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SHORT COMMUNICATION

CHEMICAL INDUCTION OF LOW TEMPERATURE BREAKDOWN IN APPLES

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Abstract—Jonathan apples injected with acetate, mevalonate, malonate, formate, butyrate, benzoate, caffeine, amytal, adenosine triphosphate and octanol showed an increase in low temperature breakdown after storage at $-1°$. Injection of calcium resulted in a decrease in the incidence of breakdown. It is suggested that isoprenoid metabolism may be involved in the metabolic events leading to breakdown.

INTRODUCTION

When stored below 5° certain varieties of apples are susceptible to the physiological disorder, low temperature breakdown. The disorder, which appears initially as browning in the outer cortex, has long been considered to result from an alteration in the metabolism of the fruit at low temperature.[1]

Wills[2] examined the volatiles given off by apples and found that, when the loss of certain acetate esters increased, the susceptibility of the fruit to breakdown decreased. He suggested that accumulation of acetate in the fruit could be involved in breakdown. Wills, Scott and McGlasson[3] examined the role of acetate and found that, early in storage, acetic acid accumulated to higher levels in fruit that were more susceptible to breakdown but later fell to low levels in all fruit before the appearance of visible symptoms of breakdown. They were able to induce the disorder by injecting acetic acid into the core of fruit but it appeared only after storage for many weeks. It was concluded that acetate itself was not causing breakdown but that the disorder was a result of its effect on the metabolism of the stored fruit.

In this paper we have attempted to determine which aspect of acetate metabolism might be involved in breakdown, by injecting a large number of substances that can be formed from acetate. We have also injected the major volatile compounds of apples, other compounds which have been associated with breakdown, namely oxalacetate,[4] acetaldehyde,[5] sorbitol,[6] and compounds containing nitrogen and phosphorus,[7] and salts of several

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mineral elements present in the fruit. The fruits were stored for 1 week at $-1°$, and each injected with 80 μmole of the substance used and then stored at $-1°$ for 3–9 months before being inspected for breakdown.

TABLE 1. INCIDENCE OF BREAKDOWN IN APPLES INJECTED WITH VARIOUS CHEMICALS

Compounds affecting breakdown	% Bd.	Sig. diff.	Comps. not diff. from control	% Bd.	Solvent control	% Bd.
Expt. 1						
R-Mevalonate	59	+++	Pyruvate	42	Water	30
Malonate	53	++	L-Malate	36		
Formate	49	++	Acetaldehyde	36		
Acetate	48	++	*cis*-Oxalacetate	35		
Butyrate	47	+	L-Serine	34		
			L-Glutamate	34		
			Propionate	32		
			Acetoacetate	31		
			D-3-Phosphoglycerate	24		
			Isopentyl acetate	45	Ethanol	33
			Isopentanol	40		
			Butyl acetate	38		
			Hexyl acetate	37		
			Ethyl butyrate	32		
			Hexanol	31		
Expt. 2						
Benzoate	69	+++	Thiourea	30	Water	18
Caffeine	49	+++	α-D-Glucose-1-phosphate	26		
Amytal	43	++	L-Phenylalanine	26		
Acetate	40	++	Sodium carbonate	24		
A.T.P.	36	+	Potassium carbonate	23		
			Phosphoric acid	23		
			Shikimate	19		
Expt. 3						
Acetate	61	++	Sorbitol	43	Water	41
Expt. 4						
Octanol	52	+++	Octyl acetate	24	Ethanol	12
			Hexanoic acid	23		
			Ethyl hexanoate	21		
			Hexanol	16		
			Heptanol	16		
			Hexyl butyrate	14		
			Hexyl acetate	14		
			Hexanol	5		
Expt. 5						
Acetate	59	+	Hydrochloric acid	48	Water	42
Calcium chloride	24	—	Ammonium chloride	46		
			Magnesium chloride	40		
			Potassium chloride	39		

(+++), (++) And (+) indicate that the compound produced significantly more breakdown than control fruit at 0·1%, 1% and 5% levels respectively and (–) indicates less breakdown than control at 5% level.

RESULTS AND DISCUSSION

The results (Table 1) show that a number of substances were effective in increasing the the incidence of breakdown. Mevalonate was the most effective of the substances associated with acetate metabolism. Malonate was also effective, but a number of other compounds had no significant effect under the conditions used. Both acetate and mevalonate are precursors for isoprenoid synthesis and the greater activity of mevalonate perhaps points to isoprenoid involvement in breakdown. Octanol, which was effective at the same level as mevalonate, might affect isoprenoid metabolism as could malonate. Octanol has been shown to behave like a monoterpene in its feedback inhibition of prenyl transferase[8] and malonate was found to be an intermediate in the synthesis of mevalonate in animal systems.[9]

No explanation can be advanced for the activity of other substances that increased breakdown, though the effect of caffeine, amytal and adenosine may be due to a requirement for nitrogen in breakdown processes in view of the increase in breakdown observed following high nitrogen levels of fertilization of the tree during growth.[7] The effect of calcium in reducing breakdown confirms the work of Perring[10] who found lower levels of calcium in fruit susceptible to breakdown, and Rasmussen,[11] who reduced breakdown by spraying with calcium during growth.

Work is proceeding in this laboratory to determine the importance of isoprenoid metabolism, and also purines and benzoate, in the production of breakdown.

EXPERIMENTAL

Fruit was harvested in 1969 and 1970 from commercial orchards in N.S.W., Australia, and randomly distributed into units, each of 25 fruits. Fruits were injected in the core areas with 80 μmole of the free acids or pure compounds, dissolved in 0·2 ml of H_2O or EtOH, except for D-3-phosphoglycerate, L-glutamate, benzoate, shikimate, L-phenylalanine and amytal which were injected as sodium salts, and acetoacetate and *R*-mevalonate which were injected as the methyl ester and as the *RS*-lactone respectively. As only the racemate of mevalonate was available, 160 μmole of the mixture was injected into the fruit. The amount of ATP added was 80 μmole with respect to the concentration of phosphorus, equivalent to 27 μmole of ATP.

TABLE 2. STORAGE DATA OF APPLES INJECTED WITH VARIOUS CHEMICALS

Expt.	Time at −1° (weeks)	No. of fruit per treatment	S.E. of mean (angles)
1	13	200	±3·0° on 144 d.f.
2	40	100	±3·4° on 39 d.f.
3	18	125	±2·0° on 12 d.f.
4	16	100	±4·1° on 30 d.f.
5	16	100	±3·3° on 21 d.f.

After storage at −1°, the fruits were kept at 20° for 7 days after which they were examined for the occurrence of breakdown. The percentages of fruit affected with breakdown were transformed to angles by the arcsin transformation and standard errors were calculated. The levels of statistical significance were determined by *t*-test. Table 2 gives the standard errors for the groups of injections and the time each group was stored at −1°.

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