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INCORPORATION OF [^{14}C]ACETATE INTO APPLES IN RELATION TO DEVELOPMENT OF STORAGE BREAKDOWN

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Key Word Index—*Malus sylvestris*; Rosaceae; Jonathan apple; [^{14}C]acetate; lipids; non-volatile compounds; storage breakdown.

Abstract—[2-^{14}C] Acetate was injected into the core of Jonathan apples that had been stored at −1° under high and low relative humidity conditions to induce a difference in susceptibility of the fruit to develop storage breakdown. Incorporation of [^{14}C] into the total non-volatile compounds in the flesh tissue and the neutral lipids was initially greater in the fruit that was more susceptible to breakdown, but after *ca* 25 days the amount of [^{14}C] in the fruit of lower susceptibility had increased to a similar level. Incorporation of [^{14}C] into the semi-polar lipids was initially similar in both treatments but became greater after *ca* 15 days in the fruit that was more susceptible to breakdown, the difference was maintained during the rest of the storage period. No marked differences in incorporation into polar lipids was found throughout storage.

INTRODUCTION

Many varieties of apples develop a physiological breakdown of cortical tissue during cool storage. The condition appears as areas of brown tissue and can result in severe economic losses. The metabolic changes that precede the appearance of low temperature breakdown are not fully understood, but it has been suggested that an accumulation of acetate in the tissue of susceptible fruit during storage at low temperature is initially involved [1, 2]. The incidence of the disorder can be reduced by physical treatments, such as storage of the fruit in air of low humidity [3] and a short mid-storage warming-period [4], which result in increased losses of acetate from the fruit as volatile compounds such as low MW acetate esters [5, 6]. Later steps in the metabolic sequence that lead to the development of breakdown have been proposed involving the metabolism of acetate to mevalonic acid and non-volatile compounds in the isoprenoid pathway [7–9]. These studies, however, were based on the injection of a range of compounds into apples and subsequent examination of their relative effectiveness in inducing breakdown; no metabolic studies on the metabolism of acetate have been carried out. This paper reports a study on the incorporation of [2-^{14}C] acetate into apples during cool storage to determine areas of metabolism that may be involved in the development of breakdown. [2-^{14}C] Acetate was injected into the core of Jonathan apples that had been stored for 5 weeks at −1°, one group at high (95%) and another group at low (30%) relative humidity (RH), therefore the two groups had different susceptibilities to develop breakdown [3].

RESULTS AND DISCUSSION

The rate of incorporation of [^{14}C] into non-volatile compounds in the flesh tissue differed between the fruit stored at high and low RH (Fig. 1). Incorporation was faster into the fruit held at high RH, in fruit with the greater susceptibility to breakdown, and by 5 days had accumulated to a maximum level of [^{14}C] which was

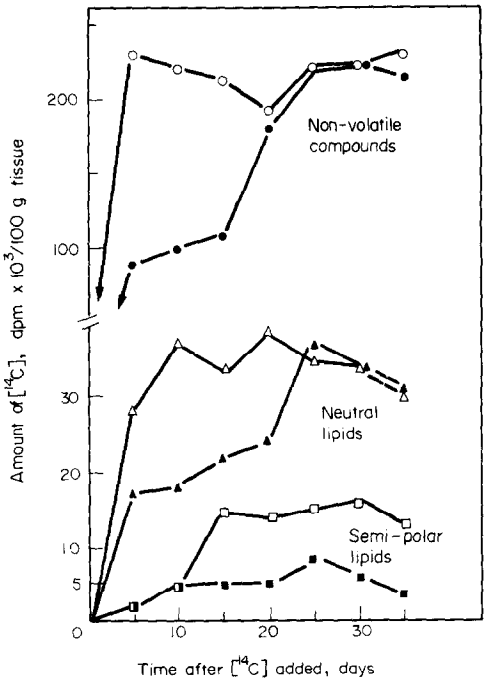

Fig. 1. Amount of [^{14}C] in non-volatile compounds and lipid fractions of flesh tissue of apples stored at high and low relative humidity. ○ = non-volatile compounds, high RH; △ = neutral, high RH; □ = semi-polar lipid, high RH; ● = non-volatile compounds, low RH; ▲ = neutral, low RH; ■ = semi-polar lipid, low RH.

maintained for the remainder of the storage period. The amount of [^{14}C] in fruit held at low RH was initially lower but increased with time until by *ca.* 25 days when the amount was similar to that in the high RH fruit. Acetate is a key intermediate in many biological pathways and the data can only be interpreted to suggest that an increased rate of water loss from the fruit causes a reduction in the rate of metabolism of acetate to many pathways, but since the amount of [^{14}C] eventually equalizes in both treatments, the end-products of the major acetate-using pathways appear to accumulate to the same extent. This equalization would seem not to be the result of a limitation in [^{14}C]acetate as the amount of [^{14}C] in non-volatile compounds only accounts for *ca* 2% of the total [^{14}C]added to the fruit. A major source of loss of [^{14}C] from the fruit would be as CO_2 but calculations based on measurement of $^{14}CO_2$ at irregular intervals on fruit held at high RH suggest that, over 30 days, this accounts for only *ca* 50% of the total [^{14}C] added.

A reduced rate of acetate metabolism in fruit with a reduced incidence of breakdown is consistent with the causal hypothesis proposed by Wills and co-workers [1, 5]. However, the hypothesis presumes that inhibition of the causal pathway will prevent the accumulation of toxic end products later in storage. The metabolic pathway leading to the development of breakdown may only be a minor pathway for the total utilization of acetate and hence effects later in storage may be hidden by metabolism into the major pathways. The possible involvement of lipid metabolism was examined by their extraction from the non-volatile tissue and subsequent fractionation on the basis of their polarity into 3 fractions; neutral lipids, which contain compounds such as hydrocarbons, esters, alcohols and free fatty acids; semi-polar lipids, containing compounds such as glycolipids; and polar lipids, containing, for example, phospholipids [10, 11]. The rate of incorporation of [^{14}C] into the neutral lipids (Fig. 1) was similar to that for the total non-volatile compounds, with the fruit at high RH having the maximum amount of [^{14}C] early in storage whereas fruit at low RH did not acquire this level of radioactivity until *ca* 25 days. The incorporation of [^{14}C] into the semi-polar lipids (Fig. 1) however differed in that the amount of [^{14}C] after 10 days was similar in both treatments but while this level was maintained in the low RH fruit for the rest of the storage period, the radioactivity in high RH fruit increased at 15 days and this increased activity was maintained during storage. The amount of [^{14}C] in polar lipids was lower than that in the other fractions, was similar in fruit from both treatments and showed no marked changes during storage; the range of values was 1.1–4.2 dpm $\times$ 10^3/100 g tissue.

At the conclusion of the experiment, the fruit had been in cool storage for a total of 9 weeks and while no visible symptoms of breakdown were present, it could be expected that some of the immediate precursors of the disorder could be present to some extent. The increased incorporation into the semi-polar lipids suggests that some glycoside or other sugar bound derivative may be involved in the development of breakdown. These types of compounds were implicated with the disorder by Wills and Scriven [12] who found that geraniol, a compound that enhances the incidence of breakdown [8], was rapidly metabolized to geranyl β-D-glucoside. They did not follow the metabolic sequence further but observed that geranyl glucoside was itself rapidly metabolized. An examination of the compounds found in the semi-polar lipid fraction and the reactivity of these compounds would thus appear to be warranted.

EXPERIMENTAL

Mature Jonathan apples were stored at $-1°$ in boxes lined with polyethylene film. Dishes containing anhydrous $CaCl_2$ (200 g) were added to half the boxes to reduce the RH. The RH and rate of H_2O loss from the $CaCl_2$ treatment was 35% RH and 0.15 g/100 g per week and in the boxes with polyethylene film only was 95% RH and 0.03 g/100 g per week respectively. After 5 weeks, fruits were injected with [2-^{14}C]acetate (5 μCi/fruit in 0.1 ml H_2O) into the core. At various intervals, 4 fruits from each treatment were removed from storage, the cores removed and a 50 g sample of flesh and skin tissue from each fruit was frozen in liquid N_2, freeze dried and ground to a powder. To determine the [^{14}C] in the non-volatile compounds, a sample of fruit flesh tissue (0.1 g) was combusted in O_2 [13], the CO_2 collected in a soln of 20% v/v ethanolamine in methoxyethanol (20 ml), an aliquot (9 ml) added to standard toluene scintillator soln (9 ml) and the amount of [^{14}C] radioactivity determined in a scintillation counter.

Lipids were extracted from the dried tissue (14 g) and separated by the methods of ref. [14]. This involved extraction with $CHCl_3$–MeOH (150 ml), dialysis, evaporation and separation of a $CHCl_3$ soln (5 ml) on a column of acid washed Florisil (10 g) with successive elution using $CHCl_3$, Me_2CO and MeOH (75 ml of each). The 3 lipid fractions were evaporated, dissolved in standard toluene scintillator (10 ml) and the amount of [^{14}C] radioactivity determined. Some fruits were held in glass jars which were ventilated with wet air at 1 l./hr. The effluent gas stream from each jar was passed through a soln of ethanolamine/methoxyethanol to collect CO_2, the amount of $^{14}CO_2$ then being determined.

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