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ABSCISIC ACID AND THE DEVELOPMENT OF STORAGE BREAKDOWN IN APPLES

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Abstract—(±)-ABA injected into the calyx of mature fruit increased the incidence of storage breakdown in Jonathan apples. The increase in disorder was greater when ABA was added early in the storage period and was proportional to the amount of ABA added. Most of the added ABA was converted into 'bound' ABA which remained at high concentrations throughout storage; however, the amount of 'free' ABA was also always higher than that in a control fruit. GA_3 decreased and geraniol increased the amount of 'free' ABA in the fruit on injection into the calyx. This could explain the ability of these compounds to respectively decrease and increase the incidence of breakdown.

INTRODUCTION

Cool storage is universally accepted as a means of prolonging the useful life of apples. However, in some varieties, cool storage causes browning affecting mainly the cortical tissue. The presence of the disorder can cause severe economic losses. The metabolic changes in the fruit that precede the appearance of the brown tissue are not fully understood but it has been suggested that the initial steps involve an accumulation of acetate in the tissue [1,2] followed by metabolism of acetate to mevalonate [3] and the smaller members of the isoprenoid pathway, with the monoterpene geraniol being very effective in inducing the disorder [4]. Gibberellic acid (GA_3) and abscisic acid (ABA) are of interest in that exogenous GA_3 will reduce the incidence of the disorder [4,5] while ABA injected into the fruit at 20 μmol/fruit enhanced breakdown [6]. This paper examines the effect of exogenous ABA on the incidence of breakdown in Jonathan apples and compares the effect with that of GA_3. It also studies changes in concentrations of ABA in the fruit in response to exogenous applications of ABA, GA_3 and geraniol.

RESULTS AND DISCUSSION

The injection of increasing amounts of ABA into apples just after harvest induced increasing levels of breakdown in the fruit during cool storage ($P < 0.001$). In the absence of ABA 30% of the fruit were affected. With 1, 4 and 8 μmol ABA/fruit breakdown was 60, 65 and 75% resp. This is in contrast with results obtained for GA_3 [5,7,8] which showed that the maximum reduction in breakdown is achieved by relatively low levels of applied GA_3 (~0.05 μmol/fruit). Higher concentrations of GA_3 merely maintain a similar reduction in disorder. When ABA and GA_3 were added separately to the fruit at different times during cool storage, both exerted their maximum influence on the fruit when added early in storage. Injection of 10 μmol ABA/fruit shortly after harvest, and after 8 and 13 weeks storage at −1° induced levels of breakdown in the fruit after 17 weeks storage at −1° that were 78, 60 and 42% respectively. Untreated fruit had 35% affected which was the level that would have been expected from fruit treated with ABA at the end of storage. Injection of 2 μmol GA_3/fruit into fruit from another orchard shortly after harvest, and after 6, 8 and 13 weeks storage induced breakdown levels after 17 weeks at −1° that were 42, 46, 52 and 60% respectively. Untreated fruit had 65% affected which would have been the level expected from fruit treated with GA_3 at the end of storage. There was a linear decrease in effectiveness when added at later times and untreated fruit had breakdown levels that would have been expected from fruit treated with ABA or GA_3 at the end of storage.

The effect of adding ABA and GA_3 on the amount of ABA in the fruit is shown in Fig. 1. The (±)-ABA was injected into the core area whereas the analysis for both free-ABA and a polar neutral form referred to as 'bound' ABA (probably abscisyl-β-D-glucopyranoside) which are the natural forms of ABA found in plants [9], were carried out using cortical and skin tissue only. Maximum concentrations of ABA were found in the flesh tissue after one week and indicate that the added ABA had diffused rapidly from the core throughout the fruit. Most of the ABA was present as the 'bound' form and was maintained at relatively high levels throughout storage. It accounted for *ca* 40–50% of the total ABA initially added to the core. The amount of free ABA fell substantially during storage but even after 13 weeks it was still present at twice the concentration of that in control fruit, which had been injected with water only. Fruit dipped in GA_3 solution showed increased ABA levels for the first week after treatment but thereafter had less ABA than controls. 'Bound' ABA tended to parallel the changes in 'free' ABA but was present in the fruit at much lower concentrations.

The injection of geraniol into the fruit enhanced the level of 'free' ABA in the fruit (Fig. 2). A change in ABA levels was observed two days after injection and geraniol produced its maximum effect after one week when treated fruit had twice the amount of ABA as control

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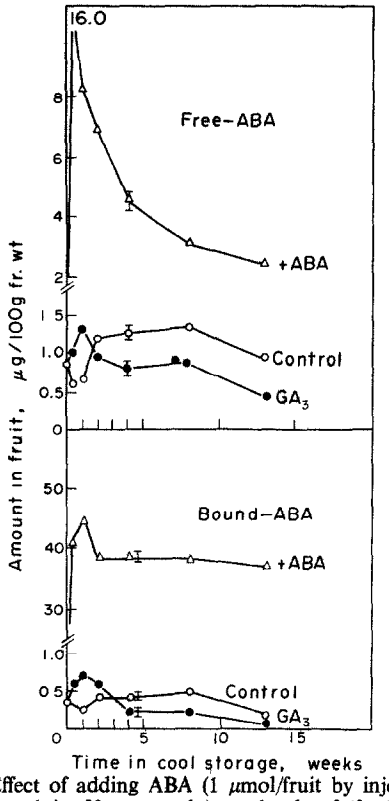

Fig. 1. Effect of adding ABA (1 µmol/fruit by injection) and GA_3 (dipped in 50 ppm soln) on levels of 'free'-ABA and 'bound' ABA in apples. (I = ±standard deviation).

fruit. The relative difference in levels between treatments was maintained throughout the storage period.

It is quite feasible that the endogenous ABA could be involved in enhancing reactions leading to cell degradation in cool stored apples since treatment with ABA is known to acceleate various senescence phenomena in a wide range of plant tissues [10]. The mechanism by which added GA_3 reduces breakdown may be by lowering the amount of ABA in the tissue. However it would seem that only a small amount of GA_3 is required to exert its maximum control over ABA concentrations since higher amounts of GA_3 do not give greater reductions in breakdown [5]. Conversely, the enhancement of breakdown effected by geraniol could be due to either a stimulation of ABA production or partial metabolism to ABA. The specific area of metabolism controlled by ABA and GA_3 seems to be at an early stage of development since both were more effective in inducing breakdown when added early in storage.

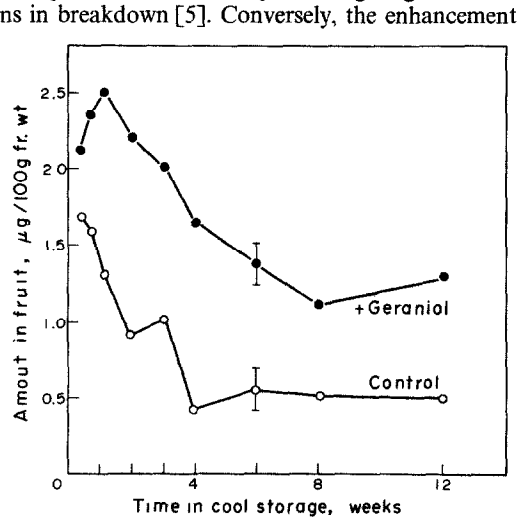

Fig. 2. Effect of injecting geraniol (10 µmol/fruit) on levels of 'free' ABA in apples. (I = ±standard deviation).

EXPERIMENTAL

Jonathan apples from N.S.W., Australia (data reported on ABA concentration study, and Fig. 1) or Otago, New Zealand (data in Fig. 2) were used. In each expt, mature fruit were harvested from commercial orchards and randomised so that the same number of fruit from each tree were distributed into each treatment unit. Treatment units that were stored for the development of breakdown each contained 25 fruit, while groups of five fruit were analysed for ABA. All fruit were stored at −1° for a few days before being treated. ±ABA, geraniol and GA_3 were injected into the calyx of the fruit in EtOH soln (0.2 ml/fruit) while aq dips of GA_3 used Berelex tablets (ICI Plant Protection Ltd). Fruit examined for breakdown were removed from cool storage after a specific period and held at 20° for 7 days to allow the development of browning. Data reported are the mean values of breakdown found in four units of fruit per treatment. The sample for analysis (50 g) was obtained by removing the core area from each fruit and selecting two longitudinal sections (each of 5 g) from each fruit. After freeze-drying and grinding, a sub-sample (2 g) was analysed for ABA by blending with MeOH (150 ml) containing BHT (0.2%) for 1 min at 0°. The extract was filtered and the vol reduced under vac at 35°. The concentrate was taken up in 0.5 M Pi buffer (pH 8.2) and the ABA fractions partitioned from the soln with various solvents according to the method of ref [11]. 'Bound' ABA was released by alkaline hydrolysis of the final acidic aq phase [11]. The final EtOAc fractions were concentrated and an aliquot was further purified on ChromAR sheet (Mallinchrodt) using BuOH–PrOH–18 M NH_4OH–H_2O (2:6:1:2) as solvent. The ABA zone was located with markers of standard ABA spotted on the sheet and visualised under UV light (254 nm). The extract was protected from UV with Al foil. The ABA zone was eluted with H_2O saturated with EtOAc. Recovered ABA was methylated using CH_2N_2 [12] and assayed by GLC using a ^3H EC detector and a glass column (2.1 m × 3 mm) of 2% QF-1 on Chromosorb maintained at 187°. Carrier gas was N_2 (flow rate 40 ml/min). Data reported are the means of values obtained from 2 units per treatment.

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