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Auxin Inhibition of Ripening in Bartlett Pears[1]

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ABSTRACT

The effect of indoleacetic acid and 2,4-dichlorophenoxyacetic acid on the ripening of intact mature-green pears (*Pyrus communis* var. Bartlett) was investigated using a vacuum infiltration technique.

The effects of indoleacetic acid and 2,4-dichlorophenoxyacetic acid at concentrations of 0.01, 0.1, and 1.0 mM each were studied on softening, degreening, and on ethylene and CO_2 evolution. Softening and degreening were inhibited increasingly in response to increased concentrations of indoleacetic acid. This inhibitory property was amplified by 2,4-dichlorophenoxyacetic acid at concentrations comparable to those of indoleacetic acid. Application of the auxins also prevented the climacteric rise in respiration, but stimulated ethylene synthesis. Despite the presence of elevated ethylene levels, the inhibitory auxin effect was predominant.

It is proposed that endogenous auxins in fruit represent a resistance factor in ripening and must be inactivated before ripening can occur.

It was proposed that endogenous factors in fruit resist the action of ethylene in the promotion of ripening (14). Auxins are among the naturally occurring constituents in fruit which could function as resistance factors in ripening (16). Consequently, it was suggested by Frenkel (9) that the decline in the level of auxins concomitant with ripening is required to sensitize fruit tissue to the action of ethylene gas. In order to verify the role of auxin as a resistance factor in ripening, the authors studied changes in ripening and in CO_2 and ethylene evolution in Bartlett pears in relation to applied auxins. The results of this study indicate that auxin could overcome the action of ethylene and inhibit ripening in Bartlett pear.

MATERIALS AND METHODS

Pears (*Pyrus communis* var. Bartlett) at the mature green stage were obtained from the pear orchard of Rutgers University, New Brunswick, New Jersey. The fruits were treated with 10 μl/l ethylene for 12 hr at room temperature prior to auxin application.

Solutions containing IAA or 2,4-D were applied to the fruit by a modified vacuum infiltration procedure (10). Intact fruits were punctured through the central cavity from the calyx end with a 20-gauge needle and were submerged in the infiltration solution. Infiltration of the solutions was regulated by the vacuum setting at which the fruits were equilibrated prior to infiltration (Fig. 1). Fruits were infiltrated at 5 ml/100g tissue. Solutions used were 0.01, 0.1, and 1.0 mM of IAA or 2,4-D in combination with 0.3 M mannitol as a carrier solution (10). The resulting auxin concentration in the fruit tissue was one-twentieth of the auxin concentration in the infiltration solution. After infiltration the fruits were kept at room temperature, and samples were taken at intervals for measurement of the ripening process and of the evolution of ethylene and CO_2.

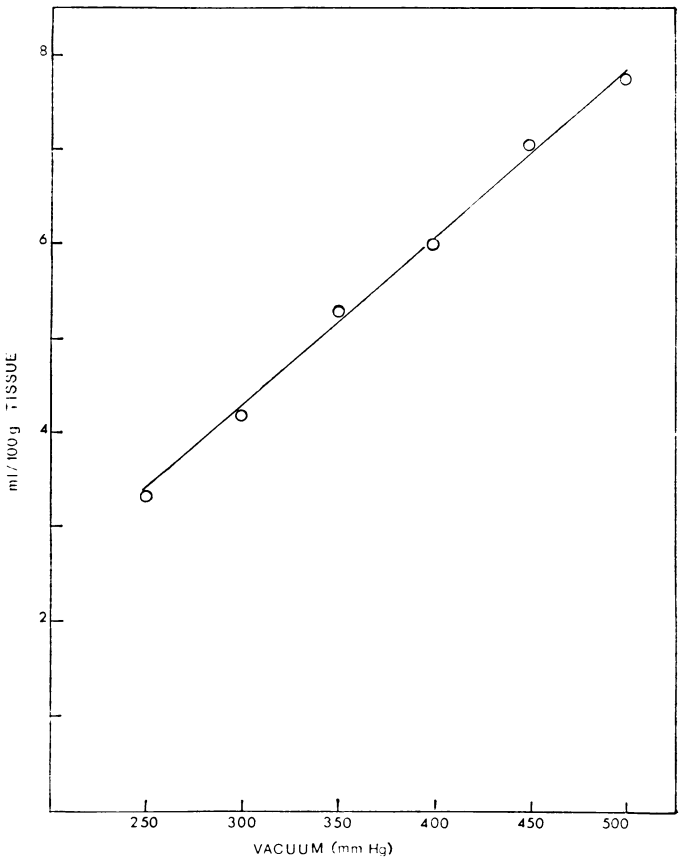

FIG. 1. The uptake of an infiltration solution by pears (ml/100 g tissue) is shown as a function of fruit equilibration at different partial pressure settings prior to infiltration. Fruits were submerged for 10 min at different vacuum regimes, followed by infiltration for 30 min at atmospheric pressure.

Softening and the breakdown of chlorophyll in the peel tissue were used as parameters of ripening. Softening was measured by a Magness-Taylor fruit pressure tester. The changes in chlorophyll were measured as previously described (9). Softening of pear tissue and degreening were measured at 2-

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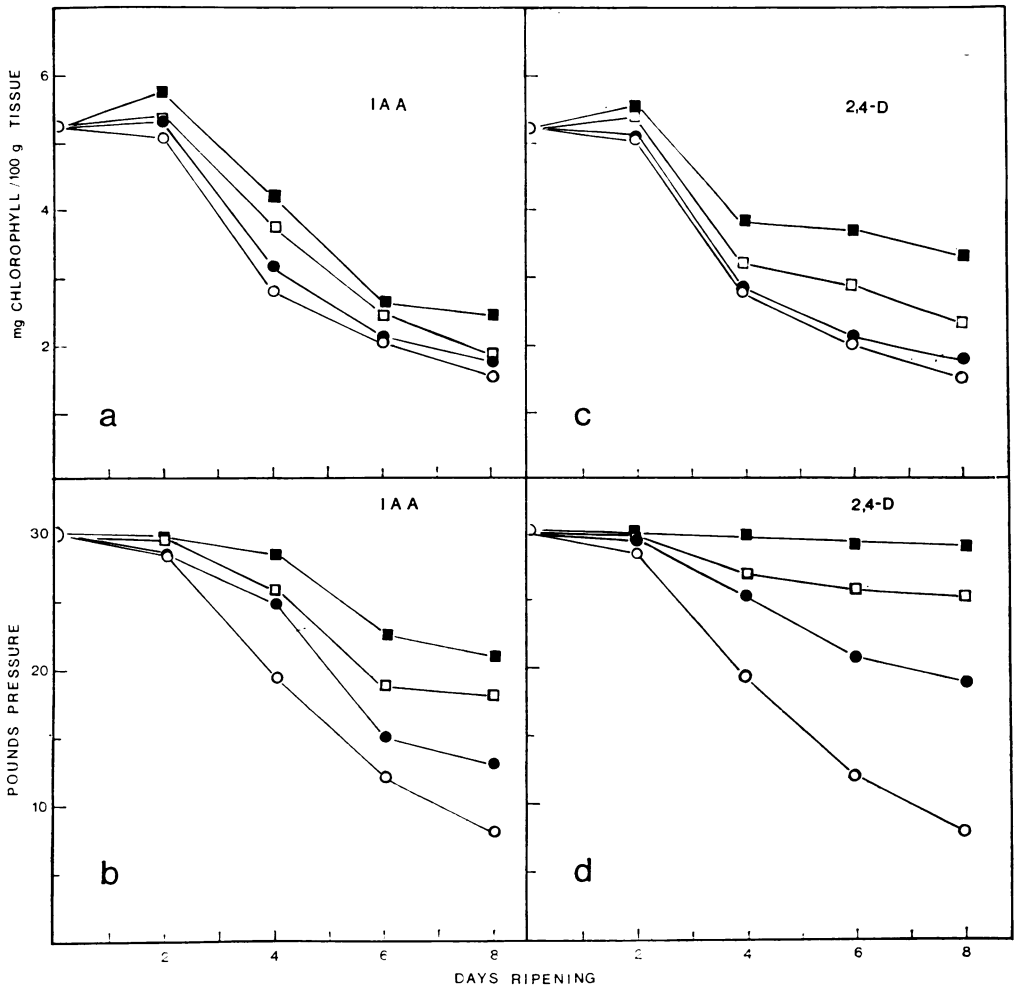

FIG. 2. The inhibition of pear ripening by auxins. The effect of IAA and 2,4-D on chlorophyll breakdown is shown in Figures 2,a and c. The effect of IAA and 2,4-D on softening is presented in Figures 2,b and d. The auxin concentrations in the infiltration solution were zero (mannitol control) (○); 0.01 mM (●); 0.1 mM (□); and 1.0 mM (■).

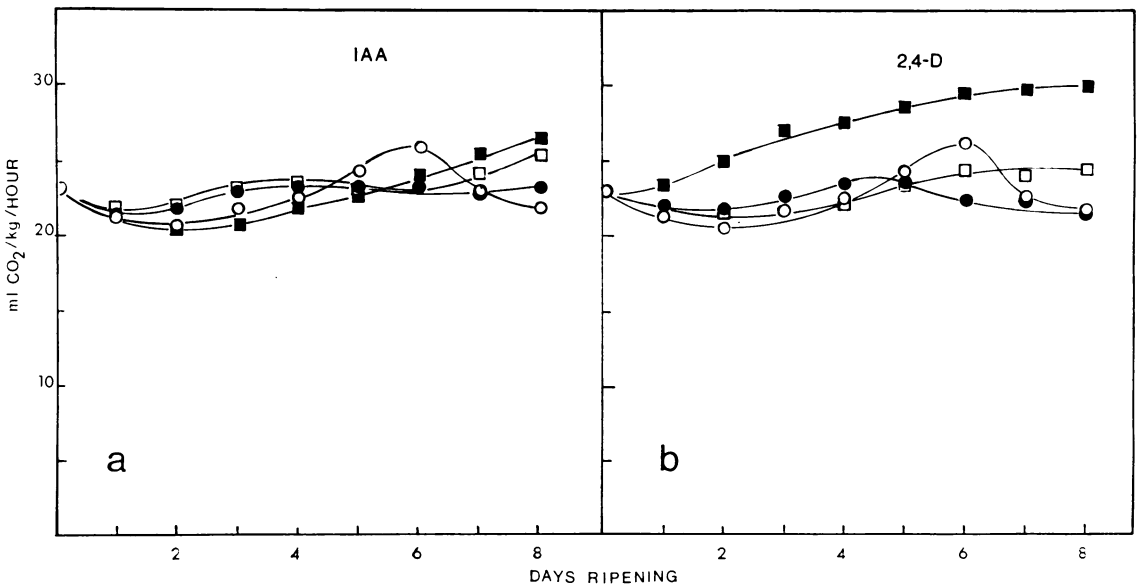

FIG. 3. The effect of IAA and 2,4-D on the respiratory pattern of pears (Figs. 3,a and b). The auxin concentrations in the infiltration solution were zero (mannitol control) (○); 0.01 mM (●); 0.1 mM (□); and 1.0 mM (■).

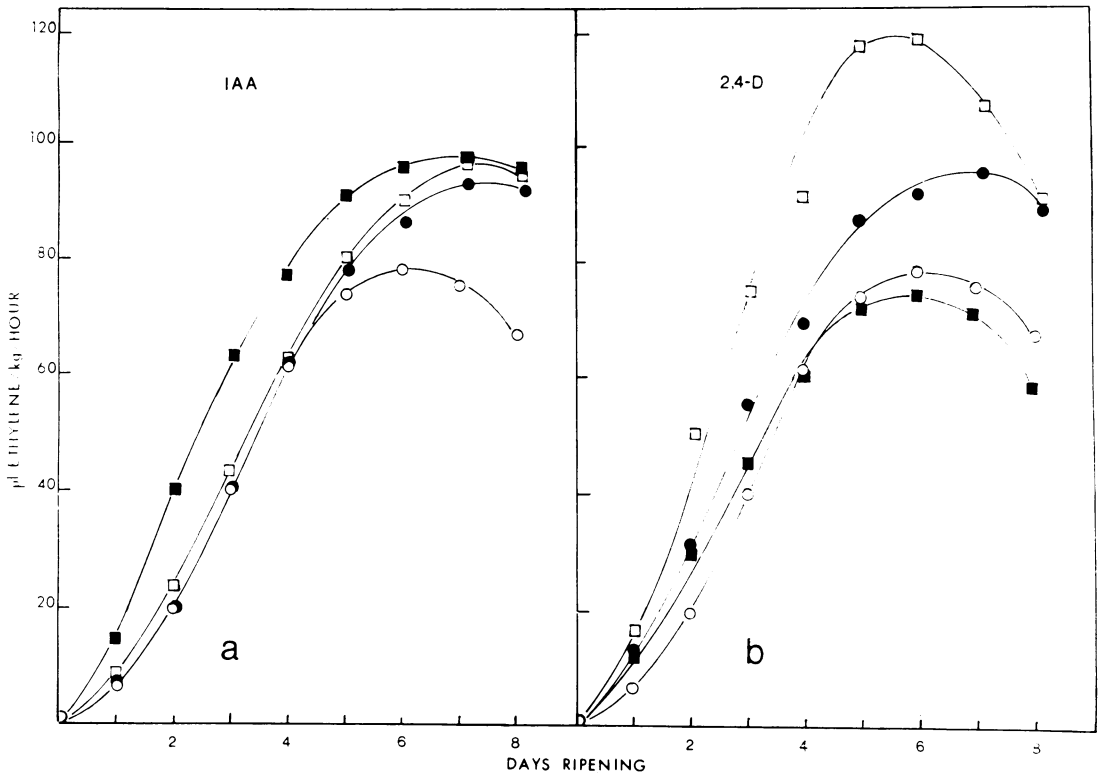

FIG. 4. The effect of IAA and 2,4-D on ethylene evolution by pears (Figs. 4,a and b). The auxin concentrations in the infiltration solution were zero (mannitol control) (○); 0.01 mM (●); 0.1 mM (□); and 1.0 mM (■).

day intervals. Ethylene and CO_2 evolution were measured at 6-, 12-, and 24-hr intervals for the remainder of the experiment. Ten fruits were used for each of the above determinations. All determinations were run in duplicate.

RESULTS AND DISCUSSION

The effects of auxin on ripening and on CO_2 and ethylene evolution are shown in Figures 2, 3, and 4. The results indicate that IAA inhibited chlorophyll breakdown and softening (Fig. 2, a and b), and that the inhibition was in proportion to the applied auxin concentration. The pattern of inhibition in chlorophyll breakdown and softening by 2,4-D (Fig. 2, c and d) was similar to that by IAA, although at comparable concentrations 2,4-D was a more effective inhibitor of ripening. Furthermore, the increase in chlorophyll content following the application of IAA and 2,4-D (Fig. 2, a and c) suggests a temporary reversal of senescence in fruit by the applied auxins.

The current study supports a previous suggestion that auxins function as a resistance factor in fruit ripening (16) and evidently must be inactivated as a prerequisite to ripening (9). Consequently, auxins, such as 2,4-D, which resist degradation (19) cause persistent and magnified inhibition of ripening. The latter observation also supports the contention that the activity of IAA-oxidase is a mode of auxin inactivation in a ripening fruit tissue (9), although other mechanisms of auxin inactivation in fruit cannot be ruled out (4).

The effect of IAA and 2,4-D on CO_2 evolution is shown in Figure 3, a and b. Although the changes in respiration in relation to changes in auxin concentrations are not as clear cut as in degreening or softening, it is evident that the respiratory climacteric was diminished. An exception was noted at a high 2,4-D concentration (Fig. 3b), which resulted in an intensified CO_2 evolution and could suggest metabolic damage. The simultaneous inhibition of the respiratory climacteric, together with chlorophyll breakdown and softening, supports the observation that in fruit with a clearly defined climacteric the respiratory rise is coupled with the expression of other ripening parameters (21). It also indicates the independence of the respiratory climacteric from auxin-induced rise in CO_2 production and ethylene evolution (see below) observed in other plant systems (15).

Figure 4, a and b, shows the effect of IAA and 2,4-D, respectively, on ethylene evolution. Increase in auxin concentrations induced progressively higher ethylene evolution with the exception of the highest 2,4-D concentration (Fig. 4b). The stimulation of ethylene synthesis in Bartlett pear by auxin application has been observed by Hanson (13) and corresponds to other instances in which ethylene evolution by fruit tissue was enhanced by auxin application (18).

However, enhanced ethylene evolution could not overcome the inhibition of chlorophyll breakdown, softening, and the respiratory climacteric by auxins. On the contrary, there was an inverse relationship between the observed levels of ethylene and the acceleration of ripening. Vendrell (23) in a similar study observed that auxins affect simultaneously a system concerned with the regulation of ethylene synthesis and a system which desensitizes the fruit tissue to the action of ethylene. The dual effect of auxin could lead therefore to ambiguous results if care is not taken to insure the penetration of the auxin solutions into the bulk of the fruit tissue. Surface applications of auxins to intact fruit result in enhanced ethylene levels but only a few peripheral cell layers are inhibited by the auxin. Under such experimental conditions the auxin treatment results in an ethylene effect and will actually cause an acceleration of ripening (18). Penetration of auxin solutions into the fruit as a result of vacuum infiltration assured that the inhibitory effect of auxin became predominant in spite of elevated ethylene levels.

The inhibition of ripening by auxins, in spite of enhanced ethylene evolution, was observed by Vendrell (23) in banana peel slices. Sacher (22) in a related study showed that auxin inhibited ripening of bean pericarp segments. Cumulatively, these observations correspond to other instances in which auxin inhibition could override the effect of ethylene as in the inhibition of leaf senescence (20) and foliar abscission (11).

The nature of the auxin-ethylene interrelation in fruit is still a matter of conjecture. However, Hall and Morgan (12) suggested that ethylene regulates auxin levels in plants by promoting the activity of IAA oxidase. This view is compatible with the suggestion that the increase in IAA oxidase activity during fruit ripening signifies a mode of auxin degradation in fruit (9). It is tempting to speculate that the role of ethylene in ripening is principally in the activation of IAA oxidase, hence the degradation of auxins, although other modes of auxin inactivation by ethylene (7) cannot be ruled out.

The inhibition of ripening by auxins is reminiscent of a similar effect by gibberellins (5) and cytokinins (6). The inhibition of ripening coincides with the retardation of other senescence phenomena by these phytohormones (24). It is therefore conceivable that the resistance to ripening which is displayed. for example, by fruit at early stages of maturity or attached avocado fruit (14), reflects the action of inhibitory levels of senescence retarding hormones in the fruit tissue. Furthermore, the decline in senescence retardants which accompanies the aging process, as in leaf senescence (2, 8, 20), flower senescence (17), or foliar abscission (3), is also part of the normal process in the tissue of fruit undergoing ripening. The role of the hormonal turnover in ripening and its interrelation to the action of ethylene will be dealt with in future studies.

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