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PAL AND ETHYLENE CONTENT DURING MATURATION OF RED AND GOLDEN DELICIOUS APPLES

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Key Word Index—*Malus domestica*, Rosaceae, anthocyanin; ripening

Abstract—Phenylalanine ammonia-lyase activity (PAL), a PAL inhibitor (PAL-IS) and fruit internal ethylene levels were measured during apple maturation in Red and Golden Delicious apples. In both cultivars internal ethylene and PAL increased at approximately the same time Apple PAL-IS extract stimulated yeast PAL activity initially, but increased yeast PAL inhibition was observed as maturation progressed PAL inhibitor levels were similar in both cultivars The increase in yeast PAL inhibition with maturation, increased red skin colour development in Red Delicious and a comparable pattern of PAL activity and inhibition in Red and Golden Delicious indicates that PAL-IS was not closely involved with the regulation of anthocyanin synthesis in apple skin

INTRODUCTION

Phenylalanine ammonia lyase (E C 4 13.5) has been extensively reviewed [1, 2] Ethylene enhances PAL activity in pea seedlings [3], carrots [4], and swede and parsnip root tissue [5] PAL activity is stimulated by, but lags behind, ethylene production in irradiated grapefruit [6]. In cultured parsley cells, ethylene alone does not induce PAL [7] A proteinaceous inhibitor of PAL activity is found in sunflower [8] and inhibitors are present also in gherkin hypocotyls [9], sweet potato [10] and apple skin [11, 12]. PAL activity is inhibited by the end product of the reaction, *trans*-cinnamic acid [13]

In apple skin, ethylene production is associated with anthocyanin accumulation and a concomitant increase in PAL activity in response to maturation, wounding and temperature [14–16]. Low temperatature and light stimulate anthocyanin accumulation in apple skin, increase PAL activity, and lower PAL inhibitor activity [11]. Faragher and Chalmers [17] concluded that while PAL influences anthocyanin accumulation in apples, there are other controlling factors Tan [12] concluded that low temperature stimulates PAL activity and decreases PAL inhibitor, thus increasing anthocyanin synthesis. The objectives of this study were (i) to describe the sequence of changes of PAL, PAL-IS and ethylene levels in apple skin during fruit maturation on the tree, (ii) to compare these changes in an anthocyanin producing Red Delicious apple with a non-anthocyanin producing Golden Delicious apple, and (iii) determine if PAL inhibition is a major factor in the development of red skin colour in apples.

RESULTS AND DISCUSSION

PAL activity and internal ethylene level increased at approximately the same time in Red Delicious (Fig. 1). Stimulation of PAL activity by ethylene in apples is in agreement with the findings of others [15]. When apple PAL-IS extract was added to yeast PAL it did not greatly alter activity of the yeast PAL during the first sampling, after which time it increasingly inhibited the activity of the yeast PAL (Fig. 2). As internal ethylene and apple PAL activity increased, inhibition of the yeast PAL also increased. This increasing inhibition of yeast PAL activity with fruit maturation is in contrast to the conclusion of Tan [11] that PAL-IS is responsible for regulation of anthocyanin accumulation in apple. The use of yeast PAL in the assay is desirable due to its high specific activity [8], however, yeast PAL may not clearly reflect the effect of the inhibitor(s) on all PAL isozymes It has been reported that there may be more than one type of

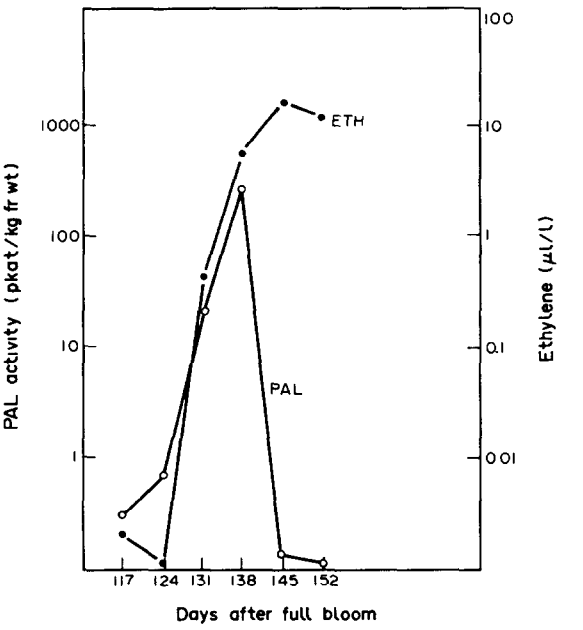

Fig 1 Changes in internal ethylene concentration and PAL activity of the skin of Red Delicious apples during maturation

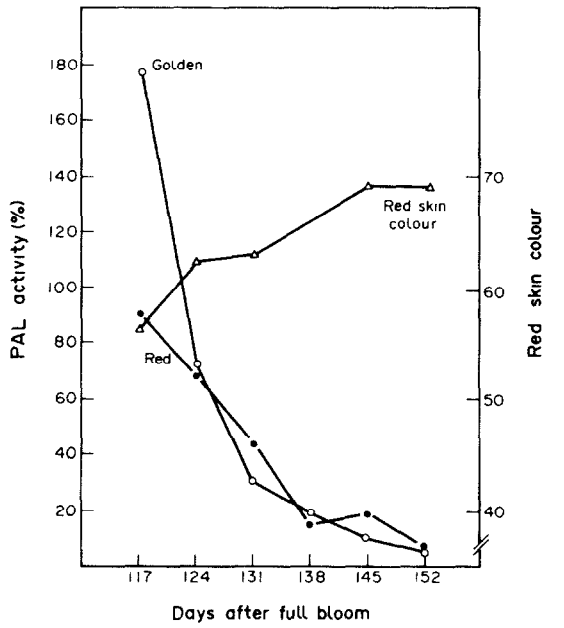

Fig 2 PAL activity of the yeast *Rhodotorula glutinis* as influenced by Red or Golden Delicious PAL-IS extract and the per cent of the total Red Delicious apple skin that exhibited red colour during maturation

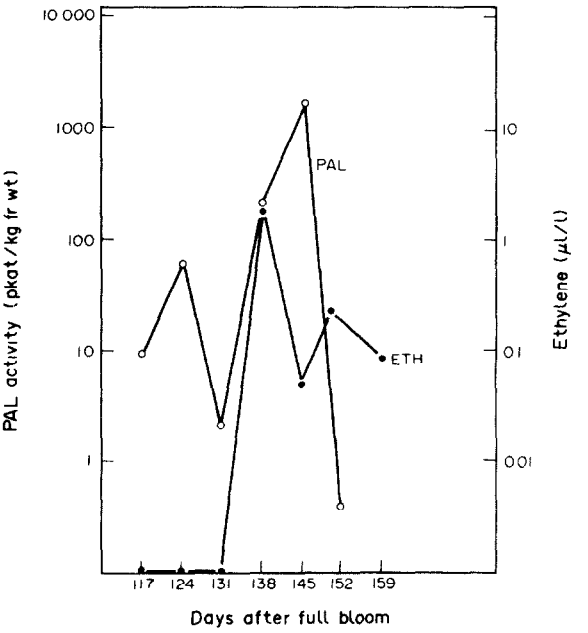

Fig 3 Changes in internal ethylene concentration and PAL activity of the skin of Golden Delicious apples during maturation

inhibitor in sunflower [8] and, perhaps, this also occurs in apple skin. The high PAL activity during the increase in internal ethylene had little effect on red colour development, since the skin gradually developed red skin colour, even when PAL levels were low (Fig 2)

In Golden Delicious PAL activity and internal ethylene both increased during maturation (Fig 3) Golden Delicious often do not exhibit the consistant increase in ethylene production that occurs in Red Delicious However, the onset of ethylene production did occur at approximately the same time as an increase in PAL activity Apple PAL activity was comparable between Red and Golden Delicious The apple PAL-IS initially stimulated the yeast PAL, but as maturation progressed, the yeast PAL activity decreased (Fig 2) Overall PAL-IS levels were similar in Red and Golden Delicious apples during maturation (Fig 2) The similarity of PAL and PAL-IS activity between Red and Golden Delicious would imply that other steps in anthocyanin synthesis control red skin development The apple PAL-IS seems to be effective on the yeast PAL, but its role in regulation of *in vivo* PAL activity seems questionable

EXPERIMENTAL

Plant material Malus domestica cv Starkrimson Delicious or cv Golden Delicious were harvested from commercial N C orchards Fruit was selected for uniformity of size, freedom from defects and similar position on the trees Analyses were conducted within 1 day of harvest The time period covered by this study represents the time period during which the apples were initially immature, then attained horticultural maturity, and commercial harvest was begun

PAL assay 5 g of skin from 3–5 apples was collected by cutting strips of skin at each 90° from stem and calyx Skin was ground in 10 ml 50 mM sodium borate buffer, pH 8 8 with 5 mM 2-mercaptoethanol, 1 mM EDTA, and 0 25 gm PVPP The homogenate was centrifuged for 30 min at 20000 *g* and the supernatant collected The 30–50% $(NH_4)_2SO_4$ fraction from the supernatant was obtained The resulting pellet was redissolved in the extraction buffer minus PVPP, then dialysed against 50 mM borate buffer, pH 8 8 The total assay vol was 1 ml and contained 50 μl 0 02 M phenylalanine in buffer, 300 μl 50 mM sodium borate buffer with 5 mM 2-mercaptoethanol and 200 μl extract The blank contained all of the above except phenylalanine The assay was read at 290 nm at 25 over 1 hr

PAL-IS was extracted and assayed by the methods of Tan [11] against PAL enzyme from the yeast, *Rhodotorula glutinis*

Internal C_2H_4 was extracted under vacuum from 25 apples by the methods of Saltveit [18] Duplicate experiments were conducted with similar results and the average of the 2 experiments is presented

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