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Effect of Aminoethoxy Analog of Rhizobitoxine on Ripening of Pears[1]

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ABSTRACT

Ripening reactions in pears (*Pyrus communis* L.) were differentially affected by an aminoethoxy analog of rhizobitoxine (L-2-amino-4-[2-aminoethoxy]-trans-3-butenoic acid) (AAR). Ethylene production of both 'Anjou' and 'Bartlett' pears was inhibited by AAR. Decrease in firmness, increase in protein N and soluble pectin were delayed by AAR in 'Anjou' but not in 'Bartlett' pears. While loss in malic acid was retarded in 'Anjou' pears, rates of citric acid accumulation and malic acid reduction were not affected by AAR in 'Bartlett' pears.

Ripening is necessary for a pear to develop full flavor. The failure to ripen is one of the factors which limit the storage life of a pear (19). However, ripening inhibitors are essential to prolong the shelf life and to control problems such as premature ripening (20). Gibberellic acid and Alar (succinic acid-2,2-dimethyl-hydrazide) counteract the accelerated rate of pear ripening (11, 20). Cycloheximide inhibits protein synthesis and ethylene production (4). Other reported ripening inhibitors in pears include indoleacetic acid, 2,4-dichloro-phenoxyacetic acid, and alpha (*p*-chlorophenoxy)isobutyric acid (2, 3). Recently, Lieberman and his co-workers (9, 13) found that rhizobitoxine, which is a metabolic product secreted by bacterium *Rhizobium japonicum* growing symbiotically in the root nodule of soybean, inhibited ethylene production of apple tissues by blocking the conversion of methionine to ethylene. They also reported that a series of structural analogs of rhizobitoxine had similar inhibitory properties (10). These compounds have great potential in retarding ethylene-induced senescence and may have practical applications. A study was initiated to determine the effect of the aminoethoxy analog of rhizobitoxine on ripening of 'Bartlett' and 'Anjou' pears.

MATERIALS AND METHODS

'Anjou' and 'Bartlett' pears were harvested at commercial maturity based on the firmness of 6.4 and 8.2 kg pressure test, respectively, from mature trees at the Mid-Columbia Experiment Station, Hood River, Ore. Both varieties of pears were stored at −1.1 C in perforated poly-lined boxes for 2 months to ensure an adequate cold requirement for overcoming the ripening resistance (7, 14, 17). After removal from storage, fruits were treated with 1000 ppm of aminoethoxy analog of rhizobitoxine (L-2-amino-4-[2-aminoethoxy]-*trans*-3-butenoic acid) by 3-min dipping or by vacuum infiltration (5). Control fruits were similarly treated with distilled H_2O. After treatment, the fruit remained at 20 C for ripening.

Ethylene production was measured daily at 20 C. Organic acid, protein N, soluble pectin, and firmness were determined before the treatment and then at 2-day intervals thereafter. Methods for these analyses and determinations were described previously (19).

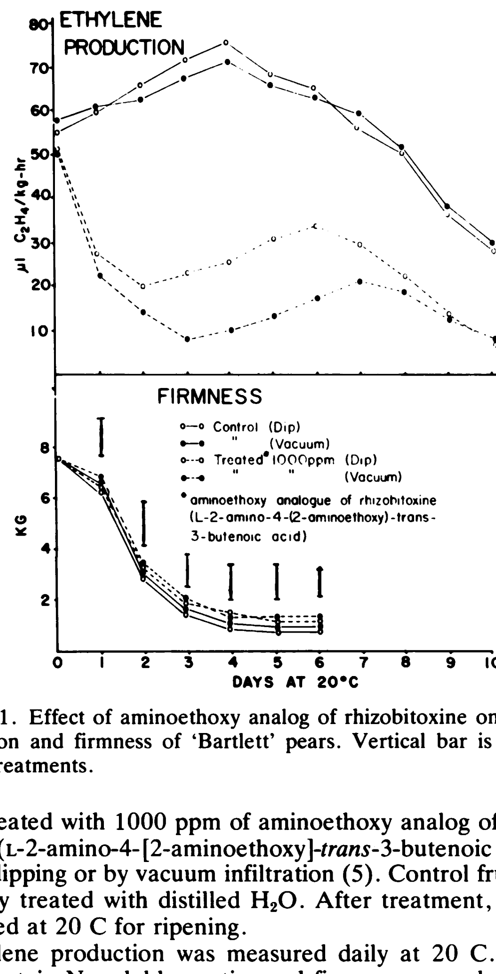

Fig. 1. Effect of aminoethoxy analog of rhizobitoxine on ethylene production and firmness of 'Bartlett' pears. Vertical bar is LSD .05 among treatments.

RESULTS

A varietal difference and an independent response of various individual ripening reactions were shown by the treatment with AAR.[3] The magnitude and rate of ethylene production in 'Bartlett' pears were suppressed but softening was not affected by AAR (Fig. 1). The vacuum infiltration method was slightly more effective than the dipping method in inhibiting ethylene production, but 'Bartlett' pears treated by both methods softened at the

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[3] Abbreviation: AAR: L-2-amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid.

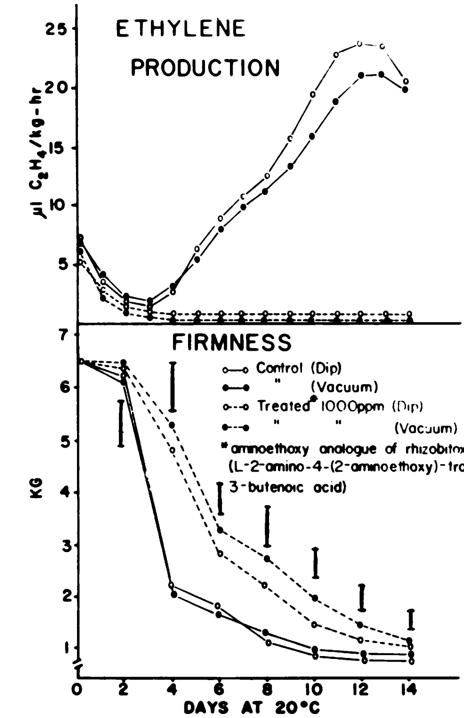

FIG. 2. Effect of aminoethoxy analog of rhizobitoxine on ethylene production and firmness of 'Anjou' pears. Vertical bar is LSD .05 among treatments.

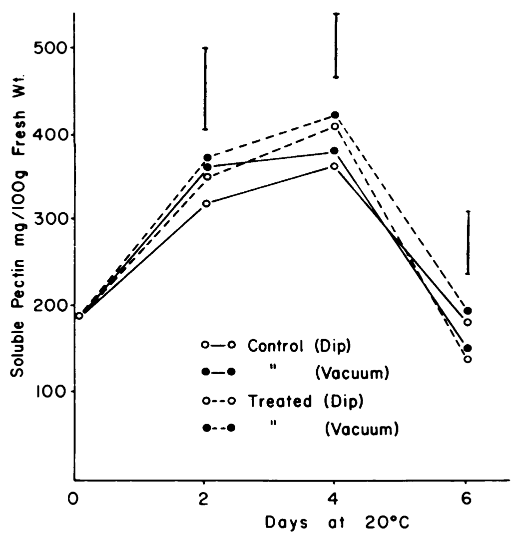

FIG. 3. Effect of aminoethoxy analog of rhizobitoxine on soluble pectin of 'Bartlett' pears. Vertical bar is LSD .05 among treatments.

same rate as the untreated fruits. In 'Anjou' pears, the increase in ethylene production was markedly inhibited by AAR (Fig. 2). The level stayed very low except at the beginning. Decrease of firmness in treated 'Anjou' fruits was delayed but eventually reached eating ripe stage. The initial high level of ethylene must have triggered the softening mechanism of the treated fruits and the process proceeded even though ethylene was subsequently inhibited by AAR.

Soluble pectin of AAR-treated 'Bartlett' pears increased at the same rate as control fruits (Fig. 3). The content reached a peak in 4 days and then declined. Little difference was also found in the rate of protein N rise between treated and untreated 'Bartlett' pears (Fig. 4). Accumulation in citric acid and decrease in malic acid in 'Bartlett' pears during ripening did not appear to be affected by AAR treatment (Fig. 5). Increase in both soluble pectin and protein N were retarded by AAR treatment in 'Anjou' pears (Figs. 6 and 7). Loss of malic acid, which is the predominant organic acid in 'Anjou' pears, was delayed by AAR treatment (Fig. 8). Inhibition by AAR was much greater on ethylene production than on other parameters of ripening in both varieties of pears.

DISCUSSION

These results indicated that various individual ripening processes were affected differently by AAR treatment and each process reacted independently. The experiments also demonstrated the differences between the ripening response of 'Anjou', a long keeping and slow ripening winter pear, and 'Bartlett,' a short lived and fast ripening pear, as affected by AAR.

Rhizobitoxine was shown to be an inhibitor of β-cystathionase, an enzyme in the pathway of methionine biosynthesis in higher plants (6). Because the inhibition of ethylene synthesis

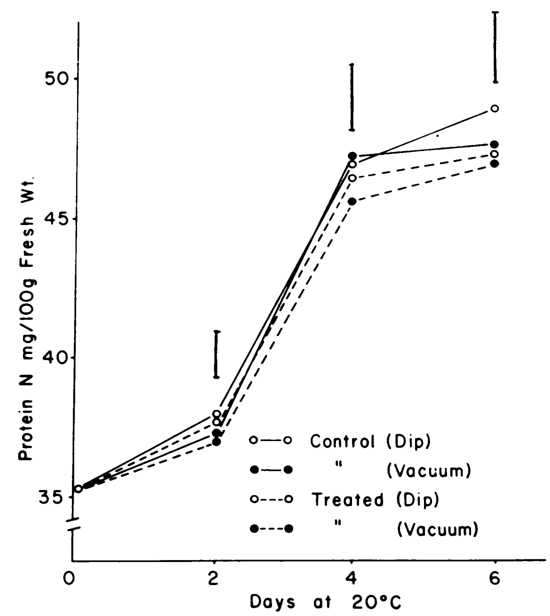

FIG. 4. Effect of aminoethoxy analog of rhizobitoxine on protein N of 'Bartlett' pears. Vertical bar is LSD .05 among treatments.

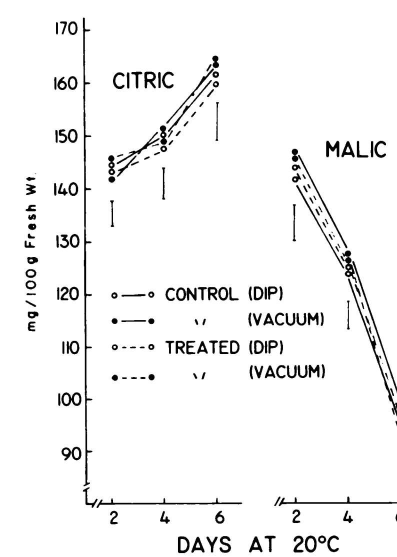

FIG. 5. Effect of aminoethoxy analog of rhizobitoxine on organic acids of 'Bartlett' pears. Vertical bar is LSD .05 among treatments.

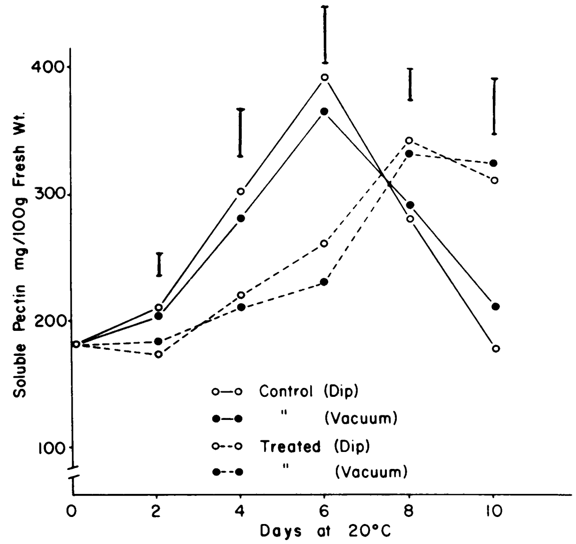

FIG. 6. Effect of aminoethoxy analog of rhizobitoxine on soluble pectin of 'Anjou' pears. Vertical bar is LSD .05 among treatments.

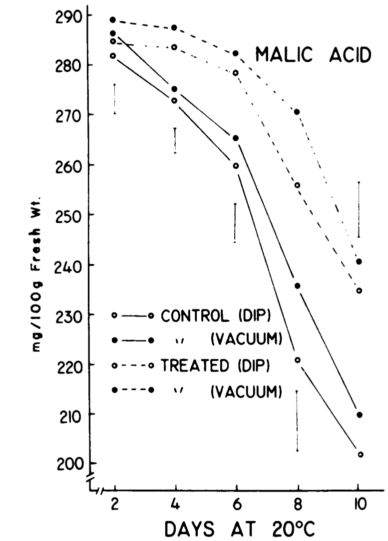

FIG. 8. Effect of aminoethoxy analog of rhizobitoxine on malic acid of 'Anjou' pears. Vertical bar is LSD .05 among treatments.

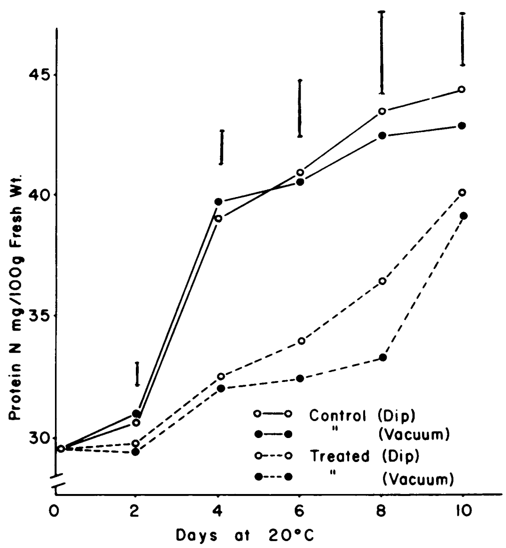

FIG. 7. Effect of aminoethoxy analog of rhizobitoxine on protein N of 'Anjou' pears. Vertical bar is LSD .05 among treatments.

cannot be relieved by added methionine, Lieberman and coworkers (10, 13) concluded that rhizobitoxine interfered with ethylene biosynthesis by blocking the conversion of methionine to ethylene and that the inhibition is nonreversible. As high as 99% of the ethylene evolution in aging rib segments of morning-glory flowers was inhibited by AAR (8). Since ethylene production in pears was greatly inhibited by AAR and since AAR is thought to be a specific inhibitor of ethylene synthesis in the methionine pathway (10), this study also showed that methionine is the major, if not the only, source of ethylene during ripening in pears.

Many changes occur during ripening along with the increase in ethylene synthesis. Dependency of these reactions on ethylene is unclear because it is very difficult to remove ethylene without concomitant inhibition of these reactions. As specific inhibitors of ethylene synthesis from methionine, rhizobitoxine and its analogs offer a good opportunity to study the behavior of these processes in the absence of ethylene.

Increasing evidence (1, 4, 12, 18) demonstrates that these different individual processes occur more or less at the same time during ripening but independently of one another. The climacteric rise in respiration may represent merely a collective result for energy requirement and may not be a phenomenon closely associated with ripening in fruit (9, 15, 16, 21). Separation of the various individual processes would help to clarify mechanisms involved in fruit ripening. The differential effects on ethylene production and other ripening events in pears by treatment with AAR in this study indicate that there were differences in the sensitivity of various reactions to ethylene and ethylene inhibitors. Treatment with AAR did not exert the same degree of inhibition on various ripening reactions. The differential responses to this inhibitor suggest that these processes can proceed separately and independently. Better manipulation of ripening quality may be possible by retardation of certain undesirable processes and promotion of other desirable reactions.

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