

## Effects of Temperature and Daminozide on the Induction of Ethylene Synthesis in Two Varieties of Apple

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Received September 3, 1987; accepted December 28, 1987

**Abstract.** Factors affecting the induction of ethylene synthesis in apple were investigated through their effect on the delay in ethylene production (DEP) in single fruits after harvest. DEP in Cox apples was not affected by temperature from 1 to 25°C, but in Golden Delicious, DEP was shorter below 12°C and above 20°C. An orchard treatment with daminozide increased DEP in apples held below 15°C or treated with ethylene after harvest. Experiments in which the temperature was changed after ethylene treatment showed that ethylene was less active at 5°C than at 15°C but that response to it could be equally rapid. The results suggest that endogenous ethylene is not regulatory prior to the onset of rapid ethylene synthesis.

Ethylene is generally considered to be the hormonal regulator of ripening of climacteric fruits, but the mechanism by which the rise in their rate of ethylene synthesis is induced is not known. McGlasson et al. (1978) argue that ethylene itself induces the rise; low concentrations present in the preclimacteric fruit become effective through an increase in the sensitivity of the tissue to ethylene. If confirmed, this would be an example of regulation of plant development through a change in sensitivity to a hormone rather than a change in hormone concentration (Trewavas 1982).

Firn (1986) has developed this concept and distinguishes three aspects of sensitivity: receptor concentration or "receptivity," receptor affinity, and response capacity. Most of the published data on ethylene and fruit ripening cannot be used to discriminate between these possibilities.

McGlasson (1985) has restated the theory as an increase in receptivity during fruit development. According to this view, the delay in ethylene production (DEP) observed in a fruit represents the time required for receptors to develop; the primary regulator of the onset of ethylene synthesis is not ethylene, but the factor that causes the production of receptors.

A somewhat different concept emerges from the work of Peacock (1972) on banana fruit. Treatment with a low concentration of ethylene had no immediate effect but caused the onset of rapid ethylene synthesis to occur earlier than in untreated fruit. This suggested that receptors were present for some time prior to the natural onset of ripening. The time of onset would be influenced by the ethylene experienced by the fruit and by its response capacity. The changes in sensitivity reported by Peacock (1972) can be interpreted as changes in response capacity. This interpretation retains ethylene as a regulator of the onset of rapid ethylene synthesis and other aspects of ripening in climacteric fruits.

In contrast to the effects of ethylene on bananas, the DEP of immature apples was not reduced by ethylene levels of 100–1000  $\text{mm}^3/\text{m}^3$ , whereas these concentrations reduced DEP later in development (Knee et al., 1987). Since immature fruit responded to higher ethylene concentrations, both receptors and response capacity were present, and the results suggest that receptor affinity increased with fruit development. The experiments described in this paper were further attempts to determine the regulatory role of ethylene in the induction of its own synthesis in apples.

An earlier paper showed that DEP was shorter at 3°C than at 20°C in Golden Delicious apples but was relatively little affected by temperature in Cox apples (Knee et al. 1983). The inhibitory effects of daminozide on the ripening of apples can be reversed by ethylene (Looney 1968), and daminozide treatment may have practical value to delay the onset of ethylene production during fruit storage (Liu 1979, Knee 1986).

Varietal differences and the effects of temperature and daminozide could not be explained by immediate effects on ethylene synthesis (Knee et al. 1983, Knee 1985). They should be explicable in terms of receptor concentration, affinity, or response capacity if the onset of rapid ethylene synthesis is controlled through the sensitivity of fruit to ethylene.

## Materials and Methods

Apple fruits were harvested from trees at East Malling and used immediately for experiments. Daminozide (1,4-butanedioic acid mono-(2,2-dimethyl hydrazide); Alar 85, Murphy Chemical Co.) was sprayed on some trees prior to harvest, as indicated below, in solution at a rate of 1.1  $\text{m}^3/\text{ha}^1$ . Except in the first experiment, all fruit were immersed briefly after harvest in a solution of metalaxyl (100  $\text{g}/\text{m}^3$ ) and carbendazim (500  $\text{g}/\text{m}^3$ ) (Ridomil MBC, Ciba Geigy) to protect against fungal infection.

Ethylene treatments were applied to groups of eight apples for 48 h after harvest, and the concentration was checked twice during this time (Knee et al. 1987). The internal ethylene concentration of untreated fruit was estimated at 0 and 48 h, and the mean of these values was taken to be the effective ethylene concentration for this fruit. Treated fruit was assumed to contain the sum of this internal and the measured external concentration (Knee et al. 1987).

After harvest and after any ethylene treatment, single fruits were held in containers with an air flow of 0.001  $\text{m}^3/\text{h}^1$ , and ethylene production was measured regularly. The time from harvest until the rise in ethylene production to 1

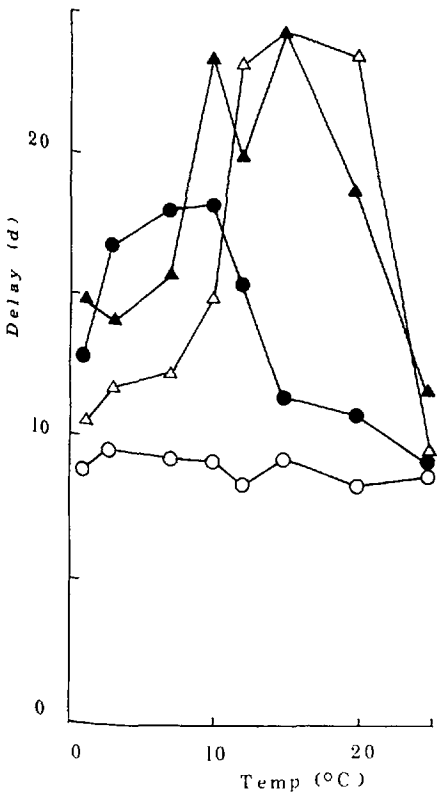


Fig. 1. Effects of daminozide and storage temperature on the delay in onset of rapid ethylene production after harvest in two varieties of apple. Daminozide was sprayed at 1.7 kg/m<sup>3</sup> on July 22, Cox apples (○, unsprayed; ●, sprayed) were harvested on September 3, and Golden Delicious (△, unsprayed; ▲, sprayed) on September 29, 1982.

mm<sup>3</sup>/kg<sup>1</sup>/h<sup>1</sup> was estimated for each fruit. (The mean for all fruits in a treatment is defined as the delay in ethylene production (DEP) (Knee et al. 1987).) There were 16 fruits per treatment in the temperature transfer experiment and eight fruits in the others. Treatment effects were evaluated by analysis of variance after logarithmic transformation of data (Knee et al. 1987).

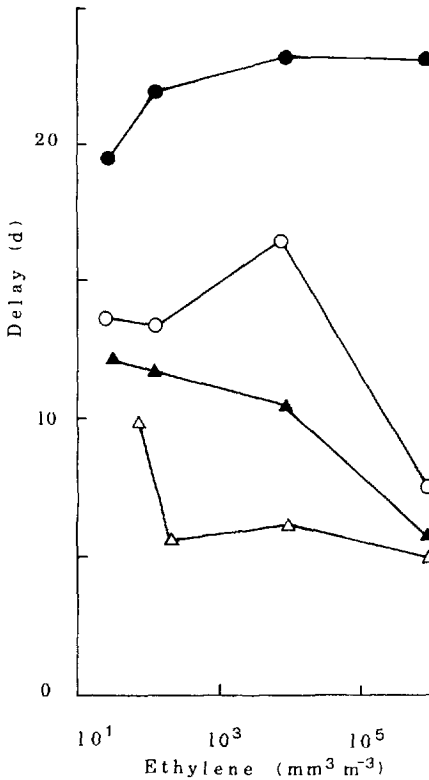
### Results and Discussion

In earlier work, the effect of three temperatures, 3, 12, and 20°C, on the DEP of apples was reported (Knee et al. 1983). To obtain a fuller account of temperature effects and to help select treatments for further experiments, apples were held at eight temperatures from 1 to 25°C. The DEP of Cox apples without daminozide treatment was virtually constant across this temperature range (Fig. 1; Table 1). In Golden Delicious, DEP decreased sharply below 12°C and above 20°C in this and another, similar experiment whose results are not shown. An orchard spray treatment with daminozide led to longer DEPs, especially in Cox, and this effect was most marked below 15°C (Fig. 1, Table 1).

**Table 1.** Analysis of variance for effects of temperature and daminozide on the onset of rapid ethylene production after harvest in two varieties of apple (Fig. 1 data).

Experimental material	Source of variation	Variance ratio	DF	<i>p</i>
Golden Delicious	Daminozide	8.6	1/112	<0.001
	Temperature	22.8	7/112	<0.001
	Interaction	3.3	7/112	<0.01
Cox's Orange Pippin	Daminozide	103.5	1/112	<0.001
	Temperature	6.0	7/112	<0.001
	Interaction	4.2	7/112	<0.001

DF is the degrees of freedom for the source of variation divided by the residual degrees of freedom. *p* is the probability of the null hypothesis.



**Fig. 2.** Effect of daminozide, storage temperature, and ethylene treatment on the delay in onset of rapid ethylene production in Cox apples. Daminozide was sprayed at 0.85 kg/m³ on July 23, and the fruit was harvested on September 9 and treated for 48 h with ethylene. ○, unsprayed at 5°C; ●, sprayed at 5°C; △, unsprayed at 15°C; ▲, sprayed at 15°C.

In earlier work (Knee et al. 1983) it was shown that the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) increased at the same time as the rise in ethylene synthesis in Golden Delicious apples at 3°C. It seemed possible that low temperature could induce ACC synthase activity and eth-

**Table 2.** Analysis of variance for effects of daminozide, storage temperature, and ethylene treatment on the onset of rapid ethylene production in Cox apples (Fig. 2 data).

Experimental material	Source of variation	Variance ratio	DF	<i>p</i>
Unsprayed at 5°C	Ethylene	12.9	3/28	<0.001
Daminozide sprayed at 5°C	Ethylene	17.8	3/28	<0.001
Unsprayed at 15°C	Ethylene	3.0	3/28	<0.05
Daminozide sprayed at 15°C	Ethylene	28.1	3/28	<0.001
Unsprayed	Temperature	123.7	1/56	<0.001
	Ethylene	10.0	3/56	<0.001
	Interaction	11.9	3/56	<0.001
Daminozide-sprayed	Temperature	68.2	1/56	<0.001
	Ethylene	37.4	3/56	<0.001
	Interaction	7.7	3/56	<0.001
Sprayed and unsprayed at 5°C	Daminozide	118	1/56	<0.001
	Ethylene	21.8	3/56	<0.001
	Interaction	7.7	3/56	<0.001
Sprayed and unsprayed at 15°C	Daminozide	410	1/56	<0.001
	Ethylene	15.1	3/56	<0.001
	Interaction	24.5	3/56	<0.001

Abbreviations as in Table 1.

ylene synthesis directly in this variety without the mediation of ethylene. To retain a role for ethylene, it would be necessary to show that some aspect of ethylene action was favored by low temperature. To retain a consistent role for ethylene in unsprayed Cox, it would be necessary to demonstrate constant ethylene action at different temperatures, but daminozide would be required to cause a temperature-dependent change in sensitivity in this variety. Temperatures of 5 and 15°C were chosen for their contrasting effects in further experiments on the interaction of ethylene, daminozide, and temperature.

There could be no ethylene-free control in experiments involving ethylene treatment of apple fruits. All of the fruit experienced the endogenous ethylene plus any exogenous treatment. Inclusion of the endogenous ethylene eliminates one source of variation in dose response curves (Goeschl and Kays, 1975), so it is more likely that the true response in relation to concentration will be detected.

Daminozide treatment made Cox apples at 15°C less responsive to ethylene concentrations between  $10^2$  and  $10^4$   $\text{mm}^3/\text{m}^3$  (Fig. 2; Table 2). Ethylene at  $10^6$   $\text{mm}^3/\text{m}^3$  overcame the daminozide effect. Thus, daminozide treatment keeps apples in the "low-affinity" state that is characteristic of immature fruit (Knee et al. 1987), and the daminozide effect on apples at 15°C is explicable in terms of ethylene action. However, at 5°C, the DEP of unsprayed Cox was reduced only at the highest ethylene level tested,  $10^6$   $\text{mm}^3/\text{m}^3$ . DEPs were longer in daminozide-sprayed fruit at all ethylene concentrations, and ethylene treatment actually delayed induction of ethylene synthesis in this fruit (Fig. 2; Table 2). Thus, at the low temperature the daminozide effect was not explicable in terms of an effect on sensitivity to ethylene. A possible explanation is

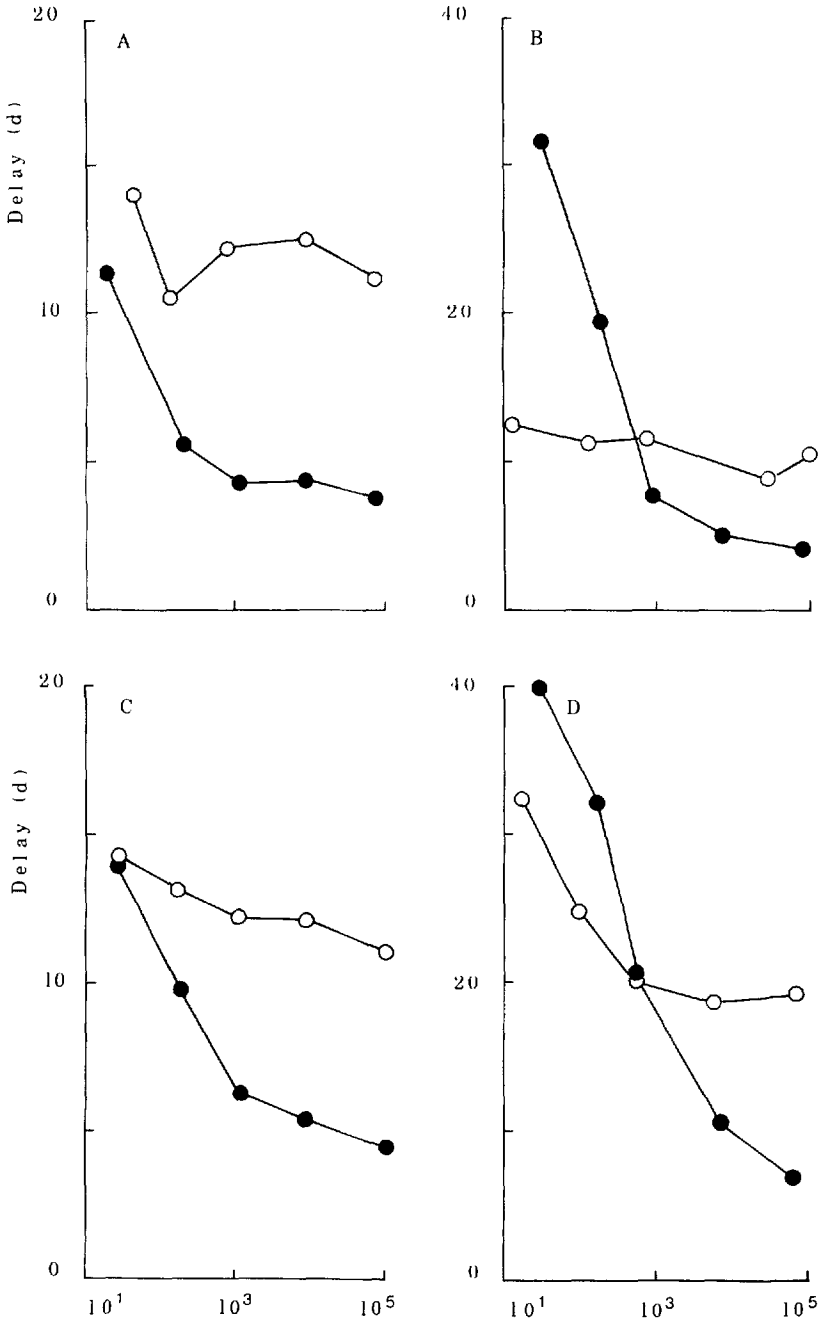


Fig. 3. continued on next page.

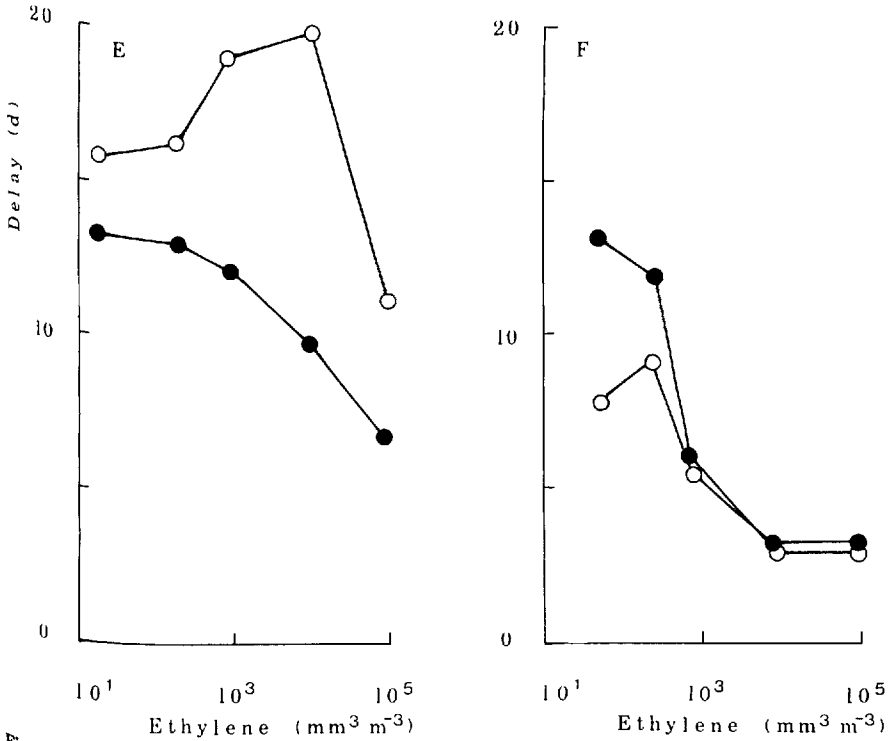


Fig. 3. Effect of temperature during and after 48-h ethylene treatments on the onset of rapid ethylene production in two varieties of apple. ●, Fruit held continuously at 15°C. ○, Other treatments: A, B, continuously at 5°C; C, D, treated at 5°C and then held at 15°C; E, F, treated at 15°C and held at 5°C. Cox apples (A, C, E) were harvested on September 4 and 11, 1984, and August 26, 1986, and Golden Delicious (B, D, F) on September 25 and October 2, 1984, and September 29, 1986.

that as the temperature was lowered, there was a transition from ethylene-induced to low-temperature-induced ethylene synthesis; daminozide might block both induction processes, independently or through an effect on a common intermediate.

The interacting effects of ethylene and temperature on the induction of ethylene synthesis were further explored in a temperature transfer experiment. The aim was to distinguish between effects of temperature on ethylene action and effects of temperature on the induction of ethylene synthesis. Because of limitations of apparatus, it was not possible to compare all treatments simultaneously, and so treatments involving varying exposure to 5°C were tested singly against similar sets of apples held continuously at 15°C (Fig. 3; Table 3). The first series involved continuous exposure to 5°C; the ethylene treatment had little or no effect under these conditions (Fig. 3A, B; Table 3). This could result from inhibition of action or response at the low temperature. When apples were treated at 5°C and then transferred to 15°C at 48 h, this should have allowed a response if ethylene action occurred at the lower temperature.

**Table 3.** Analysis of variance for ethylene effect on the onset of rapid ethylene production in two varieties of apple subjected to various temperatures during and after a 48-h ethylene treatment (Fig. 3 data).

Experimental material	Variance ratio	<i>p</i>
A. Cox continuous 5°C	2.3	>0.05
A. Cox continuous 15°C	177.4	<0.001
B. GD <sup>a</sup> continuous 5°C	4.0	<0.01
B. GD continuous 15°C	61.9	<0.001
C. Cox 5 then 15°C	3.1	<0.05
C. Cox continuous 15°C	61.0	<0.001
D. GD 5 then 15°C	6.7	<0.001
D. GD continuous 15°C	36.7	<0.001
E. Cox 15 then 5°C	17.2	<0.001
E. Cox 15 then 5°C (part)	8.2	<0.001
E. Cox continuous 15°C	18.5	<0.001
F. GD 15 then 5°C	93.9	<0.001
F. GD continuous 15°C	47.9	<0.001

The degrees of freedom were 4 for ethylene treatments and 75 for residual except in the analysis of part of experiment E, when the highest ethylene treatment was omitted, so that degrees of freedom were 3.

<sup>a</sup> Golden Delicious.

The results showed that ethylene was less effective when applied at the lower temperature in both varieties (Fig. 3C, D; Table 3). The opposite transfer, to 5°C after ethylene treatment at 15°C for 48 h, should reveal whether response reactions are limiting at 5°C. The Cox apples in this series were insensitive to ethylene below 1000 mm<sup>3</sup>/m<sup>3</sup> at 15°C (Fig. 3E) and were therefore immature (Knee et al. 1987). The apples transferred to 5°C showed delayed induction with concentrations up to 10<sup>4</sup> mm<sup>3</sup>/m<sup>3</sup> and acceleration at 10<sup>5</sup>/mm<sup>3</sup>/m<sup>3</sup> (Fig. 3E; Table 3). The Golden Delicious apples treated in this way were naturally closer to induction than those in other treatments (Fig. 3F), perhaps because they had experienced low temperatures on the tree. When ethylene-treated apples were transferred to 5°C, ethylene synthesis was induced at least as rapidly as in those held continuously at 15°C (Fig. 3F; Table 3).

The temperature transfer experiments did not provide evidence for a role of ethylene in the induction of rapid ethylene synthesis at 5°C. It is possible that endogenous ethylene was enough for a maximum response under these conditions or that ethylene was active immediately before the onset of rapid synthesis rather than earlier in induction. But, allowing these possibilities, the implication remains that ethylene is not the limiting or controlling factor in the induction of rapid ethylene synthesis.

From earlier work (Knee et al. 1987), it seems that during much of apple fruit development, the endogenous ethylene does not promote the onset of rapid ethylene synthesis; close to the natural onset, low concentrations become promotive. Low temperature and daminozide treatment maintain the fruit in a state in which ethylene is inactive. The data can be interpreted as showing



variation in receptor affinity, but it may be erroneous to infer such a fundamental molecular property from a physiological response. Ethylene is the only plant growth regulator with a clearly demonstrated role in fruit ripening. However, its role appears to be mediating rather than predominant, and it is subject to genotypic, developmental, and environmental control, as envisaged by Trewavas (1986) for plant growth regulators in general. Opportunities for practical control of fruit ripening may involve manipulation of ethylene action as well as ethylene synthesis. The use of daminozide is one approach to this objective, although there are problems in its practical adoption (Knee 1986).

*Acknowledgments.* Thanks are due J. R. Coppins, D. Farman, and S. G. S. Hatfield for their assistance with this work.

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