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## Promotion of Reddening in Unripe Strawberry Fruits by Fungal Extracts

ETHYLENE promotes ripening in many fruits<sup>1,2</sup>, and auxins cause premature colouring in pears3, apples4,5 and other fruits<sup>6</sup>. Some pome fruits redden prematurely around damaged areas.

No reports have been found of promoted ripening or reddening of strawberry fruits by ethylene, auxins or damage, and attempts elsewhere<sup>7,8</sup> and at Long Ashton, to advance ripening in strawberry by ethylene and by 2-chloroethylphosphonic acid (CEPA or Ethrel), which decomposes to yield ethylene in plant tissues, have not been successful, although tests have been done under several conditions, both in vitro and on fruit retained on the plant.

This investigation arises from the finding of several immature strawberry fruits, of the cultivar Rabunda, on which areas surrounding pathogenic infections were prematurely reddened. Lesions were pale-brown in colour, dry and firm, varying in size from about 1 to 3 cm<sup>2</sup> in area. The reddened areas surrounding the lesions formed a band up to about 1 cm in width. The remaining parts of the fruit were green and unripe at the time, but ripened later. A fungus was isolated from an infected fruit and identified as Fusarium sambucinum Fuckel, of importance on hops and stored potato but also recorded on strawberry in Europe<sup>9</sup>.

Cultures of the fungus were incubated either on potato dextrose agar for 14 d, or in Czapek Dox liquid media for 15 d at 25° C. Six agar cultures in 9 cm Petri dishes were macerated in 100 ml methanol and the liquid phase separated. The methanol was evaporated off at 40° C under nitrogen. The water fraction was made up to 100 ml with distilled water and its pH adjusted to 2.5 with 1% HCl. This water extract was partitioned three times against 50 ml of ethyl acetate and the ethyl acetate phases dried with a little anhydrous sodium sulphate. The ethyl acetate was evaporated off at 40° C and the residue preserved for bioassay. Liquid cultures were extracted in a similar way.

The residues were assayed for their ability to colour unripe strawberry fruits in situ on plants in the glasshouse. Suitable fruits were firm but not hard, pale-green in colour, and 5 to 7 d before the stage at which they would normally colour.

Individual achenes were removed from the fruits with a sterilised scalpel, and a small cavity opened up beneath the scar. The residues to be assayed were taken up in 20% ethanol in water, and 8 µl aliquots placed into freshly-opened cavities. Treatments and controls (the latter included application of 20% ethanol and extracts of uninoculated agar and untreated cavities) were replicated from twice to ten times in different tests.

Active extracts applied in the manner described induced a ring of precocious reddening in tissue round the cavity 1 to 4 d after application, the response time depending partly on the time of year. The rings of reddened tissue were 2 mm wide and up to 1 cm in diameter, the diameter being less with greater dilution of the extracts. The cavity was separated from the ring by a zone of decoloured tissue, which did not ripen. Normal ripening of the fruit occurred outside the ring, which was no longer distinguishable, 3 to 5 d later. Control cavities exhibited neither red rings nor decoloured zones: the lip and lining inside the cavity turned brown but did not rot.

The dependence of ring diameter on extract concentration and the presence of the inner zone of decoloured tissue, indicate that the extracts may have been toxic in high concentrations near the point of application but became diluted by diffusion outwards to the region where they induced pigment formation in the host tissue.

Bioassay on Avena mesocotyls indicated that the extracts contained growth-regulating substances.

In work of colleagues in this laboratory, similar rings of reddened tissue were induced by the application of several auxins. Therefore, it appears likely that the fungal extract contained auxins or possibly other unidentified substances which can initiate or promote reddening or ripening in strawberry. The failure of attempts to promote ripening by ethylene or ethylene generators, however, raises the question whether ethylene was mediating in the stimulation of reddening either by auxins or the fungal extract, even though auxins are known to stimulate ethylene evolution in several plant tissues<sup>10,11</sup> and ethylene to promote ripening in many fruits.

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## Negative Cooperativity of Phosphofructokinase as a Possible Regulator of Ripening in Banana Fruit

THE banana belongs to a group of fruits which undergo a rapid increase in respiration with ripening<sup>1</sup>. Several theories have been advanced to account for this 'respiratory climacteric'2: the most often mentioned hypotheses are (1) the uncoupling of oxidative phosphorylation, (2) a change in acceptor control by ADP, (3) a change in membrane permeability, and (4) the synthesis or activation of a rate-limiting enzyme. The uncoupler theory and the ADP to ATP ratio change have, however, been disproved, and no clear evidence has been presented in support of the other theories.

In lacatan bananas a twenty-fold increase in the fructose-1,6-diphosphate level has been measured and it has been suggested that this could indicate the activation of phosphofructokinase<sup>3</sup>. Activation of this same enzyme by an increasing concentration of inorganic phosphate has been suggested as a possible reason for increased respiratory activity in ripening