to genetic background effects. A survey of 62 tomato lines from divergent sources revealed 47 lines with relative malate concentrations between 0.4 and 1.0, 12 lines with concentrations between 1.2 and 2.0, and 3 lines with concentrations between 2.4 and 3.6. The distribution of malate concentration among these lines strengthens the concept of simple inheritance with dominance for low concentration, and indicates that more than 2 alleles are involved in malate inheritance. The lack of significant correlation ($r=0.12$) between citrate and malate concentrations among the 62 lines may strengthen the theory of independent inheritance of the 2 compounds. Because of the possibility that the genes were in the coupling phase in some lines and in repulsion in others, this evidence must be accepted with reservation.

Because tomato acidity results from mixtures of organic acids, a finding of complex inheritance might be expected from titratable acidity or pH studies unless variation is confined to 1 compound or controlled by a major factor with pleiotropic effects. As already noted, pleiotropy does not appear to occur in citrate and malate inheritance. Our results can be reconciled with Walkof and Hyde's findings (11) by assuming that the acidity variation studied was caused by a single compound other than malate, or by a malate allele not observed in this study. Davies (5) reported that citrate and malate account for most intercultivar acidic variation and that there are minimal differences in concentrations of the other acids among cultivars. Our findings agree with those reported by Davies. Galacturonate and pyrrolidonecarboxylate were present only in very small quantities in our samples, and the other organic acids were not detected.

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**Effect of Temperature on Development of Premature Ripening in 'Bartlett' Pears**

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Abstract. Premature ripening, a physiological disorder of 'Bartlett' pears, was induced experimentally by use of temperature controlled limb cages. Exposure to 65° day and 45° F night temperatures for 3-31 days prior to harvest caused an early acceleration in ethylene production and occurrence of the climacteric rise in respiration. These changes were accompanied by fruit softening, increases in soluble pectin and protein N, a more rapid decline in malic acid as well as a decrease in the rate of citric acid accumulation. Treatments not develop in fruit maintained at 75° day and 60° night temperatures during the experiment.

In 1954 'Bartlett' pears grown at higher elevations in the Mid-Columbia districts of Oregon and Washington tended to ripen on the trees and drop to the ground prior to commercial harvest. Losses varied from approximately 10 to 100 per cent, according to orchard and site. The disorder occurred during 7-8 subsequent seasons in varying degrees of severity. In 1968, this condition developed in other 'Bartlett' pear growing regions of Washington and Oregon as well as in California, with an estimated loss of 5 million dollars (13). Since no disease or insect has been found to be associated with this abnormal development, it is assumed to be of a physiological nature and has been referred to as "premature ripening".

The disorder has several progressive stages of development.

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1. Received for publication June 19, 1970. Technical paper No. 2906. Oregon Agricultural Experiment Station.
2. Mid-Columbia Experiment Station, Hood River, Oregon. This study was supported by U.S.D.A. Cooperative Agreement No. 12-14-100-9002 (51). Washington State Fruit Commission, Oregon Bartlett Pear Commission and the Hood River Traffic Association.

ripening processes. Effects of Alar and gibberellic acid treatments were also studied in relation to preventing or retarding development of the disorder.

Materials and Methods

Temperature control. Cooling and warming fruit on the tree during the course of the experiment was accomplished by use of temperature controlled limb cages constructed of Mylar plastic film on removable wooden frames, in which were mounted thermostatically controlled refrigeration or heating equipment. Other cages were equipped with blowers only for continuous circulation of ambient air. Temperatures were recorded in all cages.

The cages were installed July 25, 1969, one month prior to estimated harvest date, in an orchard in the upper Hood River valley, Oregon, where premature ripening had occurred during several previous seasons. To provide sufficient fruit for the experiment by use of composite samples, 6 cooled, 6 heated and 4 ambient cages were installed. Temperatures in the cooled cages were maintained at 65° F during the day (6 a.m. - 6 p.m.) and at 45° during the night. Heated cages were maintained at 60° at night and at 75° day temperature. Mean temperatures of the ambient air during the experiment were 68° day and 53° night.

Growth regulator treatments. In the cooled cages only, 8-10 fruits were dipped in gibberellic acid (GA3), 100 ppm, for 10 seconds. Two treatments were made, one at the beginning of the experiment and the second 9 days later, when fruits were similarly treated with Alar, 1000 ppm.

Sampling. Samples were collected at 3-day intervals for determination of firmness, soluble pectin, protein N and organic acids, and at 3, 12, 23 and 31 days after start of the experiment for determination of respiration rates and ethylene production. Composite samples of 8 pears were prepared by taking fruit from all cages of each treatment.

Analytical methods. Determination of firmness and preparation of samples for analyses have been described previously (15). Soluble pectin was determined according to Carre' and Haynes (3) and protein N by the Lowry et al. procedure (17). Malic and citric acids were separated by partition column chromatography (14) and quantitatively determined with a Waters organic acid analyzer.

Fruits for measurement of CO2 and ethylene production were placed in 2-gal plastic pails fitted with inlet-outlet tubes and air-tight lids. Air flow was maintained at 200 ml/min and temperature at 70° F. Rates of respiration were determined twice daily with a Beckman infrared CO2 analyzer. A 1-ml air sample was collected in a gas sampling syringe from the effluent of the respiration chambers and ethylene determined with a Varian Model 1200 flame ionization gas chromatograph, using a 5-ft Porapak Q column.

Results and Discussion

Respiration of fruits from all treatments picked 3 days after the start of the experiment showed similar declining rates for the first 6-7 days following picking (Fig. 1). Between the 12th and 17th days, however, the respiration rate of the previously cooled fruit approximately doubled. The rates of all other fruits, including those of the cooled sample treated with GA, continued at the low level. Ethylene production was below the detectable limit (.075 µl/kgm-hr) in all treatments for 14 days. Subsequently, an increase occurred only in the cooled sample (Fig. 1).

The second samples were picked 12 days after start of the experiment. The cooled fruit developed the climacteric rise in respiration beginning on about the 4th day after picking (Fig. 2). All other samples maintained a low steady rate of respiration. Ethylene evolution was detected analytically only in the cooled fruit. In this sample, production increased to 90 µl/kgm-hr between the 4th and 11th days, then declined rapidly.

After 23 days' treatment, the climacteric rise in the cooled fruit developed immediately after picking and reached the peak on the 6th day (Fig. 3). Respiration rates of the other samples, however, remained at a low level for 4-5 days following picking, and did not increase to the climacteric peak until the 10th and 11th days. Ethylene production of the cooled fruit was 1 µl/kgm-hr on the first day following picking, increased to a peak value of 86 µl/kgm-hr on the 6th day, then declined. In all other treatments, detectable increases not only began later but peak values were considerably lower than those in the previously cooled fruit.

Fig. 3. Effect of temperature, GA and Alar on attached 'Bartlett' pears, during enclosure in limb cages for 23 days, on respiration and ethylene production after picking.

By the end of 31 days, pears in the cooled cages had developed the typical symptoms of premature ripening and many had ripened and dropped. Respiration of the cooled fruit was near the climacteric peak on the 1st day following picking (Fig. 4). Respiration rates of all other samples remained low for 3-4 days then increased to the climacteric maxima. The climacteric peak occurred 10 days later in the heated than in the cooled sample and 4 days later than in the other samples. Ethylene production of the cooled fruit was approximately at the peak on the 1st day after picking. In the other samples, no emanation could be detected until after 2-4 days, and the peak values reached were much lower.

Throughout the 31-day period of cool treatment, the changes in soluble pectin, firmness, protein N and organic acids, determined at 3-day intervals, were comparable to those normally associated with postharvest ripening (8, 11). Soluble pectin increased slowly for the first 18 days, then approximately doubled in concentration from 90 to 180 mg/100 gm fresh weight during the following 16 days, while in the heated fruit the increase was from 80 to only 90 mg in the same period of time (Fig. 5). Increases in the other treatments were intermediate between these 2 extremes. These changes in soluble pectin were accompanied by decreases in firmness, the cooled fruit decreasing in pressure test from 22 to 12.5 lbs and the other samples from 22 to 18.5-17 lbs.

Protein N in all lots showed comparable decreasing trends during the first 21 days (Fig. 6). Subsequently, there was a sharp increase in the cooled fruit, while the decline continued in all other samples.

Changes in citric and malic acids according to treatment were observed as early as 3 days following installation of the limb cages (Fig. 7). In the cooled fruit there was the least change in citric acid, which increased from 4 to 46 mg/100 gm fresh weight, while in the heated fruit, the citric acid content increased to 154 mg during the 31-day period. In all other treatments increases were intermediate between the values for the cooled and heated fruits. Malic acid decreased steadily in all samples, being most rapid in the cooled and slowest in the heated pears.

These and the other data show that exposure of 'Bartlett' pears to cool temperature treatment beginning one month prior to harvest brought about an early development and acceleration of the biochemical and physiological changes normally associated with maturation and ripening. As a result, premature ripening occurred on the tree prior to anticipated time of normal harvest. The early increase in ethylene production and occurrence of the climacteric rise in respiration showed that ripening was initiated in the fruit on the tree following exposure to cool temperature.

Treatments with GA and Alar tended to counteract the accelerating effect of cool temperature on maturation and ripening. This was shown by the retarding effect on the rate of changes in citric and malic acids, soluble pectin, protein N, ethylene production and respiration. These effects were obtained by GA3 applied at start of the experiment as well as by GA3 and Alar treatment 9 days later. Although GA has been reported to hasten the softening of Bartlett pears (9), Scott and Leopold (22) have shown gibberellin and ethylene to have opposing effects on ripening, and Dostal and Leopold (5), Abdel-Gawad and Romani (1) and Russo et al (21) demonstrated that this growth regulator delayed ripening in tomatoes, apricots and bananas. That Alar influences maturity (4), delays ripening (16,10) and retards preharvest drop (2,6,7) in apples and pears has been reported also.
Although the critical cool temperature or length of exposure required for initiating the changes leading to premature ripening were not determined, the conditions are probably less severe than those used in this experiment. This is indicated by the observation that definite changes in metabolism developed after only 3 days' treatment. Also, previous reports indicate that the quality of 'Bartlett' pears can be adversely affected by exposure to cool temperatures during development and maturation, even though premature ripening does not develop. Thus, Magness (18, 19) observed that fruit grown in the cool coastal regions of California ripened faster after harvest, remained in prime eating condition for a shorter period of time and had more tendency for core breakdown than pears from the warm interior valleys. Similar observations were made by Putterill (20) in South Africa, who also reported low acid content in 'Bartlett' pears to be correlated with low mean daily temperatures. The condition of the fruit reported by these authors is indicative of advanced maturity and suggests that quality and storage life can be affected by cool temperatures not sufficiently severe to result in premature ripening.

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Attempting Chemical Induction of Haploidy Using Toluidine Blue¹

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Abstract. Earlier references reported that toluidine blue would prevent the division of the generative nucleus in developing pollen tubes. These references suggested that a concomitant event might be stimulation of the embryo without fertilization resulting in the practical production of haploid seed. A test of this premise in tomatoes and corn is described herein. Haploids were not produced in excess of spontaneous levels. The probable cause is that fertilization of the diploid fusion nucleus does not occur. Thus there is no endosperm to provide a food supply for the embryo.

Chemical induction of haploidy has been reported in frogs (1) and mice (6) from treatment of the male sperm with the vital dye, toluidine blue. The dye appeared to inactivate the sperm nucleus without disrupting other functions of the cell. Treated sperm apparently stimulated the egg to develop without a genetic contribution to the developing embryo. Toluidine blue prevented the division of the generative nucleus of Vinca rosea (11) and Tradescantia paludosa (7) in pollen cultured in vitro. The purpose of this study was to investigate the potential use of toluidine blue for inducing haploidy in species having binucleate pollen, such as Lycopersicon esculentum L., and in species having trinucleate pollen, as Zea mays L. Both bi- and trinucleate pollen was used in the event that the presence or absence of sperm nuclei before addition of chemicals would effect the stimulation in the egg sac. The frequencies of dye-induced haploidy could be compared with the natural frequencies reported for the two species.

Materials and Methods

Tomato. A tomato line homozygous for green stem (aa) and heterozygous for cut leaf (Cc) constituted the female parent. The ‘Marglobe’ (AA, CC) was used as a pollen source. Pollen from several freshly opened flowers was collected on a depression slide, moistened with a drop of the aqueous solution from several freshly opened flowers was collected on a

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1 Received for publication June 26, 1970. Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 522.
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