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# ACETIC AND PROPIONIC ACIDS, INDUCERS OF RIPENING IN PRE-CLIMACTERIC GOLDEN DELICIOUS APPLES

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**Key Word Index**—*Malus pumila*, Rosaceae, pre-climacteric apple fruit, induced ripening, acetic acid, propionic acid, ethylene formation, start of ripening

**Abstract**—When treated with acetic or propionic acid vapours, intact pre-climacteric Golden Delicious apples started ripening prematurely Experiments with [2-$^{14}$C]propionic acid showed that a small part of the added acid is transformed into [$^{14}$C]ethylene, and that ripening probably begins in these circumstances as a result of the artificial increase in the endogenous ethylene level It may be that in unripe apples the small amount of evolved ethylene is mostly derived from simple organic acids, so that the moment at which its triggering concentration is reached depends on the available supply of acids As this, in turn, is a function of the state of maturity of the fruit, there must be a direct relationship between the start of ripening and the degree of maturity Thus, even in very unripe apples, catabolic processes are at work or potentially present because of the modified $\beta$-oxidation of [1-$^{14}$C]propionic acid observed

## INTRODUCTION

The special role played by ethylene in ripening has long been recognized (see reviews [1, 2]), although its mode of action is still a matter of speculation The main source of ethylene is L-methionine, which is transformed into the alkene by way of 1-aminocyclopropane-1-carboxylic acid (ACC) [3, 4] Yang proposed that, prior to ripening, ACC-synthase is first formed, so as to produce an adequate supply of ACC, from which starter ethylene would be formed (system 1 ethylene) The latter could induce higher activity in the enzyme which converts ACC into ethylene, and this would lead to massive ethylene production (system 2 ethylene), and to the start of visible ripening [3] In this connection it is interesting to note that whereas application of ACC to tissues from root, stem, leaf, inflorescence and fruit of 16 plant species greatly enhanced ethylene production [5], this effect was absent with pre-climacteric apple and cantaloupe tissues [3, 4] Ethylene formation may also be triggered by external factors such as wounding, flooding, certain chemicals and radiation (see refs [1–4, 6])

Previous experiments in our laboratory showed that addition of propionic and butyric acid vapours to intact, pre-climacteric, immature Golden Delicious apples led not only to an increase in $CO_2$ production, but also to premature ripening of the fruits The fact that a reference sample, when treated with ethylene 7 days after the start of the experiment, started ripening and respiring in a manner very similar to that of the acid-treated fruit suggested that the acids might have an influence on ethylene production The hypothesis was formulated that sufficient amounts of alkene might be formed from small carboxylic acids (cf system 1 ethylene [3]) to trigger the normal ethylene production (cf system 2 ethylene [3]) [7] This supposition could imply that a mechanism such as the acrylic acid pathway for ethylene formation from propionic acid as proposed by Shimokawa and Kasai [8] might be operative in living tissue, an idea which is challenged in the recent literature [1, 9]

It is thus clear that the validity of a ripening hypothesis centred around the influence of simple organic acids on the behaviour of pre-climacteric apples depends on the answer to several questions Among them does addition of the acids generate ethylene, and if so, is it not the result of a wound-like effect?, how much ethylene is evolved during the induced ripening phenomenon?; can ethylene be formed directly from propionic acid?, is the large $CO_2$ production at the start of the experiments derived directly or indirectly from propionic acid, and is modified $\beta$-oxidation active in unripe apples?, how does butyric acid exert its effects, is it by wounding, or by way of propylene, or is the acid first transformed into propionic or acetic acid, both of which might be precursors to ethylene?

Therefore, pre-climacteric immature Golden Delicious apples were treated with acetic acid, two different amounts of propionic acid, [1-$^{14}$C]propionic acid (as vapours) and ethylene, and their respiration and ethylene production were followed as a function of time In another set of experiments, pre-climacteric Golden Delicious apples (from two sources) were treated with different amounts of [2-$^{14}$C]propionic acid, and ethylene and [$^{14}$C]ethylene elimination were determined

## RESULTS

Depending on the substances used, and on the amounts applied, respiration of the fruits followed different courses (Fig 1) At the start, $CO_2$ production of apples treated with 100 $\mu$l propionic acid (100 P-apples) tripled, compared to the blank, while the effect was much less pronounced with acetic acid (100 A-apples) or ethylene (E-apples), and was absent with 10 $\mu$l propionic acid (10 P-apples) All curves passed through a minimum between days 5 and 8 Thereafter, the climacteric rise started and

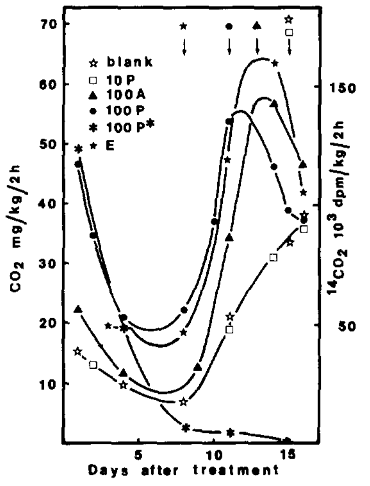

Fig 1 Respiration of pre-climacteric, immature Golden Delicious apples (± 1 5 kg) (September 1981) treated with 100 µl acetic acid (100 A), 100 µl propionic acid (100 P), 100 µl [1-$^{14}$C]propionic acid (100 P*), 10 µl propionic acid (10 P) or ethylene (E) Ethylene was applied on day 2 The start of aroma evolution detectable by smelling is indicated by arrows

reached a maximum between days 11 and 14 for the 100 P-, 100 A- and E-apples For the 10 P-apples and the blank, however, respiration was still increasing only slowly on day 16 On addition of 100 µl [1-$^{14}$C]propionic acid, $^{14}CO_2$ was set free (Fig 1). It decreased quickly between days 1 and 8, remained almost constant between days 8 and 11 (during this period $CO_2$ and ethylene elimination increased strongly), and then decreased slowly further Total radioactivity from $^{14}CO_2$ and [1-$^{14}$C]propionic acid (recovered in potassium hydroxide) increased rapidly between days 1 and 8, rose very slowly until day 11, after which the rate of elimination picked up again (Table 1) The latter was probably due to trapping of aroma esters, as activity in a dimethyl formamide scrubber installed after the potassium hydroxide scrubber started rising at that moment too

Large differences in ethylene formation and in the moment at which aroma evolution starts were observed between treated fruits 100 P, 100 A and E and the 10 P-apples and the blank (Fig 2) Application of 100 µl and 10 µl propionic acid to immature apples gathered in September 1981 resulted in the production of measurable amounts of ethylene after 1 day, which disappeared after 3 days under the analytical conditions used Thereafter, ethylene elimination increased again for the 100 P-apples, well ahead of the 100 A-ones, and far in advance of the 10 P sample and the blank The E-apples were the first to produce a weak aroma (on day 8), followed by the 100 P-fruits (on day 11), the 100 A-ones (on day 13) and finally by the 10 P-apples and the blank (on day 15) (Figs 1 and 2)

Table 1 Recovered radioactivity after addition of 45 531 × 10$^3$ dpm [1-$^{14}$C]propionic acid to 1 52 kg apples

| | Day No | 10$^3$ dpm | % Radioactivity |
|---|---|---|---|
| In 10 M KOH | 1 | 8156 | 18 |
| | 4 | 12 439 | 27 |
| | 8 | 16 766 | 37 |
| | 11 | 17 062 | 37 5 |
| | 15 | 19 152 | 42 |
| | 18 | 20 001 | 44 |
| DMF | 18 | 81 | 0 2 |
| Fruits | 18 | 3089 | 7 |
| Vessel* | | | 13 |

*Based on a small-scale blank experiment recovered in potassium hydroxide 68 5 %, from vessel 13 %

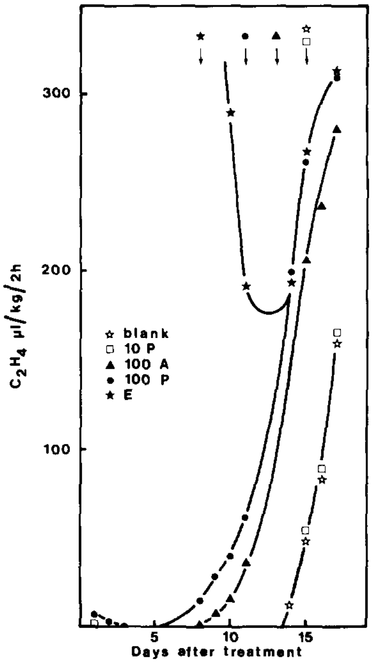

Fig 2 Ethylene production of pre-climacteric, immature Golden Delicious apples (± 1 5 kg) (September 1981) treated with 100 µl acetic acid (100 A), 100 µl propionic acid (100 P), 10 µl propionic acid (10 P) or ethylene (E) Ethylene was applied on day 2 The start of aroma evolution detectable by smelling is indicated by arrows

Pre-climacteric apples collected in September 1982 were first treated with 10 µl [2-$^{14}$C]propionic acid and 2 days later with a supplementary 100 µl A second batch of pre-climacteric fruits from another orchard was treated with 50 µl [2-$^{14}$C]propionic acid Each time, [$^{14}$C]ethylene was set free (Fig 3) Addition of 10 µl of the acid yielded only a limited quantity of alkene (amounting to a fraction of that produced endogenously), which decreased moreover after 2 days (Table 2) But when 50 or 100 µl of the acid was used, [$^{14}$C]ethylene elimination was much higher (of the same order of magnitude as total ethylene production), and showed a maximum While [$^{14}$C]ethylene formation then declined, total ethylene increased, and the 'visible' ripening process set in

## DISCUSSION

From Figs 2 and 3 it may be deduced that the observed premature ripening is the end-result of the effects which added organic acids have on the ethylene balance in

Table 2 Ethylene production by pre-climacteric Golden Delicious apples (1982) after treatment with [2-$^{14}$C]propionic acid

| | | Blank | Treated | |
|---|---|---|---|---|
| Days | Added amount (µl) | Ethylene (µl/kg per 2 hr) | [$^{14}$C]ethylene (µl/kg per 2 hr) | Total ethylene (µl/kg per 2 hr) |
| 0 | 10 | | | 0 2 |
| 1 | | | 0 03 | 0 2 |
| 2 | 100 | | 0 02 | 0 2 |
| 3 | | | 0 4 | 0 5 |
| 6 | | | 0 9 | 2 |
| 7 | | | 0 5 | 10 |
| 8 | | | 0 3 | 30 |
| 0 | 50 | 0 5 | | |
| 1 | | 0 9 | 0 4 | 0 5 |
| 3 | | 0 07 | 0 7 | 0 7 |
| 5 | | 0 2 | 0 2 | 0 5 |
| 6 | | 0 2 | 0 06 | 1 3 |
| 7 | | 0 07 | 0 03 | 3 4 |
| 8 | | 1 5 | 0 03 | 11 2 |
| 9 | | 10 | 0 03 | 32 |
| 12 | | 34 | 0 06 | 167 |

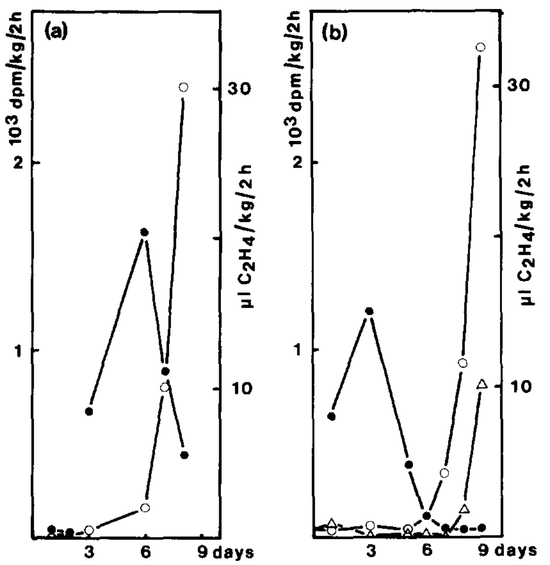

Fig 3 (a) Total ethylene and [$^{14}$C]ethylene production by pre-climacteric Golden Delicious apples (September 1982) after treatment with 10 µl [2-$^{14}$C]propionic acid on day 0, and on day 2 with a supplementary 100 µl ○, Total ethylene, ●, [$^{14}$C]ethylene (b) Total ethylene and [$^{14}$C]ethylene production after treatment with 50 µl [2-$^{14}$C]propionic acid ○, Total ethylene, ●, [$^{14}$C]ethylene, △, total ethylene of untreated samples

apples, since they advance by several days the moment when significant ethylene production starts (The formation of measurable amounts of ethylene immediately after application of propionic acid in 1981, which was absent in 1982, remains unexplained) That the involvement of propionic acid is direct and not the consequence of wounding is shown by the experiments with [2-$^{14}$C]propionic acid However, no information whatever can be obtained about the way [$^{14}$C]ethylene is formed under the experimental procedures used The transformation might be performed by the acrylic acid pathway, as proposed by Shimokawa and Kasai [8] The fact that ripening is also induced by acetic acid, from which the alkene might also be formed by a modification of the same pathway [10], might be interpreted in favour of this mechanism

The apples used in the experiments with [2-$^{14}$C]propionic acid produced small quantities of ethylene from the first day on The rate of elimination stabilized at ±0 2 µl/kg per 2 hr, after which it increased to 1 5 µl, starting autocatalytically the voluminous ethylene production which precedes visible ripening (Table 2, blank) The effect of 50 or 100 µl added propionic acid seems to reside in enlarging the (endogenous) ethylene level artificially to a correspondingly increased elimination of 0 5–0 7 µl/kg per 2 hr (which may be equal to or larger than the trigger concentration), so that autocatalytic ethylene production from ACC is started immediately when this becomes possible As the amounts of ethylene derived from propionic acid depend, furthermore, on the available supply of acid (Fig 3a), and coincide sometimes with total ethylene elimination (Table 2), it is possible to propose a ripening sequence in which propionic acid (and tentatively acetic acid) plays a role [7]

In this model, a situation is envisaged in the growing apple, where at the start catabolic processes play only a minor role, although they may be potentially present, and may exhibit their activity when given the right substrates This is illustrated by the modified β-oxidation [11] of [1-$^{14}$C]propionic acid in these very unripe apples (Fig 1) Nearing maturity the importance of the catabolic processes probably increases Some cases are described in literature where changes in membrane permeability, as an example of catabolic activity, precede other changes of ageing, e g ethylene evolution ([12–14] and refs cited therein, [15]) At that moment, acetic or propionic acid may become available in larger concentration, and part of it may be transformed into ethylene When at a further stage of development of the fruit even larger amounts of ethylene are produced in this way, it may reach the threshold concentration for triggering 'normal' ethylene formation and start visible ripening According to this model, the start of ripening is thus linked directly to the degree of maturity of the fruit, and can only take place when all circumstances are prepared for successful ripening

In connection with the foregoing hypothesis, ripening induced by treatment of pre-climacteric apples with butyric acid [7] may be explained by assuming that the acid is degraded into acetic acid by $\beta$-oxidation, and that the latter is the active species This corresponds with the analogous way in which apples react to treatment with butyric and acetic acids Moreover, in the headspace of apples treated with butyric acid, only ethylene could be detected, and no propylene was ever found (which was mentioned as a possibility previously [7])

Also unexplained is the remarkable difference in respiration of apples treated with 100 $\mu$l propionic acid and with 100 $\mu$l acetic acid (Fig 1) (and also with butyric acid [7]), in that the former triggers $CO_2$ production at the start of the experiment, and leads to a respiration maximum which is attained earlier than when the latter is used Since after application of [1-$^{14}$C]propionic acid only a fraction of total $CO_2$ is composed of $^{14}CO_2$ (3 7 $\mu$mol $^{14}CO_2$/kg per 2 hr vs 0 8 mmol $CO_2$/kg per 2 hr), it is clear that modified $\beta$-oxidation cannot account for this phenomenon However, when compared to the influence of ethylene addition, where this effect, although smaller (Fig 1), is present, it might be that the acids are transported more readily than ethylene to the location itself where 'climacteric $CO_2$' is produced and where its production may be stimulated Being a better precursor of ethylene than the other acids, propionic acid would thus affect respiration to a larger extent at the start of the experiments

## EXPERIMENTAL

Pre-climacteric, immature Golden Delicious apples (55–65 mm) were collected on 2 September 1981 in the test orchards of Glabbeek (Belgium) Before treatment, batches of *ca* 1 5 kg were stored in desiccators of *ca* 8 l at 17° under a continuous air stream of 150 ml/min for 5 days, after which a soln of the acids (10 or 100 $\mu$l in 1 ml $H_2O$) was put directly into the vessel One batch was treated with 400 ppm ethylene for 24 hr after a 7 day storage in air $CO_2$ was determined as described before [7] The expt with [1-$^{14}$C]propionic acid (100 $\mu$l, 15 2 $\mu$Ci/mmol) (The Radiochemical Centre, Amersham) was performed under the same procedures as above The eluting air was continuously passed through two scrubbers containing 100 ml 10 M KOH each, and one containing DMF Before collecting $^{14}CO_2$, the air stream was increased to 400 ml/min for 1 hr, after which it was led at the same rate through two scrubbers with saturated $Ba(OH)_2$ and one with DMF, for 2 hr The $Ba^{14}CO_3$ was filtered off, thoroughly washed with $CO_2$-free $H_2O$ and treated with excess 0 1 M HCl (back-titration of which yielded total $CO_2$) $^{14}CO_2$ was swept into 5 ml Carbosorb (Packard) with air Residual radioactivity in the fruits was estimated by homogenizing several apples with $H_2O$, and by determining the activity of the filtrate, and of the tissue left on the filter after pyrolysis

In September 1982 fruits were obtained from the 'Rijkstuinbouwschool' in Vilvoorde (Belgium) and from a commercial grower in Zomergem (Belgium) The apples were immediately placed in the desiccators (see above) After putting an aq soln of [2-$^{14}$C]propionic acid (10 $\mu$l + 100 $\mu$l, 50 $\mu$l, 2 ml $H_2O$, 17 9 $\mu$Ci/mmol) (The Radiochemical Centre, Amersham) on the bottom of the vessels, an air stream of 150 ml/min was applied, dispersing the acid as it evapd The eluting air was led continuously through two scrubbers containing 5 M NaOH, and through one containing 5 ml 0 2 M $Hg(ClO_4)_2$ in 2 M $HClO_4$ at 0° for ± 24 hr Activity from [$^{14}$C]ethylene was determined by mixing 1 ml of the soln with 10 ml Instagel (Packard) As a control of the identity of [$^{14}$C]ethylene, 3 ml of a soln of the collected Hg complex (393 dpm) was treated with 4 M LiCl at room temp The freed alkene was removed by a very slow air stream, and then led through an ice-cold trapping soln as above Recovered activity was 287 dpm (73 % recovery) Total ethylene production was monitored by direct sampling (2 ml) of the air leaving the second NaOH-scrubber, followed by GC analysis on an alumina column In 1981 the detection limit was 0 3 nl $C_2H_4$ and in 1982 0 03 nl

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