Table 2. Average length of vegetative shoots in 1969 on 'Delicious' limb units and 'Golden Delicious' trees treated with Ethrel in fall of 1968.

	Concn of Ethrel applied (ppm)	Length of vegetative shoots (cm)		
		Date applied		
		9-20-68	9-30-68	10-16-68
Delicious	0		32a[1]	33a
	250		15b	17b
	500		11b	13b
	1000		7c	7c
Golden Delicious	0	44a[1]		
	250	46a		
	500	33b		
	1000	21c		

[1]Unlike letters in the same column indicate significant difference at the 5% level.

similar to theirs but more pronounced, and may have been intensified by the cold temperature in the fall and winter of 1968, and different age trees.

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Ethylene Levels in Tomato Fruits Following Treatment with Ethrel[1]

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Abstract. **Internal ethylene concentrations in green tomato fruits rose to a maximum of 30 to 70 ppm within 2 to 5 hours of dipping in a 10,000 ppm solution of Ethrel (2-chloroethylphosphonic acid), then fell to approximately 5 ppm after 24 hours. A second rise in ethylene concentration was associated with fruit ripening, and was observed both in treated fruits and in untreated fruits which ripened as a result of removal from the plant. Incubation of Ethrel with homogenates of green tomato fruits likewise resulted in evolution of ethylene. Autoclaving or boiling the homogenates did not affect activity, indicating that release of ethylene from the chemical was non-enzymatic.**

Recent experiments by ourselves and others (6,8,9) have shown that 2-chloroethylphosphonic acid (Ethrel) stimulates ripening of banana and tomato fruits. The similar effects of this chemical and of ethylene, and the production of ethylene by Ethrel-treated pea stem sections, led Warner and Leopold (10) to conclude that Ethrel promoted ripening by stimulating ethylene production. Cooke and Randall (3) subsequently reported that Ethrel induced flowering in pineapple, and they attributed activity to non-enzymatic release of ethylene from the chemical itself. Hartmann, et al. (5) noted the evolution of ethylene from Ethrel-treated olive leaves, and Edgerton and Blanpied (4) and Warner and Leopold (11) have reported the gradual release of ethylene from aqueous solutions of Ethrel when the pH is raised to 4.9 and above. Our investigations were designed to determine the effects of Ethrel upon ethylene levels in treated tomato fruits, and to assess the role of fruit enzymes in the release of ethylene.

Experiments with intact fruits. Six plants of a tomato breeding line closely related to the 'Fireball' cultivar were grown in a greenhouse until the fruits on the second cluster were 4 to 6 cm in diameter. The plants were then transferred to the laboratory (23 C) where the fruits were treated and analyzed for ethylene. One green fruit was removed from each of the first 2 clusters on each plant, leaving a similar fruit attached. One of the 2 detached fruits and 1 of the 2 attached fruits from each plant were then held for 2 min in a 10,000 ppm solution of Ethrel (Amchem formulation 66-329), containing 0.1% Tween 20 as a surfactant. Detached fruits were left in the laboratory during the course of the experiment, while the plants with attached fruits were returned to the greenhouse after the first 24 hours, and moved to the laboratory only during sampling.

Fruits of the 'Fireball' cultivar were used in a second experiment. Mature green fruits were harvested from vines growing in a field plot at Geneva, and were taken directly to the laboratory for treatment and ethylene analysis. Ethrel was again used at 10,000 ppm.

Internal ethylene concentrations were determined as described by Burg and Burg (1), with minor modifications. Gas samples (0.2 to 1.0 cc per fruit) were withdrawn from the interior of the fruits with a gas-tight syringe while holding the fruits under water, and were chromatographed on a Beckman GC-5, using a 2-foot column of aluminum oxide at 50 C. Helium was used as carrier gas at a flow rate of 20 cc per min, and ethylene was detected with a flame ionization detector. With this system, the retention time of ethylene was 1.12 min, and less than 0.1 ppm was detectable in a 1 cc sample of gas. Reagent grade ethylene was used as a standard by preparing appropriate dilutions with air and plotting log peak height vs. log concentration. Before concluding that an observed peak was ethylene, gas samples were tested by injection into bromine water, which absorbs ethylene, and into 0.1 N NaOH, which does not (1).

Internal concentrations of ethylene in control and treated fruits (experiment 1) are graphed in Figure 1A. Ethylene levels did not exceed 3 ppm in untreated fruits during the first 5 days, while the levels in treated fruits

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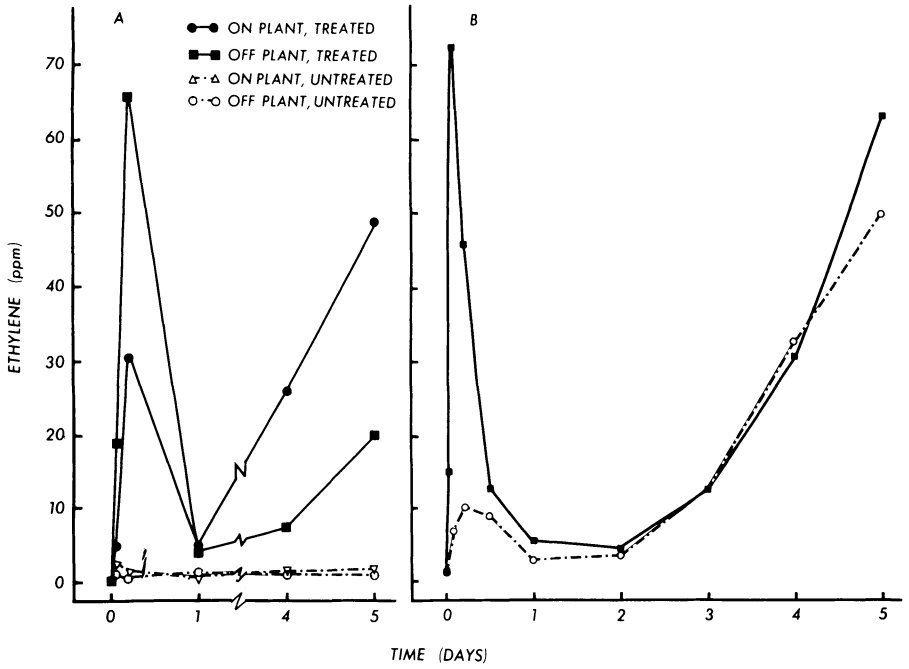

Fig. 1. Effect of Ethrel (10,000 ppm) upon ethylene levels in tomato fruits. A - intact and excised fruits grown in a greenhouse; B - excised fruits grown in the field to the mature green stage. Treated fruits dipped in aqueous solution of Ethrel at 0 time.

rose shortly after treatment, reaching maxima of 30 ppm for attached fruits and 65 ppm for detached fruits after 5 hr. The concentration of ethylene then fell, reaching a level only slightly above that in untreated fruits after 24 hr. After 4 days, treated fruits showed a secondary rise in ethylene, associated with onset of ripening. Eight days after treatment Hunter Color Difference Meter values (a/b) for attached fruits were + 0.87 for treated fruits vs. - 0.54 for untreated fruits; for detached fruits + 0.91 (treated) vs. - 0.67 (untreated). The initial rise and fall in ethylene levels in treated fruits supports the view that the chemical itself is the source of ethylene (3, 11). If the increased release of ethylene were due solely to a stimulation of ethylene biosynthesis, one might expect the level of ethylene to remain high, rather than to decline after 24 hours.

The 66-329 formulation of Ethrel contains not only 2-chloroethylphosphonic acid, but also the anhydride and the mono-2-chloroethyl ester of the acid. The second rise in ethylene production could possibly have been the result of a more gradual enzymatic conversion of the ester to the acid, with subsequent release of ethylene, as suggested by Cooke and Randall (3). This is not likely, however, for in the second experiment untreated fruits began producing large quantities of ethylene 3 days after harvest, and the rate of production was indistinguishable from that occurring in treated fruits at this time (Fig. 1B). Both treated and untreated fruits showed similar patterns of lycopene synthesis and rate of softening (data not shown). Therefore, the second rise in ethylene production is associated with the natural process of fruit ripening, while the first is a result of treatment with Ethrel.

Burg and Burg (2) have estimated that the internal level of ethylene necessary to induce fruit ripening lies between 0.04 and 0.5 ppm. Although our experiments were not designed to establish the threshold, the data suggest that higher levels, on the order of 5 ppm, may be necessary for ripening of tomatoes to occur. When control fruits contained 3 ppm ethylene, ripening did not occur (Fig. 1A); when they contained 10 ppm, ripening ensued (Fig. 1B).

Experiments with homogenates. To determine whether enzymes played a role in the release of ethylene from Ethrel, green tomatoes were homogenized in distilled water in a Waring blendor, and portions of the homogenates were boiled or autoclaved to inactivate enzymes. Autoclaved samples remained sterile during the course of the experiment. Twenty cc of homogenate were added to 50 cc Erlenmeyer flasks, the flasks were closed with rubber septa, 1 cc aliquots of Ethrel were injected into designated flasks to give a final concentration of 500 ppm, and gas samples were removed at intervals and analyzed for ethylene as above.

Ethylene levels in the air space above homogenates incubated with and without Ethrel are presented in Table 1. The results indicate that boiling or autoclaving does not reduce the capacity of the homogenate to evolve ethylene when Ethrel is added. Furthermore, a considerable amount of ethylene is evolved when Ethrel is injected into a buffer solution approaching the pH of the homogenate. However, the fact that greater quantities of ethylene are evolved from homogenates than from a buffer solution at a similar pH suggests that chemical constituents in the homogenates may interact with Ethrel to increase the rate of conversion to ethylene. The small quantities of ethylene produced by the homogenates in the absence of Ethrel are insufficient to account for the difference observed. The low activity of non-autoclaved tomato fruit homogenates in evolving

Table 1. Ethylene evolution by tomato fruit homogenates.

Preparation[1]	Ethrel (66-329) 500 ppm	pH of mixture	Total C_2H_4 evolved (μl x 10^{-3})
Experiment 1			
Water	+	3.0	11.4
Homogenate	–	4.3	3.8
"	+	4.1	380.0
Boiled homogenate	–	4.4	3.8
" "	+	4.2	388.0
Experiment 2			
Buffer	+	3.7	445.0
Homogenate	–	3.9	15.2
"	+	3.8	1090.0
Autoclaved homogenate	–	4.2	80.0
" "	+	4.1	1380.0

[1]Experiment 1 - 11.4 gm tissue per 20 cc homogenate; 12 hr. time; 2 replicates.
Experiment 2 - 15.1 gm tissue per 20 cc homogenate; 22 hr. time; 3 replicates.

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ethylene, even when appropiately buffered, has been previously reported (7).

Treatment of tomato fruits with Ethrel results in a rapid rise in internal ethylene to a point far above the threshold for stimulating fruit ripening. Although the level of ethylene subsequently drops, it remains above the threshold for autostimulation of ethylene biosynthesis and fruit ripening. The data obtained from experiments with homogenates support the conjecture (3) that non-enzymatic release of ethylene from 2-chloroethylphosphonic acid is the basis of the biological activity of this chemical.

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Storage Response of Green and Bleached Lima Beans *(Phaseolus lunatus L.)*[1]

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Abstract. **Green and bleached beans designated as high and low vigor lots were stored at 21C and 50, 70, and 90% relative humidity. At the end of 36 months, no significant loss in viability occurred in either lot at 50% relative humidity. No significant loss of viability was found in the high vigor lot stored at 70% relative humidity but the low vigor lot lost all viability. Both lots were worthless for planting purposes when stored at 90% relative humidity for 3 months.**

Differences in seed longevity among species and between cultivars in the same species in storage have been shown (1, 2). Initial vigor of seeds in these studies was not determined but may have affected their responses to storage conditions.

Lima bean seedling establishment has been shown to be associated with vigor. Horticulturists designate bleached lima bean seeds as lacking in vigor, as compared to green or unbleached seeds (6). Lack of vigor in bleached seeds is most apparent when imbibition temperatures are below 15C (3, 4). Differences among varieties in their response to imbibition temperatures have been demonstrated (5). The possibility that the vigor classification of lima beans would also affect their storability led to this investigation.

Two lots of 'Thorogreen' lima bean seeds were provided by Ben Fish and Son Seed Company, Santa Barbara, California. Seeds of Lot No. L366 were harvested in the fall of 1964. These were green and were designated as a high vigor lot. Seeds of Lot No. L199, harvested in the fall of 1962, were white-seeded and had a germination of 93%. Until sent to the National Seed Storage Laboratory, they were stored in a warehouse at Crows Landing near Modesto, California whose climate is characterized by high summer temperatures and very low relative humidities and cool winters with occasional periods of high humidity. Lot L199 was designated by the company as having low vigor. Both lots were received by the National Seed Storage Laboratory in June 1965 and were immediately placed in storage. Samples of both lots were stored at approximately 21C at three different relative humidities, 50, 70, and 90%. High and low vigor will be used herein to identify the green and bleached seeds, respectively.

Germination tests consisted of planting four 50-seed replicates on rolled germination toweling moistened with tap water. The planted seeds were held at a 20 (15 hours) to 30C (9 hours) daily temperature alternation. The seedlings were evaluated on the 9th day. Only normal seedlings, those capable of producing normal plants under favorable conditions, are included in the germination data.

Seeds from the 90% relative humidity (RH) storage were removed from storage and tested for viability at 2 and 6 months. All other tests were at 3-month intervals, except that the first test at 21C and 50% RH was after the first 6 months of storage.

The germination percentages as affected by the different storage conditions are presented graphically in Fig. 1. No loss in viability occurred in the high vigor lot at 50% RH. A regression coefficient of .0079 for the low vigor lot indicates that the 11% difference between the first and final test of the low vigor lot is not significant. In fact, two lots of the initial test and two of the final had germinations of 72%.

Differences in storability are apparent in the other two regimes. A loss of only 5% viability occurred in the high vigor lot at the end of 18 months when stored at 21C and 70% RH. The low vigor lot was worthless for planting purposes during this same period. The loss in viability was equally rapid for both lots at 21C and 90% RH. Green-seeded limas may retain near

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