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Effects of Etiolation, Stem Anatomy, and Starch Reserves on Root Initiation of Layered *Malus* Clones[1]

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Abstract. **Four *Malus* dwarfing rootstock clones showed a range of rooting success when propagated as layers. Root emergence was largely confined to a nodal position near lateral buds. Anatomical studies revealed nodal rooting to be closely associated with the parenchymatous bud and leaf gaps of the stem. The highest starch concentration was in these tissues and the outer ring of pith cells. Etiolation during layering increased stem starch and decreased the degree of sclerification of the cortex. Rooting success was negatively correlated with degree of sclerification. Etiolated stem cuttings rooted in 7 days under mist propagation, while non-etiolated cuttings failed to root, indicating that etiolation provided a stimulus to root initiation in the non-differentiated, starch-rich gap areas. Inter-relationships of stem etiolation and internal carbohydrate reserves were associated with the rooting process.**

The layering propagation method of *Malus* dwarfing rootstock clones incorporates stem etiolation as an aid to rooting. The increased propensity of etiolated plant material to root and the concomitant inhibition of the rooting process by light is clear (7, 12, 20). The etiolation effect is most pronounced when exclusion of light begins during early stages of shoot development.

A central question is whether etiolation promotes formation of root primordia, or merely stimulates elongation of preformed primordia. Some evidence suggests etiolation promotes root initiation (8, 13), whereas a few readily-rooted plants will produce latent primordia under normal growth conditions (3, 11).

The effect of etiolation on root emergence from layered shoots merits attention. A characteristic pattern of appearance of adventitious roots at nodes and from bud and leaf gaps in etiolated material has been reported (14, 19). This phenomenon differs from the usual response in cutting propagation, where roots often appear near callus proliferation at the base of the cutting. Two types of root formation have been observed in woody cuttings: morphological roots formed on the vertical axis of the stem, and wound roots formed at the basal ends (9, 23, 25). Etiolation may cause root initiation at a very early stage of development, while cutting propagation promotes formation of initials in wound-generated meristematic tissue. Under this scheme, layering as a form of propagation would not constitute true regeneration, but extension growth of already-differentiated initials.

The purpose of this research was to study the effect of stem etiolation and starch reserves on adventitious root formation in different *Malus* rootstock clones.

Materials and Methods

Four dwarfing rootstock clones with a range of size control and rooting potential were chosen for this study: Malling (M) 9, M 26, M 2, and Malling Merton (MM) 106. Rooted shoots were obtained from beds of layered stock of uniform age, growth conditions, and cultural treatment. Mounding of the shoots throughout the growing season assured early etiolation of the developing tissues. Soil in the stooling beds was sandy loam and periodic supplemental irrigation was provided.

Rooted shoots (1.0 cm diam) were removed in Nov. as experimental material. Unmounded shoots were cut at ground level as controls. The average no. of roots per shoot and typical point of emergence of roots from the stem were determined for each clone. All shoots were stored at 2°C and 85% relative humidity until needed for rooting tests. In Feb., a portion of the etiolated stems with no visible rooting was prepared as cuttings, to be compared with non-etiolated cuttings of the same clones. The dormant cuttings were placed in an intermittent mist chamber with no exogenous rooting hormone supplied.

Two determinations (Sept. and Oct.) of tissue starch levels were made in 10 etiolated and 10 non-etiolated shoots per clone, using the anthrone reagent (6). A complete stem segment was used in each case. Absorbance readings were converted to mg starch per g fresh tissue. Three replications per clone and 2 readings per replicate were made spectrophotometrically (Bausch and Lomb Spectronic 20).

Starch distribution in etiolated fresh tissue was determined in 40 μ sections. The sections were placed in boiling water for 30 sec, decolorized in cold methanol, and stained with dilute iodine solution (10). The cross sections were mounted, examined microscopically, and photographed. Further anatomical studies included sectioning of fixed (FAA) stem sections stained with safranin–fast green.

Results

Root production. The rootstock clones varied in the no. of roots produced per layered shoot. Roots emerged primarily at a nodal position near a bud. This was consistent with all layered clones, although there was a trend to more nodal rooting in the difficult-to-root clones (Table 1). Non-nodal roots typically emerged some distance from the bud, either above or below, on the opposite side of the stem, or through a lenticel.

Roots mostly emerged in close proximity to, or through, the bud and adjacent leaf scar at the node, suggesting a stem tissue

Table 1. Rooting response and degree of sclerification of 4 *Malus* rootstock clones propagated by layering.

Clone	Roots per shoot	Roots at nodal area (%)	% sclerification[z]	
			Non-etiolated	Etiolated
M 26	13.4 a[y]	91.8	54.6	44.0
M 9	17.5ab	94.7	56.2	43.4
M 2	27.4c	88.5	50.5	40.5
MM 106	58.6d	86.3	34.8	30.1
Avg		90.3	49.0	39.5*

[z]% of phloem rays blocked externally by fibers.
[y]Means separation by Duncan's multiple range test, 5% level.
*Significant at 5% level.

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Fig. 1. Nodal rooting of M 2 showing two or more roots emerged (arrows) from the leaf gap at the points of meristematic activity of the bud.

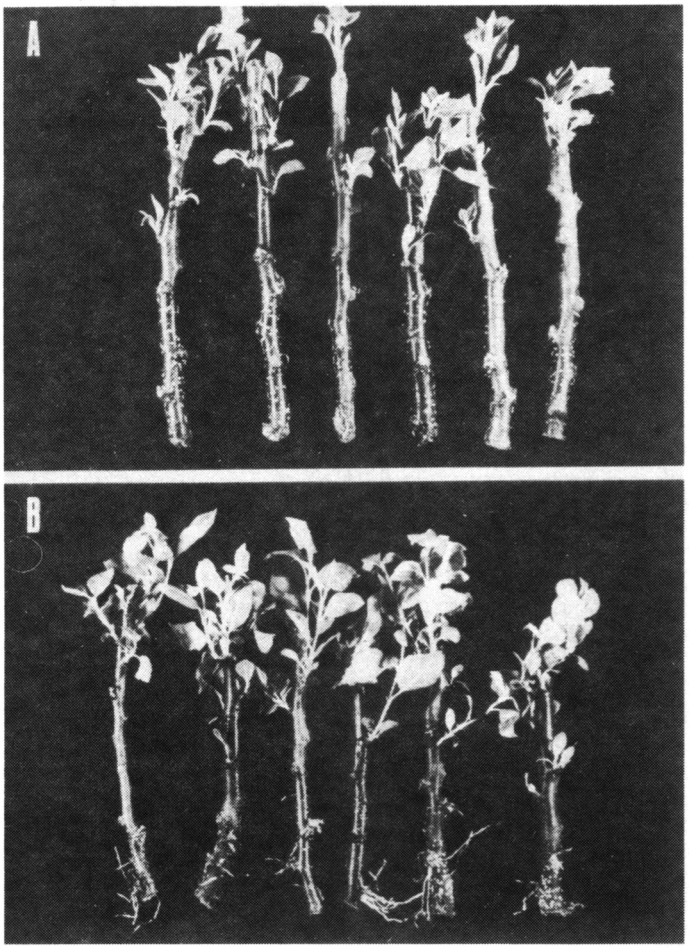

Fig. 2. Random M 2 cuttings A: from nonlayered and B: from layered shoots after being exposed to 4 weeks in greenhouse mist bed.

influence on root initiation (Fig. 1). The same pattern held true for all 4 clones, with M 2 and MM 106 producing a greater number of roots at each node.

Propagation by layering differs significantly from cutting propagation. The basal stem tissues from which roots arise are etiolated during the growing season by mounding, which provides an optimum envionment for root emergence and elongation. Rooted shoots with an extensive root system and with active leaves remain attached to a mother plant. No exogenous source of root-promoting hormone is applied, and no wounds are made to promote superficial meristematic activity. Therefore, roots apparently arise from internal tissue with sufficient food reserves, hormone supply and meristematic activity.

Etiolation. There was a striking difference in rooting of etiolated and non-etiolated portions of shoots used for cuttings (Table 2). Both percentage rooting and no. of roots per shoot were higher in the etiolated cuttings. Although cuttings were dormant when placed under mist, roots appeared within one week. The non-etiolated shoots had no roots after 4 weeks (Fig. 2). This would indicate that etiolation enhances formation of root initials in the stem which then emerge under proper environmental conditions. These results confirm previous work on rooting of etiolated materials and substantiate the success of layering techniques (8, 13, 15).

Except for "burrknot" formation (24), there is no clearcut information on pre-formed root primordia in *Malus* prior to etiolation. It is evident that root initiation in layering is dependent on exclusion of light during early stages of stem development so that, with proper temperature and humidity, root emergence and growth will occur. It must be emphasized that in layering only a portion of the growing stem is etiolated and not the entire plant.

Sclerification. A definite trend of greater sclerification in the difficult-to-root clones was found (Table 1). The % sclerification was negatively correlated with degree of rooting (–0.94). A significant decrease (10%) in sclerification occurred in the etiolated shoots as compared to non-etiolated shoots, similarly grown, corroborating other reports (1, 2, 5, 16, 22).

It is difficult to determine whether fiber tissue is associated with rooting in a cause or an effect manner. Stem sclerification as a barrier to rooting is closely tied to tissue juvenility and, therefore, difficult to consider in a purely anatomical sense. However, it is doubtful that the maximum recorded 56% complete ring of fibers could block root emergence in layers to a significant degree, since the fiber ring was not present across the bud area where most roots emerged (Table 1).

Influence of internal starch. Consistently significant variation in starch was found in the clones and in the etiolated vs. non-etiolated conditions (Table 2). There was no correlation between clonal starch levels and number of roots per shoot.

Starch accumulation during the month of Oct. was evident (Table 2). In stoolbeds, root production continues well into late autumn, suggesting an important period for the propagation

Table 2. Rooting (%) and internal starch levels of 4 *Malus* rootstock clones propagated by layering.

Clone	Cuttings rooting (%)		Starch (mg/g fresh wt)					
			Etiolated layers		Non-etiolated layers		Avg	
	Etiolated	Non-etiolated	Sept. 26	Oct. 20	Sept. 26	Oct. 20	Sept. 26	Oct. 20
M 9	41.0	1.1	49.90	65.61	31.88	44.56	49.89a[z]	55.10a
MM 106	100.0	13.3	63.50	74.06	37.22	50.07	50.36b	62.12ab
M 2	62.0	3.0	60.22	74.25	44.90	57.50	52.56b	66.04b
M 26	87.5	6.8	84.40	93.42	49.35	71.22	66.88c	82.31c
Avg	72.6**	6.0	64.50**	76.93**	40.84	55.85		

[z]Means in a column followed by the same letter are not significantly different at the 1% level by Duncan's multiple range test.
**Significant at 1% level.

success of the difficult-to-root clones (4, 14).

In this study it appeared that the positional effect of stem tissue reserves contributed to root initiation and development. Several studies (17, 18, 19, 21, 23) have correlated rooting capacity with starch content, but none has reported the effect of etiolation on starch content.

Root initiation and stem anatomy. Microscopic examinations provided further information on the effects of etiolation and starch reserves on rooting. The extensive nodal rooting pattern was found to be connected with the starch-rich leaf and branch gap areas of the stem. Such parenchymatous areas were found behind the bud, extending into and around the pith (Fig. 3B). Areas with large starch concentrations included the parenchymatous, non-differentiated tissues, such as the pith, branch and leaf gaps, and phloem and xylem rays. Meristematic areas such as the cambium, phellogen, lateral and apical buds, and root tips showed little evidence of starch. The same was true of the vascular elements and fiber tissues of the cortex.

A distinction is often made between leaf gaps and branch gaps, which serve as connecting tissues for the developing side shoot. The tissue of importance in this study was the relatively non-differentiated cells of the gap area which contained a copious starch supply.

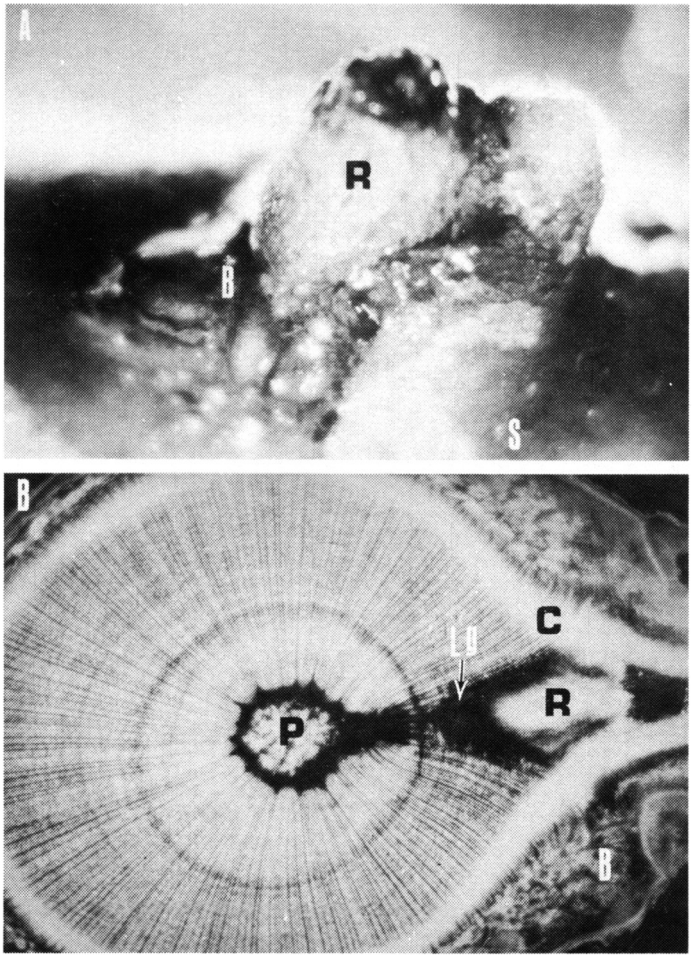

Fig. 3. A. Root emergence from node of M 2 stem showing (R) root, (B) bud, and (S) stem. B. Cross section at the node of M 2 stem showing root emergence (R), dark area of leaf gap rich in starch (LG), cambial area (C), outer phelloderm or bark (B), and pith (P).

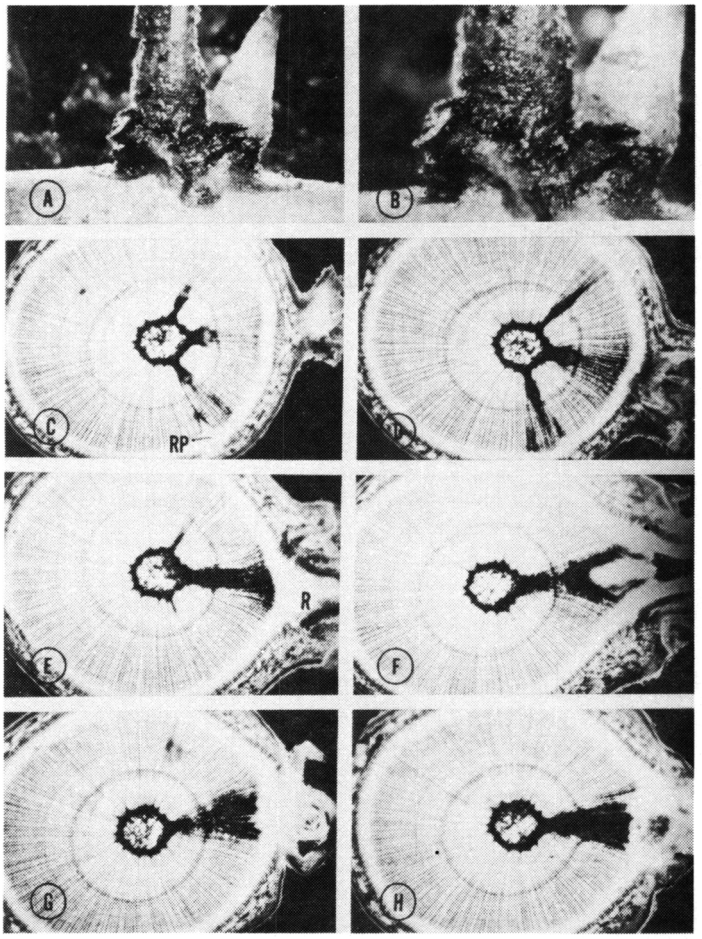

Fig. 4. Emergence of 2 roots through a bud A and B. Sequential transverse section of M 2 stem showing: C and D branch gaps and root primordium (RP); E and F new root with cambial continuity, initiated from leaf gap; and G and H position of bud with starch deposits extending into pith of cutting.

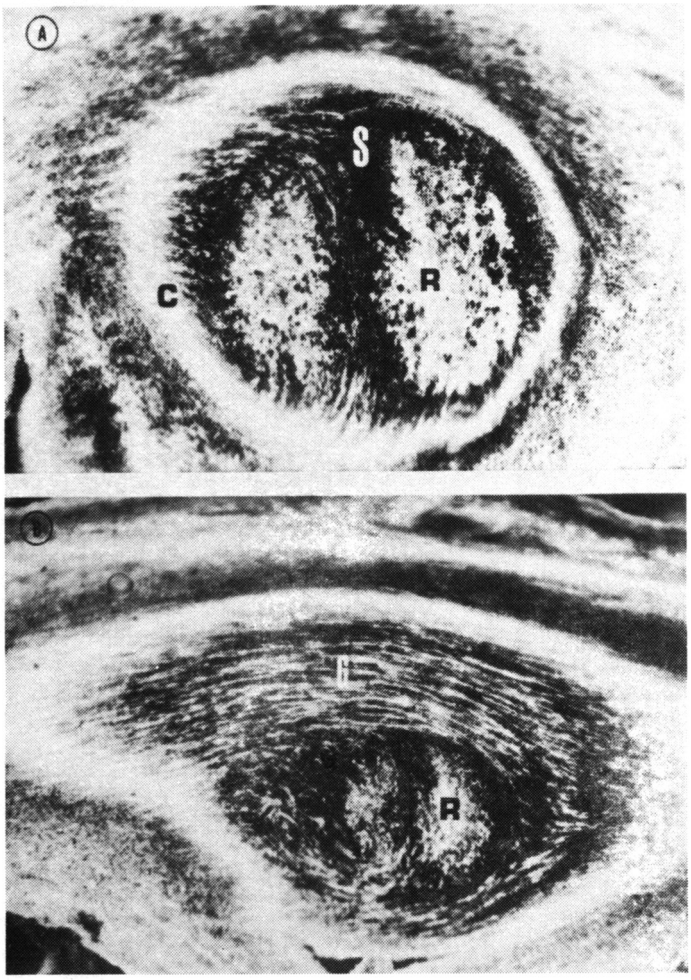

Fig. 5. Tangential sections through node of M 2 stem showing: A. Two roots (R) surrounded by cambium (C) dark and starch-rich (S) area; B. Gap area (G) and roots (R) near point of initiation.

In M 2, roots emerged from the gap area at right angles to the stem, in very close proximity to a lateral bud. The sequential illustration of stem sections depicts roots growing from the gap area slightly below the bud (Fig. 4, C to H). The two lateral traces and gaps eventually gave way to a wider central gap from which the root emerged (Fig. 4C & F). The thin cambial and phloem zones became continuous with the root at the point of initiation.

The actual point of initiation appeared to be near the cambial zone, with vascular connections leading into xylem tissue (Fig. 3B & 4F). The dark starch-filled cells of the gap were continuous with the starch-rich outer ring of the pith. The fact that nearly all the roots in the layered shoots emerged from near this point of starch deposition provides further evidence that the available starch reserves may provide an energy source for root initiation and development. The bud area also provided a particularly attractive point for emergence, because no external constricting fibers or cortical tissues were present (Fig. 3A). The production of other etiolation-induced biochemical factors by the bud are not ruled out by this study.

In tangential sections of a M 2 rooted shoot, the point of root origin was in the bud gap area. The cambial layer extended around the roots in the external section (Fig. 5A & B). The inner root tissues did not stain for starch, although starch-filled parenchyma cells were present in the xylem area of the gap. How the vascular elements of the new root established continuity with the vascular system of the stem could not be observed in microscopic tissue sections.

Discussion

Internal starch level among rootstock clones, which is presumably genetically-linked, was not correlated with relative rooting propensity. However, the localization and increase of starch in the stem seemed to favor positional root initiation. Roots were produced almost exclusively in association with the starch-rich nodal gap area of the stem. The heavy starch deposition in the non-differentiated parenchyma tissue in the leaf gap and pith areas may have provided optimum conditions for root initiation and development at this site.

The layering process and associated stem etiolation caused a significant increase in starch levels and some decrease in stem sclerification and tissue differentiation. Sclerification was negatively correlated with rooting propensity. Mounding of the clones appeared to provide environmental conditions favorable to root emergence. No doubt biochemical changes also are linked with etiolation. These might include: hormone level adjustments, production of rooting co-factors, or non-production of rooting inhibitors. This change could be influenced by the presence of the lateral bud at each node. The rapid production of roots by etiolated cuttings suggests that etiolation-induced root primordia were already present in the stems and emerged under mist conditions.

Future application of these results may provide improvements in the layering technique and more use of etiolation in rooting of difficult-to-root plants. Biochemical analyses of etiolated materials may provide further insight into the rooting process.

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Mid-summer Applications of Ethephon and Daminozide on Apples. I. Effect on 'McIntosh'[1]

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Abstract. **Applications of (2-chloroethyl)phosphonic acid (ethephon) on 'McIntosh' apple trees (*Malus domestica* Borkh.) in early August at concentrations of 125 to 500 ppm promoted the climacteric rise in respiration, increased soluble solids, fruit abscission, and red color, and also reduced flesh firmness. Results from ethephon applications in July were variable; in 1974 July applications were more effective, and in 1975 less effective, than treatments applied in early August. The effects of ethephon were reduced when 1000 ppm succinic acid-2,2-dimethylhydrazide (daminozide) was combined with the ethephon. No treatment influenced repeat bloom or set the year following application.**

A previous study (9) showed that 100 ppm and 200 ppm ethephon alone or in combination with 1000 ppm daminozide caused fruit abscission on 'Early McIntosh' apple trees when applied 30 days after full bloom. When sprayed shortly after completion of June drop, no abscission occurred and flower bud initiation was increased. It was apparent, however, that further trials were necessary before the feasibility of ethephon and daminozide sprays for flower bud initiation on bearing apple trees could be established. Here we report the effects of post June-drop applications of ethephon and daminozide on bearing 'McIntosh' apple trees.

Materials and Methods

Two blocks of bearing 'McIntosh' apple trees planted in 1964 at the Horticultural Research Center in Belchertown, Mass. were selected for this study. Different trees were used each year. All treatments were made as dilute applications and applied to run-off on whole trees with a high pressure sprayer.

1974. Ethephon at 250, 500, or 1000 ppm was applied on July 22, and to other trees at 75, 125, or 250 ppm on August 7. Each treatment was replicated 5 times in a randomized block design. On August 28 fruit samples were harvested at random from the periphery of each tree. Ten apples from each tree were tested for flesh firmness with a Magness-Taylor pressure tester (11.1 mm head) and for soluble solids content using a hand refractometer. Red color was estimated visually to the nearest 10% on 15 fruit per tree. Fruit diameter was measured on 25 fruit per tree. Drop was recorded at 3- to 6-day intervals from July 22 to Sept. 11, and drop on each date is presented as the percentage of the total crop on each tree.

1975. Ethephon alone at 125 or 250 ppm and ethephon at 125, 250, or 500 ppm combined with 1000 ppm daminozide was applied on July 17 or August 11. Each treatment was replicated 7 times in a randomized block design. Forty fruit on 2 representative limbs of each tree were tagged prior to treatment. Drop of these tagged fruit was recorded at 3- to 4-day intervals and the percent drop calculated for each date. Fruit diameter measurements were made prior to treatment and again on August 26 and 30 tagged fruit per tree. A 1½ box fruit sample was harvested at random from the periphery of each tree on August 25 and again, for the July treatments, on Sept. 3. One box of fruit from each tree was stored at 0°C in air for 20 weeks. Flesh firmness and soluble solids were determined as in 1974. Red color was estimated to the nearest 10% on 25 fruit per tree. Respiration was determined as CO_2 evolution from 1 kg of fruit measured daily for up to 11 days at 21°, using an MSA Model 200 infrared gas analyzer to monitor constant-flow (10 liters/min) effluent air. Gas samples for ethylene determination were obtained by collecting internal gas samples under water with a syringe. Gas samples from 3 fruit were combined and the ethylene in a 1-ml sample was determined on a Varian Aerograph 2700 gas chromatograph equipped with a flame ionization detector using an activated alumina column.

Samples were removed from storage in Jan. and 10 fruit from each box were tested for flesh firmness. Fruit were examined for storage disorders after remaining for 7 days at 21°C.

The influence of treatments on flower bud initiation was determined by measuring limb circumference and counting blossom clusers on 2 tagged limbs per tree. Fruit set on tagged limbs was determined in July the year following treatment.

Results

Red color development. All midsummer applications of ethephon and daminozide increased red color, except for 125 ppm ethephon alone applied in July, 1975 (Tables 1 and 2). The addition of daminozide to the ethephon treatments in 1975 did not further increase red color development. We observed that the July, 1974, treatments markedly increased red color within 7 to 10 days after their application, whereas the July, 1975, treatments had minimal influence on color until mid-August when weather was conducive for red color formation. The

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