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Influence of Rootstocks and Fertilizers on Ethylene in Apple Fruit during Maturation and Storage

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Abstract **Internal ethylene of attached fruit of 'Starkspur Golden Delicious' apple as influenced by 6 rootstocks: Seedling, Malling (M1) 1, Malling Merton 106 (MM 106), M 7, OAR 1, M 26, at 2 levels each of soil-applied K and N were measured during maturation for 2 years (1980 and 1981). Ethylene evolution of detached fruit as a result of these treatments also was measured after 2.5 months of storage at 0°C in 1981. Internal ethylene in the attached fruit was less than 0.1 $\mu l \cdot liter^{-1}$ in late September and early October and began to rise between 9 Oct. and 15 Oct. Internal ethylene increased in all treatments, almost at the same time in 1980. In 1981, ethylene in the fruit on OAR 1 began to increase 9 days later than in the other rootstocks. However, levels of ethylene in the fruit were relatively low on OAR 1 and high on M 26 as compared to those on other rootstocks in late October 1980 and 1981 and during poststorage 1981 samplings. Ethylene levels in fruit from other rootstocks were similar. Because of these variable effects of rootstocks, and the effects of a low field temperature in reducing internal ethylene levels, field sampling of internal ethylene levels was an unreliable indicator of the proper harvest time, as measured by other maturity indices. No consistent influence of K or N applications was found in the internal ethylene of the attached fruit; however, high N applications increased ethylene evolution after storage.**

Ethylene has been studied in tissues of various higher plants, and particular attention has been given to the role of ethylene in the onset of the fruit climacteric. Many researchers have studied the precursors and pathways of ethylene biosynthesis (1, 8). Others have studied the action of ethylene on the physiology of fruit ripening (2, 3, 9) and the triggering ethylene concentration (3). Dilley (4) has designated 6 ranges of internal ethylene and recommended appropriate storage duration and marketing strategies for fruit with ethylene in each range. In our research, internal ethylene levels of attached and ethylene evolution of detached 'Starkspur Golden Delicious' apple fruit on various rootstocks in combination with N and K fertilizer treatments from a high density orchard were studied to determine possible effect on maturation as determined by the rise in internal and evolved ethylene. Such a study could add information about the rootstock effect on maturity and prediction of storage life.

Materials and Methods

A 'Starkspur Golden Delicious' orchard was established at the Lewis-Brown Horticulture Farm, Corvallis, in 1972. Trees were planted 0.61 m apart within rows and 2.43 m between rows. The design was a split-split-plot arrangement with 6 rootstocks (Seedling, M 1, MM 106, M 7, OAR 1 and M 26) as main plots (blocks) and 2 levels of K as sub-plots and 2 levels of N as sub-sub-plots, with 6 replications per treatment in 1980. In 1981, the design was reduced to a split plot, with 6 rootstocks as main plots (blocks) and 2 levels of N as sub-plots with 6 replications per treatment. OAR 1 is a new rootstock selected at Oregon State Univ. Details of the planting and cultural practices are presented elsewhere (6).

One average size fruit from each tree (4 trees per replication) at about 1.65 m height was selected and tagged for identification. All fruit were located nearing a horizontal line on the south side of each hedgerow, in order to minimize the variation due to fruit size and location in each tree. In 1980, 96 fruit were sampled for ethylene from the trees on each rootstock (6 replications × 2 levels of K × 2 levels of N × 4 trees per replication) and 288 fruit from each level of K and N fertilizer (48 or 96/2 fruit for each level of K or N from each rootstock × 6 rootstocks). In 1981, a total of 48 fruit for each rootstock (6 replications × 4 trees per replication × 2 levels of N) and 144 for each level of low or high N fertilizer (24 or 48/2 fruit for each level of N from each rootstock × 6 rootstock) were tested at each time of ethylene sampling.

Ethylene samples were taken several times from the same apple starting in mid-September in 1980 and early October in 1981 through early November of both years. Coincident with the regular sampling, 2 additional tagged fruit of a same size and location on each tree were punctured in the same manner as the original fruit. If the original experimental fruit abscised, it was replaced with one of the multi-punctured fruit. Comparison of fruit with a single puncture with multi-punctured ones showed no significant difference in internal ethylene.

Internal ethylene was sampled near the mid-line between stem and calyx each time, and the needle puncture holes were marked. The next sample was taken a few days later, about 2 cm away from the previous mark on the same diameter. Needles (2.5 cm, 27 gauge, bevel tipped) were inserted to the core line (about 2 cm) then withdrawn about 0.75 cm (to prevent juice extraction) and after 5 sec about 1.2 ml of the internal gas of the intercellular space was collected in plastic syringes. Needles were sealed by insertion into a rubber septum. Each syringe was identified as to location, treatment and tree number, and the syringes then were immediately placed in a cooler at about 15°C and transported to the laboratory. One-milliliter samples were injected

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Table 1. Percentage of 'Starkspur Golden Delicious' apple fruit grouped by internal ethylene classes at 3 maturity dates as influenced by rootstocks in 1980 and 1981.

Root-stock	1980 ethylene classes[z] at 3 Dates									1981 ethylene classes at 3 Dates								
	14 Oct.			22 Oct.			28 Oct.			16 Oct.			21 Oct.			28 Oct.		
	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
Seedling	100	0	0	95	3	2	59	36	5	90	2	8	88	0	12	42	17	41
M 1	99	1	0	93	5	2	68	23	9	98	0	2	80	10	10	34	27	39
MM 106	100	0	0	99	0	1	59	36	5	96	2	2	90	6	4	51	6	43
M 7	97	3	0	91	4	5	42	37	21	92	2	6	76	4	20	36	8	56
OAR 1	96	3	1	95	4	1	75	21	4	100	0	0	98	2	0	57	29	14
M 26	99	0	1	89	7	4	39	47	14	77	11	12	71	6	23	10	19	71

[z]Classes of internal ethylene ($\mu l \cdot liter^{-1}$); low <0.5, medium = 0.5–5, high >5.

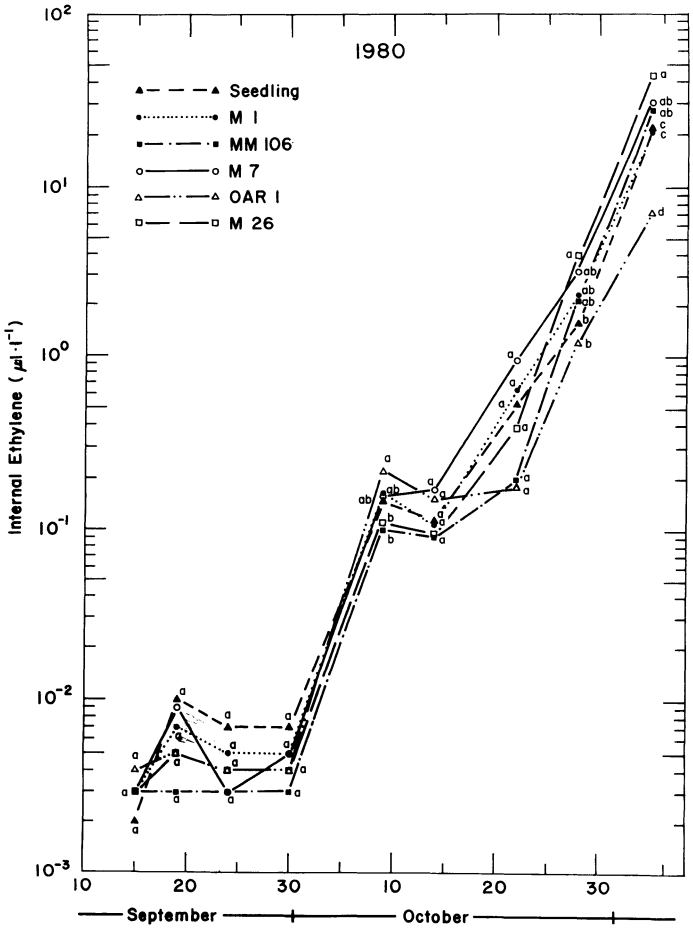

Fig. 1. Internal ethylene ($\mu l \cdot liter^{-1}$) during maturation of 'Starkspur Golden Delicious' apple fruit as influenced by rootstock in 1980. Rootstock mean separation within sampling dates by Duncan's multiple range test, 5% level.

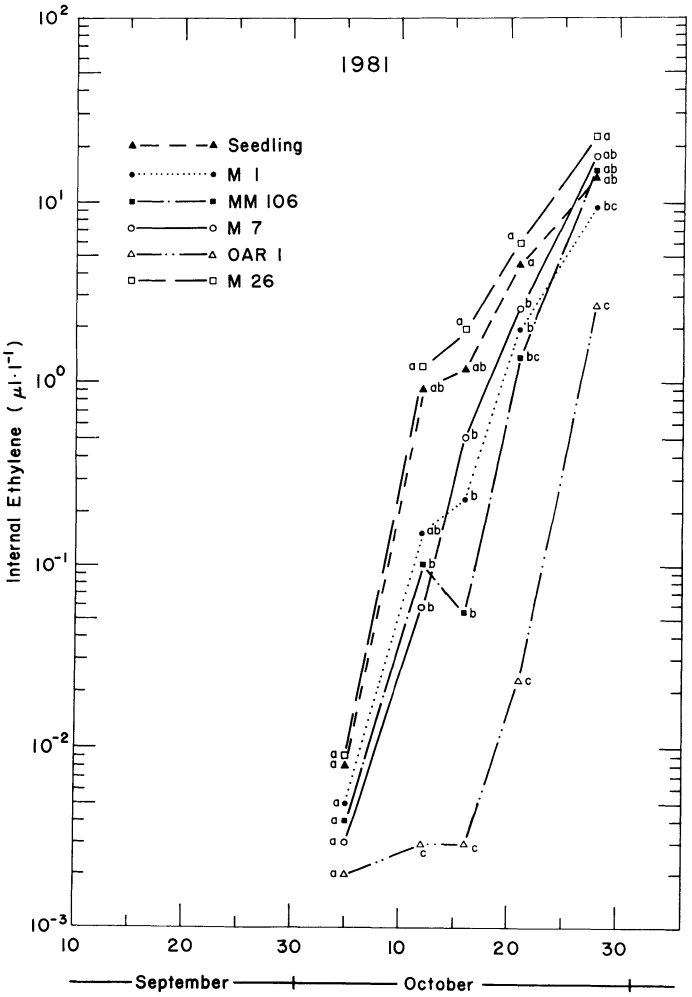

Fig. 2. Internal ethylene ($\mu l \cdot liter^{-1}$) during maturation of 'Starkspur Golden Delicious' apple fruit as influenced by rootstock in 1981. Rootstock mean separation within sampling dates by Duncan's multiple range test, 5% level.

into a gas chromatograph (Carle Analytical Gas Chromatograph Model 312). After every sampling, syringes and needles were washed, rinsed, and vacuum treated overnight to remove any absorbed ethylene and to prevent growth of fungi.

Three internal ethylene classes were designated and percentages of fruit from each rootstock falling in each of the low (<0.5 $\mu l \cdot liter^{-1}$), medium (0.5–5 $\mu l \cdot liter^{-1}$) and high (>5 $\mu l \cdot liter^{-1}$) categories were calculated for 3 different sampling times after the internal ethylene began to change.

In addition to internal ethylene, ethylene evolution of duplicate 9-fruit composites of each treatment, harvested at the commercial harvest date (10 Oct.), was measured in late December after 2.5 months at 0°C. This measurement continued during the period of ripening at 20°. Apple fruit were placed in 4-liter glass respirometer jars at 20° with an air flow of 200 $ml \cdot min^{-1}$. A 1-ml gas sample taken from the outlet tube was injected into a gas chromatograph equipped with a flame ionization detector to measure ethylene.

Analyses of variance (ANOVA) were computed from direct (rather than transformed) data, as the distributions were normal

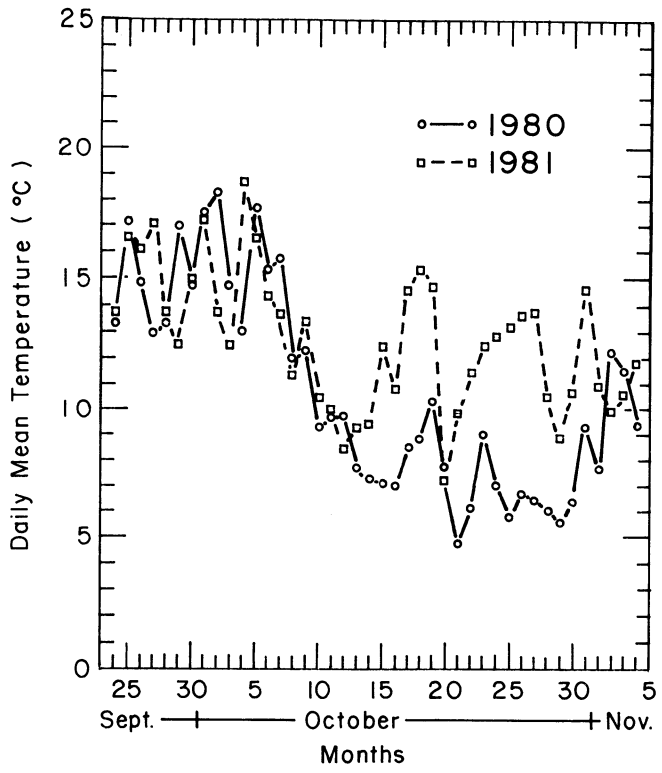

Fig. 3. Temperature fluctuations (°C) in September, October, and November of 1980 and 1981.

in all parts of this research. In the text, * indicates that the correlation coefficient (r) is significant at 5% level.

Results and Discussion

General trends. Results of preliminary tests showed the accumulation of ethylene in the flesh can be detected at the same time as in the core cavity, but the concentration of ethylene in the core cavity was about 30% higher than in the flesh. Recommendations of Dilley (4) were based on the core ethylene concentrations. We would have taken somewhat higher classes of ethylene in Table 1 if we had taken samples from the core instead of the flesh. Nevertheless, the lower concentration of internal ethylene measured by our method would not affect the experiment because our goal was to study relative differences among treatments rather than absolute amount of ethylene.

The changes in internal ethylene during maturation of 'Starkspur Golden Delicious' apple fruit in 1980 and again in 1981, are shown in Fig. 1 and 2. In general, internal ethylene began to rise between 30 Sept. and 12 Oct. in both years, and the increase was logarithmic over time, at least during the maturation period. Prior to 30 Sept. 1980, ethylene was less than 0.01 $\mu l \cdot liter^{-1}$ and nearly constant at that low concentration (Fig. 1). Results in 1981 were similar, but intensive sampling was begun only after a few samples started to show greater than 0.01 $\mu l \cdot liter^{-1}$ ethylene. Fruit harvested at these early stages in both years (2 Oct. 1980; and 30 Sept. 1981) were immature, not only with respect to internal ethylene, but also with respect to standard eating and marketing qualities (5). Furthermore, a substantial increase in the fruit weight (about 18 g/fruit or a 12% increase) occurred between the early and late harvests (7), suggesting that delaying fruit harvest could increase yield at a time when eating quality is still improving. Although the fruit harvested on 15 Oct. 1980 and 10 Oct. 1981 had better quality

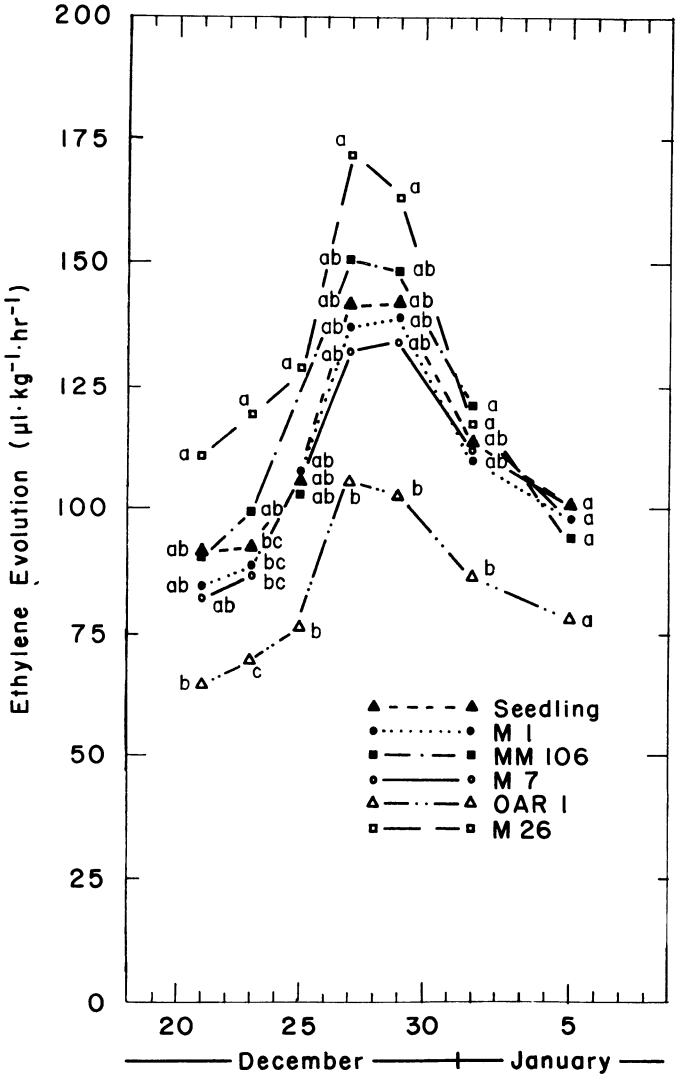

Fig. 4. Ethylene evolution ($\mu l \cdot kg^{-1} \cdot hr^{-1}$ during ripening at 20°C of 'Starkspur Golden Delicious' apple fruit harvested 10 Oct. 1981 and stored at 0° until late Dec. 1981 as influenced by rootstock. Rootstock mean separation within dates by Duncan's multiple range test, 5% level.

than fruit of previous and subsequent samplings (5), the percentage of fruit with medium and high classes of internal ethylene were low on these harvesting dates (Table 1). In 1980, there was a gradual progression in the increase in internal ethylene levels; in 1981 there was little change until 21 Oct. with a rapid change occurring during the 21 Oct. to 28 Oct. period (Table 1). At the same calendar date (28 Oct.), fruit were more advanced in maturity in 1981 than in 1980 (Table 1). Possibly the higher temperature after mid-October 1981 (Fig. 3) stimulated internal ethylene synthesis.

After storage at 0°C for 2.5 months and ripening at 20°, evolved ethylene increased to a maximum after 1 week, then declined for fruit from most rootstocks (Fig. 4) and fertilizer treatments (Fig. 5) in 1981.

Effect of weather. Some irregularities in the internal ethylene curves for both 1980 (Fig. 1) and 1981 (Fig. 2) are worth highlighting. Internal ethylene samples drawn on 14 Oct. 1980 showed a decrease, and those drawn on 16 Oct. 1981 showed a decreased rate of increase, compared to previous and subsequent samples in all rootstocks except for M 7. This difference was

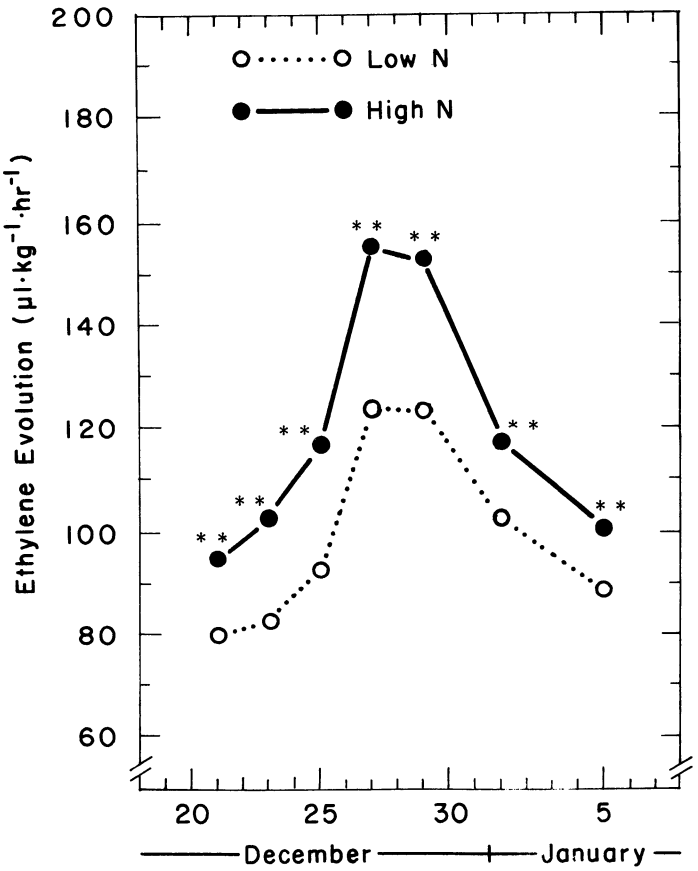

Fig. 5. Ethylene evolution ($\mu l \cdot kg^{-1} \cdot hr^{-1}$) during ripening at 20°C of 'Starkspur Golden Delicious' apple fruit harvested Oct. 10 1981 and stored at 0° until late Dec. 1981 as influenced by N fertilizer. Low level of N is significantly different from high level within dates at 1% (**).

Table 2. Total internal ethylene of attached fruit over several maturity dates in 1980 and 1981 and total evolved ethylene of detached fruit over 7 days of ripening at 20°C after 3 months of 0° storage of 'Starkspur Golden Delicious' apple as influenced by rootstock.

	Total internal ethylene[z] ($\mu l \cdot liter^{-1}$)		Total evolved ethylene[y] ($\mu l \cdot kg^{-1} \cdot hr^{-1}$)
Rootstock	1980	1981	1981–1982
Seedling	23.7 c[x]	20.0 ab	789.5 b
M 1	24.2 c	11.8 bc	764.5 b
MM 106	29.6 bc	16.0 b	807.0 b
M 7	37.7 ab	20.6 ab	755.5 b
OAR 1	9.1 d	2.7 c	583.0 c
M 26	42.9 a	31.4 a	909.0 a

[z]Sum of 5 days in 1980 (9, 14, 22, 28 Oct. and 4 Nov.) and 4 days in 1981 (12, 16, 21, 28 Oct.).
[y]Sum of 7 days (21, 23, 25, 27, 29 Dec. 1981 and 1 and 5 Jan. 1982).
[x]Rootstock mean separation within columns by Duncan's new multiple range test, 5% level.

related to low temperatures in the orchard between 11 Oct. and 16 Oct. (Fig. 3) in both years. This effect of temperature on internal ethylene is genuine, and it implies that caution must be exercised in assigning fruit to certain storage strategies based exclusively on one sampling of internal ethylene in the low range, as the fruit could, in fact, be more advanced in development than is indicated by internal ethylene. These results also could indicate a problem associated with using ethylene as an indicator of harvest time. A similar effect of temperature was observed by Walsh and Kender (10) in the internal ethylene of 'McIntosh' apple shoot and spur tissues.

Effects of rootstocks. Internal ethylene was very low (<0.01 $\mu l \cdot liter^{-1}$) in fruit from all rootstocks in early October, and no significant rootstock differences were observed up to 9 Oct. 1980 and 12 Oct. 1981 (Fig. 1 and 2). After these dates, significant differences were observed among rootstocks at most sampling dates (Fig. 1 and 2). These differences, however, are more clearly shown by calculating the total of measured internal ethylene levels over the periods of 14 Oct. through 4 Nov. 1980 and 12 Oct. through 28 Oct. 1981 (Table 2).

The rise in fruit internal ethylene on OAR 1 rootstock was lower than for the other rootstocks in both 1980 (Fig. 1) and 1981 (Fig. 2) and less pronounced in 1981 than 1980. Internal ethylene of these fruit rose 9 days later than other rootstocks in 1981. Also, total internal and evolved ethylene of fruit on OAR 1 was significantly lower than all rootstocks other than M 1 in both years (Table 2). Furthermore, trees on OAR 1 consistently had the highest number of fruit in the low class on 28 Oct. 1980 and on all sampling days of 1981 (Table 1). However, fruit on OAR 1 had higher soluble solids and yellower skin color than comparable fruit from other rootstocks (5). This indicates that internal ethylene may not be a reliable test of harvest maturity for fruit on this rootstock. The relatively small fruit size in the trees on OAR 1 rootstock, found to be a characteristic of this rootstock in a 4-year evaluation, may be responsible for a lower ethylene level (for size-ethylene association: $r = 0.53$*), perhaps by influencing diffusion rate.

Fruit on MM 106 occasionally tended to have lower internal ethylene at early sampling dates than most other rootstocks, except OAR 1 (Fig. 1, 2).

Fruit from M 26 had high internal ethylene in late 1980 (Fig. 1) and during all sampling periods of 1981 (Fig. 2). Total internal ethylene of fruit on M 26 was significantly higher than those on all rootstocks, other than M 7 (in both years) and seedling (in 1981) (Table 2). Also, the percentage of medium and high ethylene fruit for M 26 also was highest in late 1980 and 1981 (Table 1). These consistent observations in 1980 and 1981, as well as various quality indices (5), suggest that M 26 hastens maturation of 'Starkspur Golden Delicious'. However, drastically increased internal ethylene of fruit on M 26 in 1981 could be partially due to the light crop (6). Fruit from other rootstocks were intermediate in internal ethylene levels (Fig. 1 and 2).

Ethylene evolution after 2.5 months of storage (from the incubated fruit in the jars) confirmed the field sampling. Thus, fruit on OAR 1 had relatively low ethylene levels, whereas those on M 26 were the highest (Fig. 4 and Table 2). Fruit from other rootstocks had intermediate concentrations. Decline of ethylene evolution in fruit on all rootstocks occurred after 1 week, except in those on M 7 and M 1 in which decline started after 9 days (Fig. 4).

Effects of fertilizers. As soon as ethylene started to rise, fruit from the high K plots always had relatively higher internal ethylene during maturation, and this difference in comparison with low K fruit was statistically significant at the end of the season (data no shown). No significant influence of N fertilizer was found in 1980, but in 1981, attached fruit from the high N plots had slightly increased concentrations of internal ethylene. However, ethylene evolution in high N fruit always increased significantly in storage (Fig. 5), parallel to a slightly higher respiration

rate in these fruit (data not shown) (5). Thus, application rate of N is an important factor in maturation and keeping quality of apples and warrants further investigation. No rootstock-fertilizer interaction was observed in this research.

It would be of interest to study simultaneous seasonal changes in the roots, wood, spurs and fruit from trees on several rootstocks and to find the distribution of ethylene in the tree before and during fruit maturation. Research in this area is new and limited. Walsh and Kender (10) observed high internal ethylene in 2-year wood of spur and nonspur strains of 'Delicious' and McIntosh' early in the season, which subsequently declined. Possibly ethylene precursors or enzyme stimulators move from the shoots into the fruit. By the time spur ethylene declines, fruit begin to produce it. If such translocation were shown to exist, the ripening of attached fruits from trees on each rootstock would depend on the interactions between ripening inhibitors in the scion leaves (9) and the translocated factors which increase ethylene in the fruit.

Sampling internal ethylene of attached fruit gave patterns of increase which could be useful in predicting fruit maturity stages. However, it also is clear that fruit on certain rootstocks may behave atypically and that cold weather during maturation can lead to variation of ethylene that needs to be interpreted carefully in judging maturity based on internal ethylene. Future research on the physiology and on the ethylene producing system of fruit on OAR 1 rootstock and mechanism of N fertilizer interactions with ethylene would be of interest. Light cropping enhances earliness, and the early ethylene rise must be taken into account when determining the optimum harvest time.

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Low Temperature Germination of Celery Seeds for Fluid Drilling

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Abstract. **Celery (*Apium graveolens* L.) seeds germinated at 10°C for 14 days produced shorter and more uniform radicles (0–2 mm) than seeds germinated for 8 days at 24° (0–10 mm). Removal of seed leachates improved the germination of celery seeds in the light. Celery seeds germinated at 10° prior to sowing emerged faster, and produced more uniform plants than those not pregermihated, and were not thermodormant when incubated at 32°.**

Germination of celery seeds in the field is slow and sporadic, resulting in delayed and nonuniform stands, especially under cold soil conditions (3). Therefore, celery plants usually are started in greenhouses and transplanted into the field after 8 to 10 weeks. Greenhouses provide more favorable conditions for germination and emergence of celery seeds than direct seeding in the field. Even under greenhouse conditions, however, celery seedlings emerge over a 7–10 day period and are subject to thermodormancy (nongermination of the seeds in the dark) at temperatures above 22°C (22).

Celery seeds may be germinated prior to planting to improve uniformity and speed of emergency (12). The germinated seeds then are sown by suspending the seeds in a fluid gel and extruding them into the soil with a fluid drill.

Earlier and more uniform celery stands have been established with fluid drilling, using partially pregerminated seeds (a mixture of imbibed and germinated seeds with radicles emerged), than with dry seeding (2, 6).

Radicles of germinated seeds should be short (<5 mm) and of uniform length for effective fluid drilling. Radicles of germinating celery normally emerge over a period of 7 to 14 days, resulting in radicles of various lengths (18). Attempts to fluid drill germinated celery seeds with radicles longer than 5 mm have resulted in injury (18). A number of factors affect celery seed germination, including a requirement for light during imbibition (17, 22), thermodormancy at high temperatures (2, 4), germination inhibitors in the seed coat (18, 19), and irregular embryo maturity due to time differences in initiation of umbels, pollination of florets, and embryo development (15). If these

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