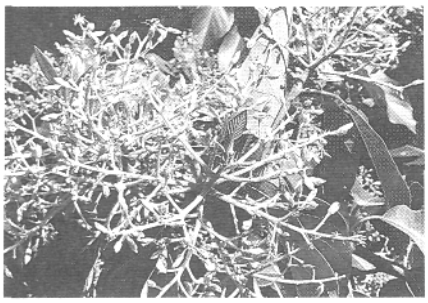

Fig 2 Avocado flowering and early fruit-set show the newly emerging vegetative flush in the centre of the panicle.

RESULTS

Rate of auxin transport

The basipetal auxin transport into the agar increased in response to the increasing auxin concentration in the donor agar block (Table 1). The quantity of extractable IAA in the 25 mm flower peduncle was almost the same as the amount of diffusible IAA from the control (zero IAA in the top agar block). Treatment with TIBA significantly reduced the amount of IAA in the receiver blocks.

TABLE 1 Diffusable IAA concentration in ng per 10 peduncles in receiver blocks of agar in response to different concentrations of IAA in the donor blocks and TIBA treatment

IAA concentrations in donor block	O TIBA	TIBA	Extractable IAA in peduncle
O IAA (control)	18,6a	14,7a	25,2a
500 ng IAA	58,5b	15,9a	31,2a
1250 IAA	86,7c	19,5a	32,7a

Values followed by the same letter do not differ significantly, at P = 0,01 and the CV = 27 per cent.

Ca uptake in response to basipetal auxin transport

The uptake of ^{45}Ca was dependent upon type of plant structure. The flower panicle took up 24 per cent, the vegetative flush from 35 to 47 per cent and the fruitlets from 19 to 21 per cent of the available ^{45}Ca (Table 2). Fruitlets took up between 19 and 21 per cent of the available Ca. In all cases treatment with TIBA reduced Ca uptake.

TABLE 2 Radioactive ^{45}Ca uptake in DPM by different avocado plant parts with and without TIBA treatment. The Ca uptake expressed as a percentage of the available ^{45}Ca in the donor block is presented in brackets

	13-09-1988		21-09-1988
	O TIBA	TIBA	O TIBA
Flower panicle	11303c (24%)	9413d (20%)	na
Vegetative flush	21407a (47%)	14220b (31%)	16019b (35%)
Fruitlet	8615d (19%)	6554e (14%)	9672d (21%)
Receiver agar	215f (0,47%)	136f (0,24%)	nd

Values followed by the same letter do not differ significantly, at P = 0,01 and the CV = 10,8 per cent.

Auxin transport from reproductive and vegetative growth flushes

The auxin transport in the expanding vegetative flushes increased from 32 ng to over 55 ng per 10 shoots (Figure 3a). Auxin transport from the flowers peaked at maximum flower opening and then declined (Figure 3c). Export of IAA showed an increasing trend with time in young fruitlets (Figure 3e). TIBA treatment reduced IAA export from all these organs (Figures 3b, d,f).

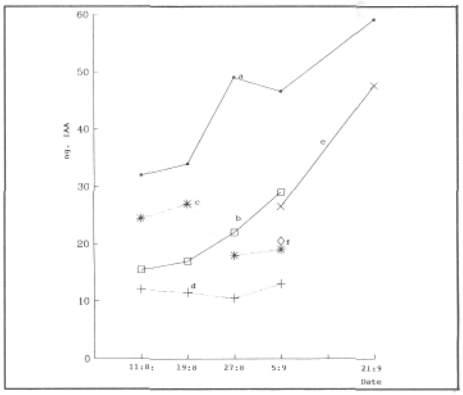

Fig 3 IAA export from various plant parts:

a = vegetative shoots;
b = vegetative shoots with TIBA;
c = flowers;
d = flowers with TIBA;
e = young fruits;
f = young fruits with TIBA treatment.

The arrow indicates maximum flowering. Vertical bars = SE of the means.
Date = day:month.

DISCUSSION

There is evidence of a strong interaction between Ca and IAA in plants at the cellular

levels (Felle, 1988). Findings indicate that a portion of the Ca^2 + transport in tomato is under the control of fruit-produced IAA (Banuelos *et al,* 1987). The authors' results show that a certain proportion of Ca allocated to the different plant structures appears to be under the control of basipetal auxin movement. This is confirmed by the reduction in Ca uptake in response to the presence of the auxin transport inhibitor, TIBA. Apparently, TIBA did not completely stop auxin transport, but appeared to be more effective when relatively large quantities of auxin were being exported. Care was taken to reduce the transpirational flow to a minimum by maintaining a 100 per cent RH, thereby reducing the transpiration dependent Ca^{2+} import.

The relative difference in the IAA export strengths of the vegetative and reproductive flushes was particularly important. Auxin transport is strongly inhibited by a group of ubiquitous phenolic compound (Jacobs & Rubery, 1988). In avocado, phenolic levels tend to be higher in fruit than those found in vegetative growth (Torres *et al*, 1987) and this could be at least partly responsible for the reduced auxin export from fruitlets, relative to the vegetative flush as found in our study. This avenue, particularly in the case of fruits which are weak Ca accumulators such as avocados, requires further investigation.

In the whole tree situation, the more vigorous the vegetative flush, the greater the production of IAA (Leopold & Koriedemann, 1975), the stronger the basipetal IAA transport (Banuelos *et al,* 1987) and the stronger the vegetative sink strength and resultant Ca allocation. ^{45}Ca uptake was higher in the vegetative flush, but declined with time, while ^{45}Ca uptake into the fruit increased. This was thought to be due to the competitive auxin export, which had begun from the young fruits (with many on a single panicle Figure 3) and the observation that although leaf expansion was continuing, no further new leaves were being produced, ie apical activity had decreased. Competition between reproductive and vegetative tissue could modify IAA export and dominance phenomena (Bangerth, 1986) and consequently contribute to lower Ca^{2+} in tissues, such as fruit with low transpiration rates.

Our findings show that flowers, and to a lesser extent young fruits, are poor IAA exporters and are weak sinks for non-transpirational Ca^{2+}. This would be to the detriment of potential fruit Ca allocated under IAA control. This is borne out by Ca analysis of fruitlets from vigorous and non-vigorous trees, which showed that fruit from non-vigorous Fuerte trees accumulated nearly twice as much Ca during the first six weeks of fruit growth (Whitney *et al*,1986) and from observations of a deterioration in postharvest fruit quality in fruit from very vigorous trees (Cutting, unpublished data). In South Africa, the soil pathogen *Phytophthora cinnamomi* has been brought under effective chemical control (Bezuidenhout *et al* 1987) resulting in excessive vegetative vigour in avocado trees. The implications of these results are obvious and important, as the largest proportion of fruit Ca is taken up during the first two months of fruit growth (Bower, 1985).

Therefore, controlling the vigour of the vegetative flush that occurs during and shortly after flowering and fruit-set, holds potential benefits for accumulation of fruit Ca and as a consequence better postharvest quality. Research to reduce vegetative vigour and/or to hold the vegetative flush for four to six weeks after flowering, is currently receiving attention.

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