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SHORT COMMUNICATION

LOW TEMPERATURE BREAKDOWN IN APPLES

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Abstract—Low temperature breakdown in apples was increased by geraniol and a number of inhibitors of isoprenoid biosynthesis which were injected into the fruit after harvest. The addition of gibberellic acid reduced breakdown.

INTRODUCTION

SOME varieties of apples when cool stored, are affected by the physiological disorder, low temperature breakdown. The disorder occurs after the fruit has been stored below about 5° and appears as areas of brown tissue in the cortex. Previous work[1,2] has shown that the incidence of the disorder is correlated with the level of acetate in the fruit. It can be increased by injecting acetic acid into the fruit and decreased by removing acetate esters from the fruit. The time lapse between the injection of acetic acid and the appearance of the disorder suggested that a metabolite of acetate was causing the disorder. Of a number of compounds metabolically derived from acetate, mevalonate, a precursor of isoprenoid biosynthesis, was most effective in increasing breakdown.[3]

In this paper we report that a number of compounds affect the incidence of breakdown when injected into Jonathan apples. Some of these compounds are products of, and others are inhibitors of, isoprenoid biosynthesis.

RESULTS AND DISCUSSION

Table 1 shows results which confirms the findings of Wills and Scott[3] that mevalonate is more effective than acetate (both at 80 μM/fruit[3]) in increasing breakdown. A number of compounds which are derived biosynthetically from mevalonate were injected into apples at a concentration of 40 μM/fruit. The smaller concentration was used as it was considered that compounds closer to the immediate cause of breakdown than mevalonate would require less material to affect the level of the disorder and result in its earlier onset. The results in Table 2 show that geraniol increased breakdown to the greatest extent and that gibberellic acid was the only compound which reduced breakdown. The sample of 'geraniol' used was found by analysis to contain 50% geraniol and 50% nerol. Since nerol itself was ineffective when 40 μM were injected per fruit, it seems that 20 μM of geraniol per fruit effectively increased breakdown.

A number of compounds which have been shown to inhibit isoprenoid metabolism in various tissues were injected into apples and their effect on the development of breakdown was studied. The compounds were injected at a concentration of 5 μM or 10 μM per

[1] R. B. H. WILLS, K. J. SCOTT and W. B. MCGLASSON, *J. Sci. Food Agric.* **21**, 42 (1970).
[2] R. B. H. WILLS and W. B. MCGLASSON, *J. Hort. Sci.* **46**, 115 (1971).
[3] R. B. H. WILLS and K. J. SCOTT, *Phytochem.* **10**, 1783 (1971).

TABLE 1. BREAKDOWN IN APPLES INJECTED WITH ACETATE AND MEVALONATE

Compound (80 μM/apple)	% Bd.	Sig. diff.*
R-Mevalonate	68	+++
Acetate	35	+
Water (control)	27	

* In this table and the following tables, (+++), (++) and (+) indicate that the compound produced significantly more breakdown than control fruit at 0·1%, 1% and 5% levels, respectively, (---) indicates less breakdown than control at 0·1% level and (ns) indicates no significant difference from control at the 5% level.

fruit. The results in Table 3 show that none of the inhibitors decreased breakdown. The greatest increase in breakdown was found with iodoacetate, while SK&F 525, SK&F 7997 and vanadium pentoxide also significantly increased breakdown.

TABLE 2. BREAKDOWN IN APPLES INJECTED WITH ISOPRENOID COMPOUNDS (40 μM/apple)

Compound	% Bd.	Sig. diff.	Compound	% Bd.
Effective compounds			Non-effective compounds (cont.)	
Geraniol	46	++	*R*-Mevalonate	29
Gibberellic acid	6	---	Nerol	32
Ethanol (control)	26		Nerolidol	35
Non-effective compounds			Phytol	25
Abietic acid	26		t-Retinol	31
L-Borneol	22		Squalene	19
Coumarin	23		α-Terpineol	31
Ethyl chrysanthemate	26		Thymol	29
Farnesol	37		D-α-Tocopherol	25

Iodoacetate has been shown to inhibit the isomerisation of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP);[4] however, it is recognized that iodoacetate also inhibits many other reactions, particularly those involving —SH groups. SK&F 525 has been shown to inhibit conversion of IPP $\rightleftharpoons$ DMAPP as well as a number of later steps in isoprenoid biosynthesis[5] while SK&F 7997 inhibits conversion of lanosterol to cholesterol.[6] Experiments using various organisms have indicated different sites of inhibition for vanadium; most at sites close to IPP.[5] Cycocel and Alar were ineffective in increasing breakdown. Paleg[7] found these formulations to be the least effective of a number of known inhibitors of cholesterol synthesis in animal tissue.

[4] H. YOKOYAMA, J. O. M. NAKAYAMA and C. O. CHICHESTER, *J. Biol. Chem.* **237.** 681 (1962).
[5] W. L. HOLMES and N. W. DITULLIO, *Am. J. Clin. Nutrit.* **10,** 310 (1963).
[6] J. BONNER, E. HEFTMANN and J. A. D. ZEEVAART, *Plant Physiol.* **38,** 81 (1963).
[7] L. PALEG, *Nature, Lond.* **225,** 1252 (1970).

TABLE 3. BREAKDOWN IN APPLES INJECTED WITH INHIBITORS OF ISOPRENOID BIOSYNTHESIS

Experiment	Compound	Amount added (μM)	% Bd.	Sig. diff.
1	Iodoacetate	5	82	+++
	SK&F 525*	5	61	+++
	SK&F 7997*	5	60	+++
	2-Phenylbutyrate	5	10	ns
	Ethanol (control)		13	
2	Vanadium pentoxide	10	76	+++
	Vanadium pentoxide	5	74	++
	Ethanol (control)		46	
3	Cycocel†	10	24	ns
	Cycocel†	5	28	ns
	Alar‡	10	25	ns
	Alar‡	5	26	ns
	Water (control)		20	

* These are Smith, Kline and French inhibitors.[6]
† Formulation of (2-chloroethyl) trimethyl ammonium chloride (CCC).
‡ Formulation of *N*-dimethylamino succinamic acid (B-995).

Our results show that some compounds which are either isoprenoid compounds or alter isoprenoid metabolism affect the susceptibility of apples to breakdown. The effect of the plant growth regulator, gibberellic acid, in reducing breakdown is of particular interest. It has not previously been shown to have an effect on any storage disorder of apples. It may prove to have commercial use in reducing breakdown, especially if it can be applied as a post-harvest dip or a late pre-harvest spray. Three groups of plant growth substances are products of isoprenoid metabolism: gibberellins, the natural cytokinins and abscisins. The natural cytokinins are of particular interest as they are partly derived from DMAPP,[8] the level of which may be affected by some of the inhibitors used in this study. We feel that the effect of these other growth substances should also be investigated.

The increase in breakdown achieved by geraniol, a natural product of apples,[10] could be due to it acting as an inhibitor of isoprenoid biosynthesis. Phosphorylated geraniol compounds have been shown to inhibit prenyl transferase in animal tissue.[9] Octanol, which was also shown to inhibit prenyl transferase, has been found previously to increase breakdown.[3] Geraniol may be a natural inhibitor of isoprenoid biosynthesis in apples which predisposes the fruit to breakdown.

EXPERIMENTAL

Jonathan apples were harvested from commercial orchards at Bilpin, N.S.W., Australia, and randomly distributed into units, each of 25 fruit. The injection technique was that described by Wills, Scott and

[8] W. J. BURROWS, D. J. ARMSTRONG, M. KAMINEK, F. SKOOG, R. M. BOCK, S. M. HECHT, L. G. DAMMANN, N. J. LEONARD and J. OCCOLOWITZ, *Biochem.* **9**, 1867 (1970).
[9] G. POPJAK, P. W. HOLLOWAY, R. P. M. BOND and M. ROBERTS, *Biochem. J.* **111**, 333 (1969).
[10] F. B. POWER and V. K. CHESTNUT, *J. Am. Chem. Soc.* **44**, 2938 (1922).

McGlasson.[1] The compounds were injected in H_2O or EtOH as these solvents do not affect the level of breakdown.[3]

Comparison of mevalonate and acetate. Eight units of fruit were injected with 80 μM of each compound in H_2O. The fruit was stored at $-1°$ for 17 weeks and examined for breakdown after a further 7 days at 20°. The percentages of fruit with breakdown were transformed to angles by the arcsin transformation and the standard error ($\pm 1{\cdot}7°$) was calculated. The levels of statistical significance were determined by *t*-test. The breakdown data from the other experiments were also similarly analysed.

Injection of isoprenoid compounds. 40 μM of each compound were injected in EtOH into fruit from six units as the pure compound, except for D-α-tocopherol which was injected as the succinate derivative, and *R*-mevalonate as the *RS*-lactone. The fruit were stored at $-1°$ for 11 weeks and examined for breakdown after a further 5 days at 20°. The standard error of the breakdown data was $\pm 2{\cdot}4°$.

Injection of isoprenoid inhibitors. Each compound was injected in H_2O or EtOH into four units of fruit at the concentrations stated in Table 3. The fruit from Experiments 1, 2 and 3 was stored at $-1°$ for 21, 13 and 17 weeks, respectively and examined for breakdown after 2 days (Experiment 1) and 7 days (Experiments 2 and 3) at 20°. The standard errors were $\pm 2{\cdot}3°$, $\pm 2{\cdot}9°$ and $\pm 4{\cdot}0°$ for Experiments 1, 2 and 3, respectively. The fruit in each Experiment was from different orchards and had widely different susceptibilities to breakdown, and therefore only qualitative comparisons can be made between the results obtained in different experiments.

Gas chromatography. The analyses of the geraniol and nerol samples were carried out on a 0·9 m $\times$ 6 mm column of S.E. -30 (5%) on acid washed chromosorb W at 100°. The carrier gas was N_2 at 20 ml/min. The column was attached to an Aerograph Hy-Fi Gas Chromatograph (Model 600-D) equipped with a flame ionisation detector. The flow rate of hydrogen was 20 ml/min and air, 300 ml/min

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