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METABOLISM OF GERANIOL BY APPLES IN RELATION TO THE DEVELOPMENT OF STORAGE BREAKDOWN

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Key Word Index—*Malus sylvestris*; Rosaceae; Jonathan apple; geraniol; geranyl β-D-glucoside; storage breakdown; core; seeds.

Abstract—Geraniol was injected into the core of Jonathan apples susceptible to storage breakdown. It was rapidly metabolized to geranyl β-D-glucoside which was transported to the flesh and metabolized into other compounds. When geraniol was injected into the flesh and prevented from diffusing into the core area, metabolism of geraniol was completely inhibited. The results suggest that some factor associated with the apple seeds is involved in the formation of the glucoside and hence with the induction of breakdown.

INTRODUCTION

Low temperature breakdown is a physiological disorder that affects a number of varieties of apples following cool storage. The disorder first appears as areas of brown flesh tissue in the outer cortex and gradually spreads towards the core and skin, but only affects the core in very severe cases [1, 2]. Kidd and West [3] considered the disorder to be caused by an alteration in metabolism of the fruit below a critical temperature rather than due to senescence. Numerous workers have since correlated various changes in metabolism occurring in the flesh at low temperature with the development of breakdown. Many of these studies have suggested that breakdown is caused by some imbalance in carbohydrate catabolism and activity of the respiratory enzyme system at low temperature that leads to the accumulation of a toxic compound(s) in the flesh [4–8].

Wills and co-workers [9–11] examined the effect of a range of compounds on the incidence of breakdown by injecting the compounds into the core area of the fruit via the calyx. The core was chosen as the injection site as it is the area of the fruit rarely affected by the disorder. They found that a number of compounds associated with the isoprenoid pathway, including geraniol, were able to induce breakdown. The disorder symptoms appeared many weeks after the addition of the compounds and were identical with normal breakdown in that browning was in the outer cortex and no disorder or injury occurred in the core area. In a subsequent study, Wills and Scott [12] found that injection of geraniol to the core of Jonathan apples, in addition to increasing the incidence of flesh breakdown, reduced the level of all volatile terpenoid compounds in the fruit but no geraniol was detected at any time in the flesh tissue. The inference was that geraniol was rapidly converted to a non-volatile compound. In this study, we injected geraniol at 20 μmol/fruit into the core and flesh of Jonathan apples stored at 0° and determined the levels of free geraniol and geranyl β-D-glucoside (Ger-glc) in the core and flesh. Ger-glc has not been reported previously in apples, but Francis and Allcock [13] showed that it was the major storage form of geraniol in roses.

RESULTS

Geraniol injected into the core area was rapidly converted into Ger-glc which was detected in both core and

Table 1. Levels of geraniol and geranyl β-D-glucoside in apples at 0° that were injected with geraniol (20 μmol/fruit) into the core area

Time after injection (hr)	Amount present (μmol/fruit)				% Recovery*		
	Geraniol	Ger-glc Core	Ger-glc Flesh	Ger-glc Total	Ger	Ger-glc	Total
0.5	13.4	0.6	0.8	1.4	67	7	74
1	10.2	0.8	0.2	1.0	51	5	56
2	8.6	0.4	2.2	2.6	43	13	56
3	6.4	0.7	5.2	5.9	32	30	62
5	5.2	1.0	0.8	1.8	26	9	35
11	4.8	0.8	1.4	2.2	24	11	35
15	—	0.8	0.4	1.2	—	6	—
24	3.8	0.2	0.6	0.8	19	4	23
48	2.0	0.1	0.4	0.5	10	2	12

Values are means of two samples.

* % of added geraniol present as geraniol and Ger-glc.

flesh (Table 1). The concentration of Ger-glc in the core was relatively constant from 0.5 to 15 hr after injection and then declined to a low level, while the amount in the flesh tissue attained a maximum value after 3 hr and declined to a relatively low level by 15 hr. The amount of geraniol present in the fruit as Ger-glc attained a maximum value of 30% of that added after 3 hr but accounted for less than 5% of the added geraniol by 15 hr. The amount of free geraniol in the fruit declined rapidly with only 50% of that injected remaining in the tissue after 1 hr, while after 48 hr, only 10% was present. Control fruit that had no added geraniol was found to contain no free geraniol (i.e. <0.1 μmol/fruit, the limit of detection) and trace levels of Ger-glc (i.e. *ca* 0.01 μmol/fruit).

When geraniol was injected into the flesh of the fruit and an aluminium foil barrier was inserted to prevent the geraniol from moving into the core area, the geraniol was not metabolized so that after seven days there was 100% recovery of free geraniol. However, when geraniol was similarly injected into the flesh of normal fruit, the level of geraniol was high (*ca* 100% recovery) for the first three days but then declined. After seven days, 30% of the added geraniol remained in the fruit.

DISCUSSION

Apples rapidly converted geraniol to the non-volatile Ger-glc when geraniol was added to the core of the fruit. However, the rate of metabolism of geraniol was greatly reduced when it was added to the flesh of normal fruit and could diffuse into the core area, and was totally inhibited when geraniol was prevented from diffusing into the core area. This indicates that some part of the core is the only area of the fruit that can metabolize geraniol and that the initial reaction is most probably the formation of Ger-glc. The Ger-glc was then transported into the flesh and fairly rapidly metabolized into other compounds.

Abscisic acid (ABA) has been suggested [14, 15] as being involved in enhancing reactions that produce breakdown since geraniol and $CuCl_2$, compounds which increase the disorder, increased the level of ABA in the apple flesh and GA_3 and $CaCl_2$, compounds which decreased breakdown, decreased ABA levels. The effect of geraniol in inducing breakdown would appear to be via Ger-glc which is either metabolized to ABA in the flesh or is converted to some other reaction product which enhances the metabolic sequence that generates ABA.

It has yet to be established whether geraniol metabolism is involved in the natural development of breakdown by apples or whether it is merely an artificial event stimulated by exogenous application of geraniol. However, the ability of the fruit to rapidly incorporate and transport relatively large amounts of geraniol suggests that endogenous geraniol metabolism is associated with breakdown. Also, geraniol has been found to induce a greater incidence of breakdown and a higher level of ABA in the fruit than when equimolar amounts of ABA were added to the fruit [15]. The inference is that the geraniol is more easily incorporated into the metabolism of the fruit than is exogenous ABA, which is mostly converted to abscisyl glucoside that remains unchanged in the fruit throughout the period of the cool storage [14, 15].

The involvement of the core region in the metabolism of geraniol raises the possibility that the seeds may have a role in the development of flesh breakdown. Breakdown is usually considered to be the result of abnormal metabolism at low temperature but it may be an integral part of the life cycle of the fruit whereby seeds that have been vernalized sufficiently during cool storage are ready to germinate. Reactions are then initiated in the core region that result in the collapse of the flesh and thus allow the release of the seeds from the fruit. Release of Ger-glc from the core may be an initiation step.

EXPERIMENTAL

Mature Jonathan apples from N.S.W., Australia were stored at 0° until treated. Fruit were injected with geraniol at 20 μmol/fruit in 0.2 ml EtOH into the core area or into the flesh. Addition to the core area was by a single injection through the calyx. Injection into the flesh tissue was at the equatorial region with 10 μmol being added to opposite sides of the fruit, *ca* 1 cm below the skin. Some fruit had the core region removed by pushing a cork borer (3 cm dia) through from the calyx to the stem. The core section was enclosed in Al foil and pushed back into the fruit. These fruit were injected with geraniol into the flesh. After various periods at 0°, a core and/or flesh sample (50 g each) were obtained from groups of 5 fruit from each treatment. The core samples included the carpels but excluded the seeds and stem. The flesh samples included the skin. Each sample consisted of 10 g from each fruit. The samples were frozen in liquid N_2 and stored in dry ice until analysis. Free geraniol was analysed by boiling apple samples (50 g) with distilled H_2O (200 ml) for 30 min. Geraniol was collected in a specially designed distillation apparatus which allowed the vapours to condense and pass through petrol (bp 40–60°, 1 ml) which was enclosed in an ice-water cooling jacket. Analysis was by GLC with a FID detector using a 3 m column of 10% SE-30 at 150° and carrier gas, N_2 at 20 ml/min. Ger-glc was estimated from the amount of free geraniol liberated following incubation of apple tissue for 16 hr at 37° with β-glucosidase (3.2.1.21) (1 mg) in acetate buffer (pH 5.2) [13].

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