AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF 2,4-D ON THE RESPIRATION RATE AND ETHYLENE PRODUCTION OF BARTLETT PEARS AND RED DELICIOUS APPLES

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After nearly 20 years of commercial use in orchards without apparent tree injury in Oregon and Washington, 2,4-D [(2,4-dichlorophenoxy)acetic acid] was reported in 1970 to cause injury to apple and pear trees in Washington. In the early 1970's, there was widespread concern among growers, researchers, and processors in the Hood River Valley of Oregon that 2,4-D might cause premature ripening of pears. Studies were conducted in 1971 to: (1) document the disappearance of 2,4-D from orchard soils; (2) analyze for 2,4-D residues in developing apple and pear fruits throughout the season; and (3) determine if 2,4-D, when properly applied to Red Delicious apple and Bartlett pear orchards, alters the ripening process of mature fruit.

Applications of 2,4-D were made at 0, 2.24, and 4.48 kg/ha. Experimental sites were in apple and pear orchards in the upper and

lower Hood River Valley near Hood River, Oregon. Soil samples were collected at 5-cm increments to a depth of 30 cm at 0, 14, 21, and 28 days following treatment. Fruit was harvested weekly from the juvenile through the mature growth stages and analyzed for possible 2,4-D residues.

Each sample was hydrolyzed with NaOH to remove 2,4-D residues. The 2,4-D aliquot was then acidified and extracted with ethyl ether. Purification of the extract was accomplished by passing it through a basic aluminum oxide column. Following esterification with a 5-ml aliquot of 11% BF_3 methanol, the methyl ester of 2,4-D was partitioned into 1 ml benzene and analyzed on a Varian 1200 gas chromatograph equipped with a microcoulometric detector.

Ethylene and CO_2 samples were collected from respiring apples and pears in a continuous air flow respiration chamber to determine if 2,4-D would cause subtle physiological or biochemical changes in the ripening process. CO_2 evolution was monitored with a Beckman infrared CO_2 analyzer. Samples for ethylene analysis were withdrawn in hypodermic syringes from the exhaust tube and analyzed in an Aerograph Model 600-D ionizing gas chromatograph.

The degradation of 2,4-D from soil samples was rapid in these experiments. Approximately 98% of it was lost after 21 days. At 28 days, no detectable levels of 2,4-D (sensitivity 0.02 ppm) were found in any samples. The 5-10 cm zone was the maximum depth at which 2,4-D was detected in the soil profile. No detectable 2,4-D residues were found in any of the apple and pear fruit samples.

When orchard location (upper or lower Hood River Valley), gas

sampling time, and application rate were statistically analyzed using a three-way factorial analysis, no significant differences ($P = .05$) were found to exist as a result of treatment level in the apple or pear orchards.

The Effect of 2,4-D on the Respiration Rate and Ethylene Production of Bartlett Pears and Red Delicious Apples

by

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INTRODUCTION

Since its introduction in the early 1940's (59), 2,4-D [(2,4-dichlorophenoxy) acetic acid] has proven to be an efficient and economical means of increasing yields of certain crops by reducing dicotyledonous weed populations, hence conserving moisture and nutrients and reducing competition. When 2,4-D was first introduced, many phytotoxic problems to non-target species occurred. The majority of the problems were related to formulation, weather conditions, and stage of growth when applied. Today, formulation of dusts or highly volatile esters of 2,4-D is rare and proper timing of application has been thoroughly investigated on a variety of crops.

The use of 2,4-D for control of broadleaf weeds in apple and pear orchards had caused no apparent injury in Oregon and Washington for nearly 20 years since it was first recommended in the early 1950's. However, in 1970, Benson (7) stated that severe 2,4-D injury had occurred to apple and pear trees in Washington. He described the disadvantages of using 2,4-D in apple and pear orchards and concluded that "2,4-D may be more hazardous than we thought a year or two ago, but we can still use it if certain precautions are made." Subsequent to his presentation, more concern about premature ripening of Bartlett pears was evident among orchardists in

the Hood River Valley and personnel at the Mid-Columbia Experiment Station near Hood River, Oregon. The implication was that some of the problems could be caused by 2,4-D applications.

Other information also was considered in the controversy over possible hazards from 2,4-D usage in orchards. Postharvest physiologists universally employ the phenomenon of climacteric rise in respiration, as well as a rise in ethylene production which precedes this rise in respiration, to recognize the initiation of ripening in climacteric fruits such as apples and pears (31, 39, 52). Hansen (38) experimentally demonstrated that dipping pears in 2,4-D hastened ethylene production. Other researchers (13, 16, 64) have reported that 2,4-D can increase ethylene production in a number of crops. The question was raised whether 2,4-D used for weed control could stimulate ethylene production and cause premature ripening.

The conclusions of Benson and the reported effect of 2,4-D on ethylene production provided the stimulus for the research reported in this thesis. The objectives were: (1) to document the disappearance of 2,4-D from orchard soils, (2) to analyze for 2,4-D residues in developing apple and pear fruits throughout the season, and (3) to determine if 2,4-D when properly applied to Red Delicious apples and Bartlett pear orchards, alters the ripening process of mature fruit.

LITERATURE REVIEW

Postharvest physiologists have studied respiration, ethylene production, and other ripening indices related to fruit ripening intensively. Of the ripening indices, ethylene production and the associated rise in respiration have received the major emphasis. Volumes of literature on these phenomena have appeared in journals and books. Excellent comprehensive reviews of ethylene and its related morphological, physiological, and biochemical processes in plants have been reported by Abeles (1), Burg (12), and Pratt and Goeschl (71).

According to Burg (12), Girardin reported the defoliation of shade trees in several German cities in 1864. Further investigation revealed that gas leaking from main lines which supplied fuel for street illumination caused the trees to defoliate; but it was not until the turn of the century that Neljubov confirmed acetylene and ethylene as two active components of illuminating gas. He first observed the unique properties of illuminating gas when pea seedlings, germinating in a gas-illuminated greenhouse, produced horizontal growth instead of the customary vertical growth. In 1910, Cousins (20) concluded from observations made aboard ship that when oranges and bananas were stored in the same hold, the oranges caused the bananas to ripen. Miller et al. (62) discovered that a common green mold which grows on orange rinds produces a high rate of ethylene. Since oranges produce very little ethylene, it appears that in the case of oranges and bananas storage, fungi growing on the oranges probably caused the bananas to ripen.

Periodically through the years, researchers have reported the utilization of chemical analysis for quantitative measurement of ethylene. In 1933, a colorimetric assay with a sensitivity for ethylene detection at 5 ppm was reported by Tompkins (75). Through gravimetric analysis developed by Christenson et al. (19) and a potassium permanganate oxidation analysis developed by Nelson (65) in 1937, it was possible to detect production at levels of 100 ppm. In 1939, Hansen and Christenson (42) developed a microbromination technique for detecting ethylene at levels as low as 25 ppm. Later, the bromocoulometric method for ethylene determination was introduced by Nicksic and Rostenbach (68) with a sensitivity of 0.5 ppm. However, until the advent of gas chromatography, ethylene detection remained a cumbersome burden for researchers in plant physiology. Gas chromatography, a simple, accurate, and sensitive method for ethylene detection, was introduced by Turner (77) in 1943. Burg and Stalwijk (17) and Heulin and Kenneth (49) employed gas chromatography to detect levels of ethylene in apples as low as 10 ppm. Later that year, Meigh et al. (60) employed flame ionization to increase the sensitivity to 1 ppm. Certain refinements in techniques reported by Bellar (5) also made it possible with gas chromatography to detect ethylene at levels as low as 1 ppb. Essentially, all ethylene research today is done with the flame ionization detector. Other methods for ethylene analysis are now considered obsolete.

In 1924, Denny (24) discovered that ethylene increased respiration rates in apples. Kidd and West (53), also experimenting with apples, found that ethylene increased respiration in mature apples.

Similar findings have been made on a wide variety of other crops which include bananas (43) and soybeans (47). In 1932, Elmer (28) reported that volatile products from apples inhibited potato sprouts. Then in 1933, Huelin and Barker (48) found that ethylene affected the respiration rate of potatoes. A group of Boyce Thompson scientists (83) in the 1930's, reported and described most of the physiological effects of ethylene known today. It was not until 1934, however, that Gane (33) conclusively showed that ethylene was produced by plants. Ethylene research received the attention of many plant physiologists from the turn of the century until the mid-1930's.

A lack of interest in ethylene research during the late 1930's was primarily due to the difficulties of assessing the rate of ethylene production on a micro basis. Bioassays and cumbersome chemical techniques were the only means for detecting and measuring ethylene production. Secondly, with the advent of the new organic herbicide, 2,4-D, in the 1940's (59), study of its effects on physiological processes became the focus of attention for many plant physiologists, and ethylene research was initiated once again.

An increase in ethylene production effected by 2,4-D has been researched by a number of workers. Morgan and Hall (64) showed that 2,4-D was responsible for an increase in ethylene production in cotton and grain sorghum. Hansen (37), in the mid-1940's, demonstrated the same phenomenon in detached mature pears, and Kang et al. (50) reported that ethylene production remained high in pea seedlings that had been treated with 2,4-D.

Blampied (10), working with apple leaves and flowers during

development, found that ethylene production was the highest in dormant buds, then decreased as the leaves and flowers expanded. Ethylene concentration again increased during senescence and abscission of leaf and flower tissues. Blampied's research also found a marked increase in ethylene production during fruit maturation. Hansen (41) and Biale (9) confirmed Blampied's experiments.

The first researchers to suggest that ethylene might be an endogenous plant growth regulator were Crocker et al. (23). They concluded that ethylene played an intricate role in the initiation of fruit ripening. Then research by Hansen (41), Kidd and West (51), and McGlasson (58) supported the experimental findings of the Crocker team.

Several physiologists have found that environmental factors affect ethylene production. An optimum temperature of 30 C for ethylene production by apples has been reported by Burg and Thimann (18) and Hansen (39). Burg and Thimann showed that as temperatures were increased to 40 C, ethylene production of apples decreased to undetectable levels and remained there. Their work also showed that the temperature effect was reversible. Hansen (40) reported similar findings when studying effects of increased temperatures on ethylene production in pears. Wang et al. (79), working with Bartlett pears in 1971, found that premature ripening of pears was correlated with low temperatures during the growing season, and that orchards at higher elevations are more adversely affected. This group found a lag period in ethylene production of Bartlett pears picked from limb cages where ambient temperatures prevailed as compared to fruit from

cooled limb cages where temperatures were maintained at 18.3 C during the day and 7.2 C at night. They found that the time during the ripening process at which detectable amounts of ethylene could be measured occurred earlier for the more mature fruit and/or when cool temperatures were maintained in the limb cages. Fidler and North (31), experimenting with three different apple cultivars at 0, 3.3, 7.2, and 12 C, found that the initiation of ethylene production was not necessarily associated with the onset of the climacteric rise in respiration. Fidler and North also reported that ethylene production increased from 140 μl/10 kg/hr at 12 C for Cox's Orange Pippin apples. Their research with Golden Delicious apples yielded similar results. These scientists (30) concluded, however, that temperature regimes alone cannot be utilized to determine the rate of cellular respiration, since quantities of intercellular CO_2 vary directly with the rate of CO_2 production.

In 1929, Kidd and West (52) were the first research workers to report that the respiration rate of apples increased during ripening. They coined the term "climacteric" which has been universally accepted by physiologists to describe the transition of growth to senescence. They further demonstrated that the climacteric occurred either with the fruit attached to or detached from the tree. Fidler and North (29) reported that the respiration rate of apples varied under different temperature regimes. Kidd and West (52) experimentally demonstrated that the rate of respiration and storage life of fruit are both functions of temperature. Kidd and West also found that fruit with higher climacteric peaks have shorter storage life.

The literature review produced documented evidence that CO_2 may promote, inhibit, or remain neutral in ethylene production. Potter et al. (70) and Burg (18) found that concentrations of CO_2 as low as 10% inhibited ethylene production in apples. Abeles (2), Ben-Yehos-hua et al. (6), and Rasmussen et al. (72), reported that CO_2 did not alter ethylene production in beans or citrus. Burg and Burg (14), using etiolated pea stem sections, found that CO_2 was a competitive inhibitor of ethylene action and prevented higher concentrations of auxin from retarding elongation. They also suggested that CO_2 delays fruit ripening by displacing the ripening hormone, ethylene, from its receptor site. Nitrogen saturation of apple tissue, resulting in low oxygen concentrations, has been reported by Burg and Thimann (18) to inhibit ethylene production in apple tissue. The process was reversed when apple tissue was removed from the saturated nitrogen environment and placed in ambient air. Even though the ability of ethylene to increase climacteric fruit respiration is well known (73, 15), very little is understood about its mode of action (15).

Abeles stated that "tree factors" are unidentified compounds produced by the parent plant which control ripening. Wilkinson (82) was the first experimenter who suggested that a tree factor could alter the climacteric peak associated with respiration by keeping the fruit in a juvenile state. Meigh et al. (60) reported that ethylene production in attached apples was less than in harvested fruit, and suggested that an inhibitor of ethylene existed.

Grover et al. (34) conducted research on the drift of 2,4-D

formulations and found that 25% to 30% of the butyl ester had drifted off the target area as a vapor one-half hour after spraying. They reported that the dimethylamine salt was not lost from the target area during the same period of time.

Adsorption coefficient is the index employed to determine the leaching characteristics of a pesticide; the higher the K value, the more readily a pesticide is adsorbed and the less readily it is leached. Hamaker and Thompson (36) reported respective K_{oc} values of 12.8, 32, and 135 for chloramben, 2,4-D, and simazine. The herbicide 2,4-D is classified as a slightly mobile compound in the soil (36). Freed and Haque (32) have classified 2,4-D as strongly adsorbed to soil surfaces. Helling and Turner (46) evaluated a number of pesticides by employing thin-layer chromatography and found that Rf values could be used to rank the mobility of herbicides. They found the mobility rankings of chloramben, 2,4-D, and simazine, when using Rf values, to be comparable to mobility rankings using K_{oc} values (36).

Most leaching studies have been conducted in soil columns (8, 74, 78, 81). In 1975, Hamaker (35) reported that the soil column methods tend to overestimate the depth of penetration of chemicals because of rapid water flow and that the herbicide fraction which trails the peak also is overlooked.

Research by Audus (3) in 1964, indicated that 2,4-D generally will decompose within approximately 3 weeks in soil with no prior 2,4-D history. He found that a lag period in 2,4-D decomposition of approximately 17 days exists in such soils. Torstensson *et al.* (76)

recently reported findings in close agreement with the work of Audus. Hernandez et al. (45) reported complete inactivation of 2,4-D in soils after 4-5 weeks. Newman et al. (66) found that 10 mg of 2,4-D per pound of soil had been decomposed after 8 days.

The bark of woody plants presents a special obstacle to the penetration and absorption of 2,4-D. Crafts (27) states that "the suberized covering and the underlying cork cambium may present almost insurmountable barriers to penetration, particularly of polar compounds applied in aqueous solution."

Radiotracer research conducted by Crafts and Yamaguchi (22) demonstrated that 2,4-D is not very mobile acropetally in plants. Other scientists have reported that retention to the plant surface (55), solution in the cuticle (21), and physical adsorption to cytoplasmic proteins (11) may interfere with the absorption and translocation of 2,4-D.

Edgerton's (25) experiments showed that McIntosh apple leaves decarboxylated ^{14}C-labeled 2,4-D. However, when Edgerton et al. (26) substituted fluorine for chlorine in the 4-position on 2,4-D, decarboxylation of 2,4-D by McIntosh apple leaves was inhibited. Other research workers (4, 61) have confirmed Edgerton's research and also concluded that the 2,4-D is metabolized soon after entering the leaves. Edgerton et al. (25, 27) found that the more slowly decarboxylation occurred, the more sensitive apples were to 2,4-D. The research of Luckwill et al. (57) evidenced a rapid 2,4-D decarboxylation reaching 57% after 92 hr from leaves of 2,4-D-resistant Cox's Orange Pippin apples. During the same period, only 2% 2,4-D

decarboxylation occurred in Brambley's seedlings. Rates of decarboxylation have been reported for strawberry (57) and bean (80) leaf tissue. Damage to 8-year-old Bartlett pear trees was reported by Larsen (54) when irrigation followed the 2,4-D application with 24 hr.

For prevention of preharvest fruit drop in 'Pineapple' oranges, Phillips and Meagner (69) and Hield et al. (46) reported excellent results from an application of a 20-ppm solution of 2,4-D and silvex (2,4,5-TP). They reported a 3-week delay in fruit maturity for trees treated with silvex and 4 weeks for trees treated with 2,4-D. Negligible residues were reported in the juice.

MATERIALS AND METHODS

Site, Varieties, Chemical Rates, and Application

The Hood River Valley near Hood River, Oregon (45.5° latitude, 121.5° longitude) was the site selected for 2,4-D experiments in Red Delicious apple and Bartlett pear orchards, each at two different elevations. The lower elevation was at 153 m and the upper elevation at 460 m. Experimental trials were established and sprayed during the 1971 growing season. The 2,4-D applications were made in July during the juvenile stage of all fruit, less than 3 cm in diameter.

Individual plots were 0.20 ha and consisted of six mature trees per plot. Treated plots were isolated by leaving untreated rows on each side of the test plots. Treatments were replicated three times in a randomized block design. The dimethylamine salt of 2,4-D was applied at 0, 2.24, and 4.48 kg/ha. Blanket applications of the chemical were made from tree row to tree row so that the suckers and tree trunks would be sprayed. Applications also were made leaving a 1-m buffer strip on two sides of each tree row, thereby reducing to a minimum the opportunity for chemical contact with the trees in these plots.

Sample Collection and Handling

Soil and immature fruit samples were collected prior to establishing experimental trials to permit analysis for possible pre-application 2,4-D contamination in the environment. These samples were meticulously harvested, placed in plastic bags, then transported to

Corvallis, Oregon, and placed in a freezer at sub-zero temperatures to await laboratory analysis. Subsequent soil and fruit samples were collected at weekly intervals for 2,4-D residue experiments, until mature fruit harvest was completed.

Twelve mature fruits were randomly harvested from lower, middle, and upper extremities of each tree in each plot. The immature fruits were used only for 2,4-D residue analysis.

Soil subsamples were collected from three different locations within each plot. The samples were removed from depths of 0-5, 5-10, 10-15, and 15-30 cm. Each corresponding depth within a plot was combined to make the sample. The procedure followed in obtaining each subsample consisted of first excavating the soil to a depth and width of 46 cm to permit removing cross sectional samples from the soil profile. Prior to sample collection, the exposed wall of the soil profile was carefully shaved from the bottom to the top. This permitted removal of possible contamination from the sampling area. After cleansing the sampling tools, the first 5-cm increment was collected in a polyethylene dustpan by holding it at the lowest point of the section being removed with a flat-bladed trowel. The sampling tools were cleansed and another sample removed. Each sample was placed in a polyethylene bag, which was labeled and sealed with a wire twist.

Chemical Application

All plots were sprayed with a Bolens 1445 tractor, designed and fabricated by the researcher for precision applications in orchards.

The tractor powered a Deming #2 pump which delivered the solution to three separate spray booms (Figure 1). Application to the plots which required 1-m buffer strips on each side of the tree row was accomplished by spraying with plugs placed behind the two end TK-3 flood jet nozzles. An application rate of 176 L/ha was obtained by maintaining a ground speed of 4.8 km/hr while operating the sprayer at 1.4 kg/cm^2. Measured volume of each nozzle was 1.44 L/min.

Although growers struggled to preserve trees in the research area which had been damaged excessively from ice and high winds during the winter prior to experiments, it became necessary for them to remove a number of trees from their orchards. One complete replication was lost in the Bartlett pear orchard in the upper valley, and three plots were lost in the Bartlett pear orchard in the lower valley. Despite several trees being eliminated from the trial areas, samples obtained were adequate to complete the experiment.

Mature Fruit Harvest and Handling

Bartlett pear maturity was ascertained when a pressure reading of 1.27 kg/cm^2 was obtained on a Baluf fruit pressure gauge. These pressure readings were made by Mr. Walt Mellenthin, Superintendent of the Mid-Columbia Experiment Station at Hood River. Mature apple harvest was initiated 145 days after full bloom, which, in 1971, was the accepted indicator for commercial harvest of Red Delicious apples. Mature fruit samples were harvested by conventional hand-picking methods and placed in plastic-lined wooden fruit lugs. The Bartlett pears were warehoused in controlled CO_2 atmospheric chambers

Figure 1. Bolens 1445 tractor equipped with sprayer for 2,4-D application in orchards.

at Diamond Fruit Growers, Parkdale, Oregon. The Red Delicious apples were placed in cold storage at the Mid-Columbia Experiment Station near Hood River. Approximately 30 days after harvest, all fruit was transported by truck to the Horticulture Department on the Oregon State University campus, returned to cold storage, and maintained at 0 $\pm$ 0.6 C as laboratory experiments were initiated.

Apparatus for Collecting Gas Samples

The apparatus used to collect gas samples from respiring fruit is shown in Figure 2 and consisted of: (1) flowboard with pyrex manometer tubes, (2) air tubes to respiration chambers, (3) respiration chambers, (4) exhaust tubing, and (5) Beckman CO_2 infrared analyzer Model 215-A.

Determination of CO_2 Produced by Respiring Fruit

A trial run using Bartlett pears was conducted to check the operation of the apparatus. When unpurged atmospheric air was used to circulate through the fruit containers, excessive deflection occurred after 36 hr on the CO_2 respirometer. Therefore, a scrubbing tower using NaOH (Figure 3) was constructed to trap atmospheric CO_2. The purged air passing through the containers was relatively CO_2-free, approximately 2.6 mg CO_2/12 L.

Fruit samples were weighed, then placed into 13.8-L plastic containers, the lids of which were lubricated with petroleum jelly to insure airtight seals. The containers were then subjected to a continuous air flow of 12 L/hr and monitored by a Matheson 620

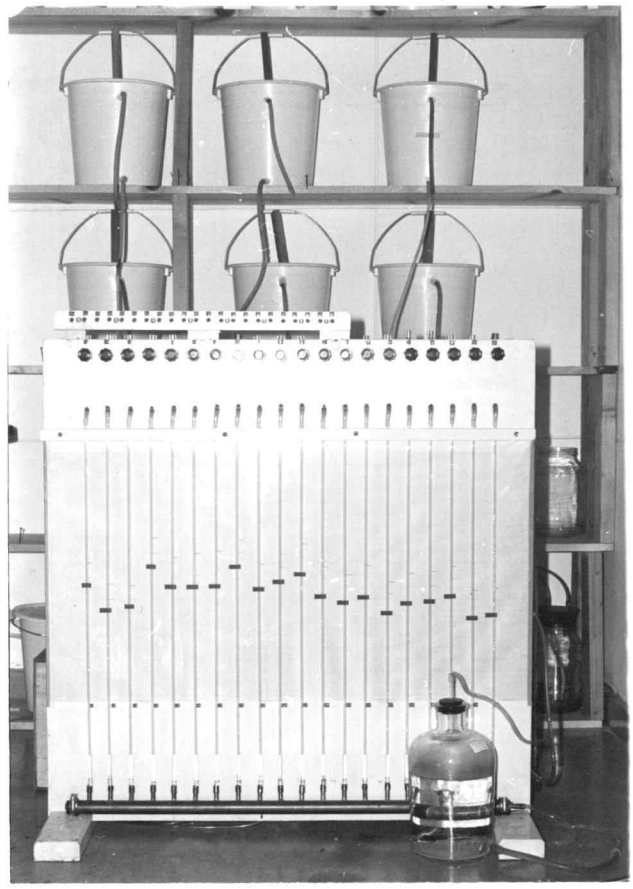


Figure 2. Apparatus for collecting CO_2 and ethylene samples from respiring apples and pears.

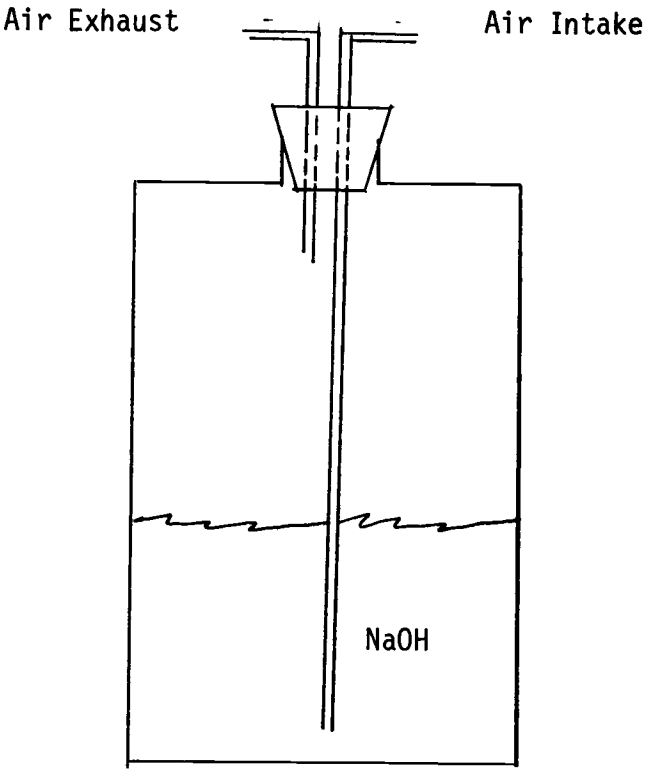


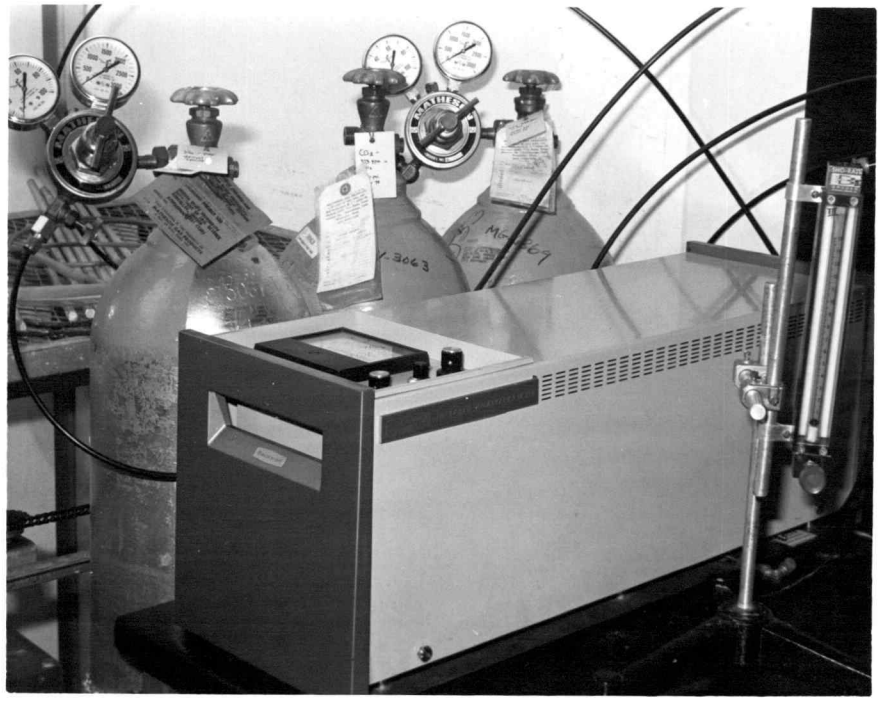
Figure 3. Schematic drawing of NaOH scrubbing tower to trap atmospheric Co_2.

flowmeter for the duration of the experiment. Air flow to the manometers on the flowboard was regulated by a pressure regulator; fine adjustments for proper air flow to the respiration chambers were made with needle valves. Room temperature was maintained at 21 C ± 1 C. A quick but accurate flow check was made possible at all times by employing a colored solution in the pyrex flow tubes. Respiration rates of both fruits were checked at 12-hr intervals or at multiples of that time period. The Bartlett pear experiment continued for 216 hr, and the Red Delicious apple experiment was maintained for 528 hr. CO_2 evolution was monitored directly by inserting the tygon tubing from the fruit container into the infrared analyzer (Figure 4). Fruit respiration in mg CO_2/kg/hr was calculated from the galvanometer deflection (Figure 2, Appendix Table 1).

Determination of Ethylene Produced by Respiring Fruit

The same apparatus employed for collecting respiration data was simultaneously used to collect ethylene samples. With the aid of hypodermic syringes, 1-ml ethylene samples were withdrawn from the surgical tubing outlets on the containers (Figure 5). Three 1-ml samples from each container were withdrawn at each test period. Each syringe needle was inserted into a large rubber stopper and transported to the Aerograph Model 600-D ionizing gas chromatograph equipped with a 1.54-ml Porapak Q resin column.

Ethylene samples were introduced into the injection port from the hypodermic syringes, and peak heights were recorded directly on a chart. Ethylene concentration in each sample was then calculated



Fiture 4. Beckman infrared analyzer Model 215-A for measuring CO_2

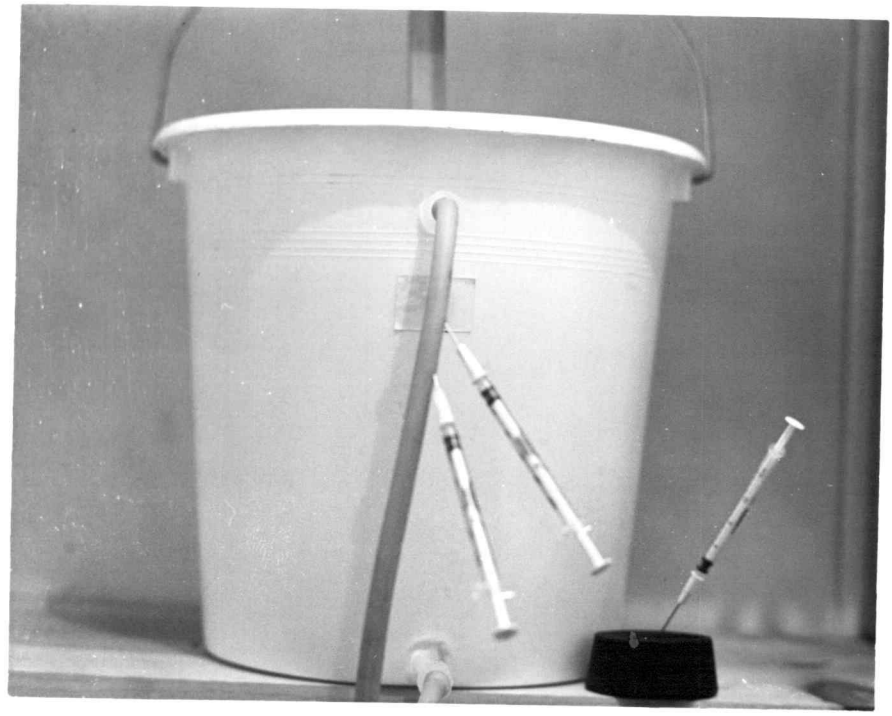


Figure 5. Collection of ethylene samples from respiration chambers.

from the attenuation used and the peak height obtained (Appendix Figure 1). It was convenient to calculate the concentration directly by using the factor 0.02405 because increased ethylene concentrations often necessitated an attenuation change. This was accomplished by using a standard curve previously constructed. The ethylene produced by the fruit was expressed in η moles C_2H_4/kg/hr.

Preparation of Samples for 2,4-D Residue Analysis

A homogenate was prepared from each weekly immature fruit harvest by pulverizing the fruit from each plot in a blender. A 5-gm portion was taken from homogenate and hydrolyzed with 1 N NaOH on a steam bath for 1 hr to extract 2,4-D residues. The slurry was centrifugated, and the supernatant was collected in a separatory funnel. The residue was resuspended two additional times in 0.1 N NaOH, and the aliquots were added to those of the initial extraction.

The supernatant was then acidified to pH 2 with 5 N H_2SO_4 and extracted with 200 ml ethyl ether, and the final 400 ml volume was concentrated to 100 ml on a steam bath.

The ether extract was purified by passing it through a 30-gm sample of basic alumina contained in a 2.5 cm pyrex glass column. The alumina column was then washed with successive 100-ml portions of ether and chloroform to elute extraneous plant materials. A vacuum was employed to remove solvents from the column, then the 2,4-D was eluted with 100 ml of a 1% $NaHCO_3$ solution.

In a separatory funnel, the supernatant was acidified with dilute H_2SO_4 and then extracted with three successive 100-ml

portions of ethyl ether. The 300-ml sample was concentrated to approximately 25 ml on a steam bath. It was then transferred to a 50-ml screw-cap volumetric flask and taken to dryness on a rotary evaporator. The sample was then ready to esterify for subsequent gas chromatographic analysis.

Esterification of the sample was accomplished by using BF_3 methanol reagent. A 5-ml aliquot of 11% BF_3 in methanol was added to the volumetric flask which contained the sample. The screw-cap was tightened, and the sample was heated for 30 min on a steam bath. After cooling, 45 ml of water was added to destroy excess BF_3 and to facilitate partitioning of 2,4-D ester into 1 ml of benzene which was also added to the flask.

After vigorously shaking the volumetric flask and contents, the two layers were permitted to separate; then the benzene extract which contained the methyl ester of 2,4-D was analyzed chromatographically.

The gas chromatograph used in this study was a Varian 2100 equipped with a microcoulometric detector. The chromatograph was equipped with a 0.32 cm x 1.83 m packed with 6% OV-1 on 100/200 mesh Gas-Chrom Q column. Oven temperature was maintained at 220 C, and the retention time of the methyl ester of 2,4-D was 3 min.

The analysis for soil residues of 2,4-D was essentially the same as the fruit analysis. After thoroughly mixing the soil in a tumbler, a 50-gm subsample was taken for analysis. The extraction, purification, and chromatography procedures were the same as those described for fruit.

To determine the efficiency of 2,4-D recovery, both fruit and soil samples were fortified with known amounts of 2,4-D. These samples were extracted and processed in the same manner as the other samples. Concentrations of 2,4-D in the fortified samples ranged from 0.01 ppm to 4 ppm. The efficiency of 2,4-D recovered was 85-89% for soil samples and 92-97% for fruit samples.

A standard solution containing 10 η gm/μl 2,4-D was used frequently when samples were injected. A series of standards were injected prior to sample introduction. Then two samples were introduced, followed by another standard.

RESULTS AND DISCUSSION

Effects of 2,4-D on Respiration of Bartlett Pears During Ripening Period

Green but mature Bartlett pears were used in this experiment to determine if 2,4-D applied at rates of 2.24 and 4.48 kg/ha to the orchards in the summer had any effect on fruit respiration. As fruit was placed in the respiration chambers, weights were recorded so that the standard measurement of respiration expressed as mg CO_2/kg/hr could be reported.

Bartlett pears showed a rapid pre-climacteric increase in CO_2 production during the first 12 hr after fruit was placed in respiration chambers, indicating that the fruit was physiologically mature and ready to ripen. This rate of increase approximated the normal increase in CO_2 evolution by untreated fruit and agreed with results obtained by other workers on fruit from orchards with no reported history of 2,4-D applications. The increase, determined photometrically, was from 9 mg CO_2/kg/hr to 35 mg CO_2/kg/hr (Figure 6, Appendix Table 1). Similar increases occurred in fruit samples taken from plots at both locations and at all designated treatment levels. After this initially rapid increase, CO_2 evolution continued but increased only gradually through the climacteric period. Approximately 60 mg CO_2/kg/hr (Figure 6) was the maximum level of CO_2 produced.

As described in the Materials and Methods section, removal of damaged trees from orchards resulted in uneven replications. In

order to analyze all available data for each treatment, a multiple t test was first employed to determine if a significant difference in amount of CO_2 evolution existed among samples within each location (sub-population) when rates of 2,4-D applied and CO_2 sampling time intervals were held constant. In addition, the t test would demonstrate any significant difference in CO_2 evolution between upper and lower valley sub-populations.

Fruit from plots treated at designated chemical rates and with both types of chemical placement produced approximately the same amount of CO_2 during any given time period (Figure 6, Table 1). Post-climacterically, the rate at which CO_2 was produced continued to gradually decline until the experiment was terminated at the end of 252 hr.

It was not feasible to graph individual CO_2 evolution readings for fruit samples from each location, application rate, and treatment method because they were so closely correlated (Table 1). Therefore, respiration data from fruits collected at each of the two locations and at the three designated 2,4-D rates and the two placement methods were averaged for each time period and then graphed (Figure 6).

The experimental data collected from respiring Bartlett pears harvested from trees growing in plot areas previously treated with 0, 2.24, and 4.48 kg/ha, either applied from tree row to tree row or by leaving 1-m buffer strips along each side of the tree rows, indicate that 2,4-D did not alter the respiration of Bartlett pears.

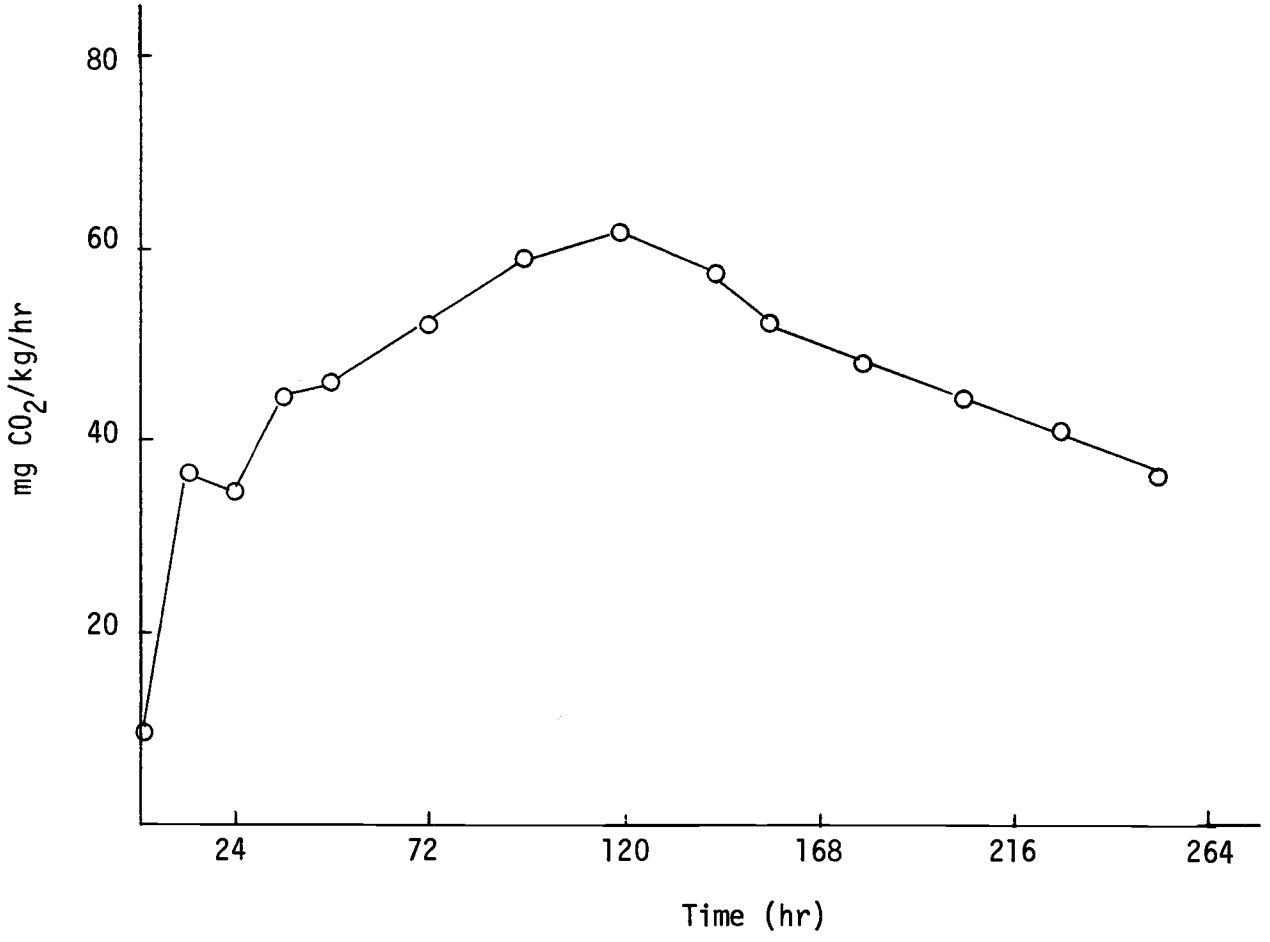


Figure 6. Respiration rates during ripening in Bartlett pears from the Hood River Valley. All 2,4-D rates and locations are combined.

Table 1. The effect of 2,4-D on respiration of Bartlett pears from the upper and lower Hood River Valley. Values are expressed as mg CO_2/kg/hr.

Sampling Time (hr)	Rate (kg/ha)				
	Check	2.24 TT	2.24 SS	4.48 TT	4.48 SS
0	9.860	9.745	9.145	10.695	9.423
12	34.808	36.740	36.773	38.393	36.398
24	32.480	35.615	34.280	36.353	33.843
36	42.035	42.933	45.495	49.248	44.400
48	44.323	45.093	47.878	47.915	46.313
72	49.583	51.448	52.875	55.265	51.445
96	58.293	60.020	60.258	60.350	57.770
120	59.443	58.043	64.150	62.763	62.820
144	54.330	56.123	57.845	59.368	59.080
156	52.123	50.728	51.960	53.575	51.948
180	47.770	46.750	48.840	49.960	48.965
204	44.118	43.398	45.453	45.570	46.280
228	37.893	38.428	38.590	40.260	39.095
252	35.530	35.403	35.423	37.368	36.415

(1) Tabulated values and means of 4 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Effects of 2,4-D on Ethylene Production in Bartlett Pears During Ripening Period

Ethylene production experiments were simultaneously conducted in conjunction with the CO_2 analysis, and consisted of withdrawing ethylene samples from the continuous air exhaust stream of the respiration chambers. Ethylene production of pears from the two test locations is compared in Figure 7. The ethylene evolved by Bartlett pears from both locations, lower and upper valley, remained below the detectable limits of 1.2×10^{-2} η moles/kg/hr during the first 24 hr. After the initial 24-hr period, all fruit tested began to produce detectable amounts of ethylene. Figure 7 shows the gradual but steady pre-climacteric increase of ethylene for the next 72 hr. Ethylene production from pears from the upper valley lagged behind ethylene production from the lower-valley pears throughout the experiment. This lag phase probably was due to a difference in maturity of the fruit at the two locations when harvested. With a difference in elevation of 308 m between the upper and lower valley, it would seem reasonable to expect a slight difference in maturity, even though the accepted test for commercial harvest of pears was employed.

The difference in ethylene production between the two groups of fruit samples (upper and lower valley locations) was found to be significant at the 20% level of confidence with the t test. Therefore, the data from each of the two groups were analyzed separately to determine if there were differences among samples within a location group at 2,4-D rates when ethylene production was sampled at

designated time intervals. With only one exception among samples from the lower elevation (Table 2), no significant differences were found to exist at the 5% confidence level. After the experiment had been in progress for 96 hr, fruit from one buffer strip plot treated at 2.24 kg/ha produced ethylene erratically. This erratic production occurred only in the 96-hr sample. An ethylene reading of 7.33×10^3 η moles/kg/hr produced from a single respiration chamber representing one replication, was considerably higher than those readings of other samples from plots treated at the same rate of 2,4-D but applied without the buffer zones between tree rows. It should be noted that this amount of ethylene produced was also significantly higher at the 1% level than that of fruit from the plots treated at 4.48 kg/ha rate. Since ethylene evolved during the time periods prior to and immediately following this erratic production fell within expected confidence levels, it appears that this erratic reading resulted from a factor other than the 2,4-D rates used or the placement methods employed in the field. An air leak around the chamber lid very likely created a high accumulation of ethylene within the chamber.

After climacteric had been reached (Figure 7), evolution of ethylene decreased at a rapid but steady rate until the experiment was terminated after 216 hr. No significant differences (LSD_{05}) in ethylene production occurred among the upper valley location samples prior to maximum ethylene evolution (Figure 7, Appendix Table 4). After maximum production was attained, evolution of ethylene decreased at a rapid but steady rate until the experiment was

terminated after the 216-hr sampling. There was, however, a significant difference in production at the 5% level (Appendix Table 4) immediately prior to experiment termination between the control samples and the 4.48 kg/ha buffer strip-to-buffer strip samples from the upper valley. At the 192-hr sampling, ethylene evolution by pears from all treated plots was significantly higher (LSD_{05}) than the evolution by pears from the untreated plots.

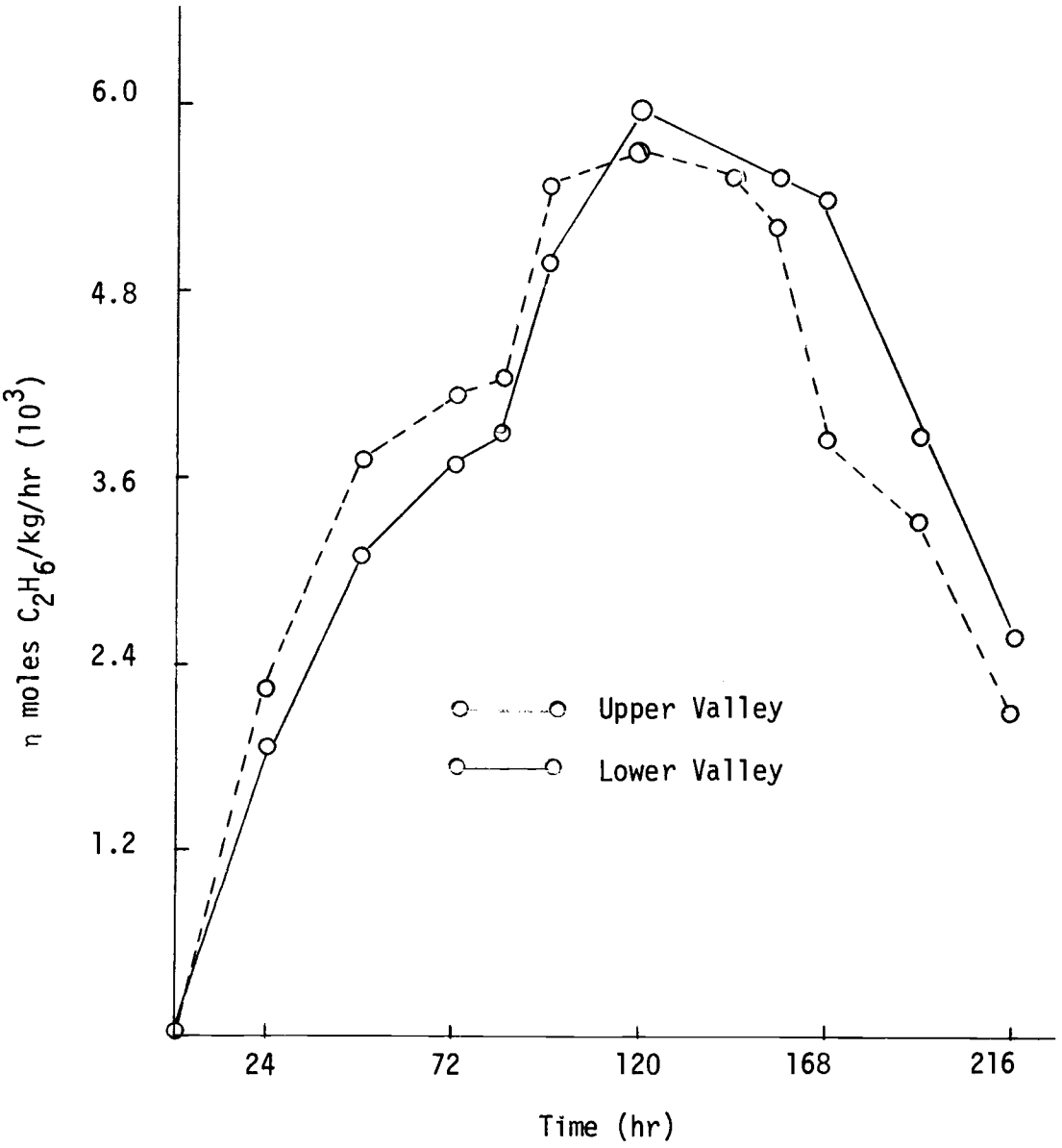


Figure 7. Ethylene production during ripening of Bartlett pears from the Hood River Valley. All 2,4-D rates are combined.

Table 2. The effects of 2,4-D on ethylene production during ripening of Bartlett pears from the lower Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
24	2.124	2.052	2.28	2.244	2.544
48	3.708	3.540	3.888	3.372	4.128
72	4.296	4.044	4.728	3.888	3.780
84	4.608	4.404	3.924	3.912	4.248
96	5.124	5.244	7.332	4.788	4.824
120	5.652	6.072	5.628	5.424	5.868
144	5.160	5.448	5.604	5.352	6.000
156	4.308	5.376	5.136	4.788	5.460
168	3.600	3.996	3.828	3.696	3.960
192	3.048	3.240	3.516	2.964	3.672
216	1.992	2.100	2.040	2.220	2.064

(1) Tabulated values are means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Table 3. The effects of 2,4-D on ethylene production during ripening in Bartlett pears from the upper Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
24	1.600	1.068	2.220	2.676	1.632
48	2.750	2.652	3.192	3.756	3.264
72	3.108	3.264	4.248	4.272	3.504
84	3.888	3.468	4.416	4.620	3.600
96	4.260	4.464	5.304	6.060	4.920
120	5.064	6.048	5.760	6.864	5.988
144	4.584	6.096	5.232	6.084	5.748
156	4.548	4.896	6.708	5.784	5.868
168	4.632	5.088	6.648	5.496	5.100
192	2.736	4.248	4.308	3.684	4.152
216	2.136	2.280	2.844	2.376	3.288

(1) Tabulated values are means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Effects of 2,4-D on Respiration of Red Delicious Apples During Ripening Period

Mature Red Delicious apples were selected for use in determining whether or not 2,4-D applied to orchards would affect CO_2 production during the postharvest fruit respiration of samples from plots treated at the 0, 2.24, and 4.48 kg/ha rates.

At inception of the experiment, the quantity of CO_2 produced by apples from upper valley location plots treated with or without the buffer strips at 2.24 and 4.48 kg/ha approximated 7 mg CO_2/kg/hr. The same quantity was obtained from those fruits harvested from check plots. Production of CO_2 rapidly increased to 29 mg CO_2/kg/hr during the first 12-hr period, an increase of 300% (Figure 8, Tables 4, 5). This percentage increase is in close agreement with the findings of other research workers (12, 17, 43) doing postharvest physiology studies on three varieties of apples from untreated orchards. Following this initially sharp increase of CO_2 produced by the respiring fruit, CO_2 production continued to slowly increase to a maximum of 34 mg CO_2/kg/hr after 96 hr. A gradual decline in CO_2 evolution followed until, at 216 hr, a level of 24 mg CO_2/kg/hr was measured (Figure 8). During the next 36 hr, respiration again increased until a peak of 27 mg CO_2/kg/hr was reached. Postclimacterically from the 288-hr sampling time, the amount of CO_2 evolved from all Red Delicious apples continued to slowly decline until the research experiment was terminated after 744 hr. Throughout the study, the rates of CO_2 evolution by apples from plots which had been treated with 2.24 or 4.48 kg/ha closely approximated CO_2

rates of fruits from untreated plots. The rate of CO_2 evolution from the lower valley fruit was slightly lower than that from the upper valley at all sampling times. However, when graphed (Figure 8), the respiration curve nearly paralleled the upper valley curve.

A multiple t test was used for comparing treatments within uneven replications of plots from the two locations, upper and lower valley. They were found to be unequal sub-populations of the valley population. At the 20% level of confidence, a significant difference in CO_2 production was demonstrated between the two location groups within each sampling interval when the chemical rates and treatment methods were held constant. Each location contained unequal replications; therefore, the t test was employed within each location. It showed that all CO_2 concentrations at a given time interval with regard to chemical treatment were within the 20% confidence level. Data were randomly deleted from each location to give equal replications so that an analysis of variance could be performed on the data. The data from each of the two locations were statistically analyzed using a prepared ANOV program at the Oregon State University Computer Center. When application rates and sampling time intervals were controlled, and with location (upper and lower valley sub-populations) as the determining variable (Appendix Tables 5, 6), no significant differences in CO_2 evolution (LSD_{05}) were found to exist at either location.

Means of CO_2 evolution determinations from the replications for each of the five treatments were so closely grouped that it was impossible to plot each treatment on the same graph. The graphical

representation of CO_2 evolved from Red Delicious apples (Figure 8) is an average of all sample means at a given sampling time for each location.

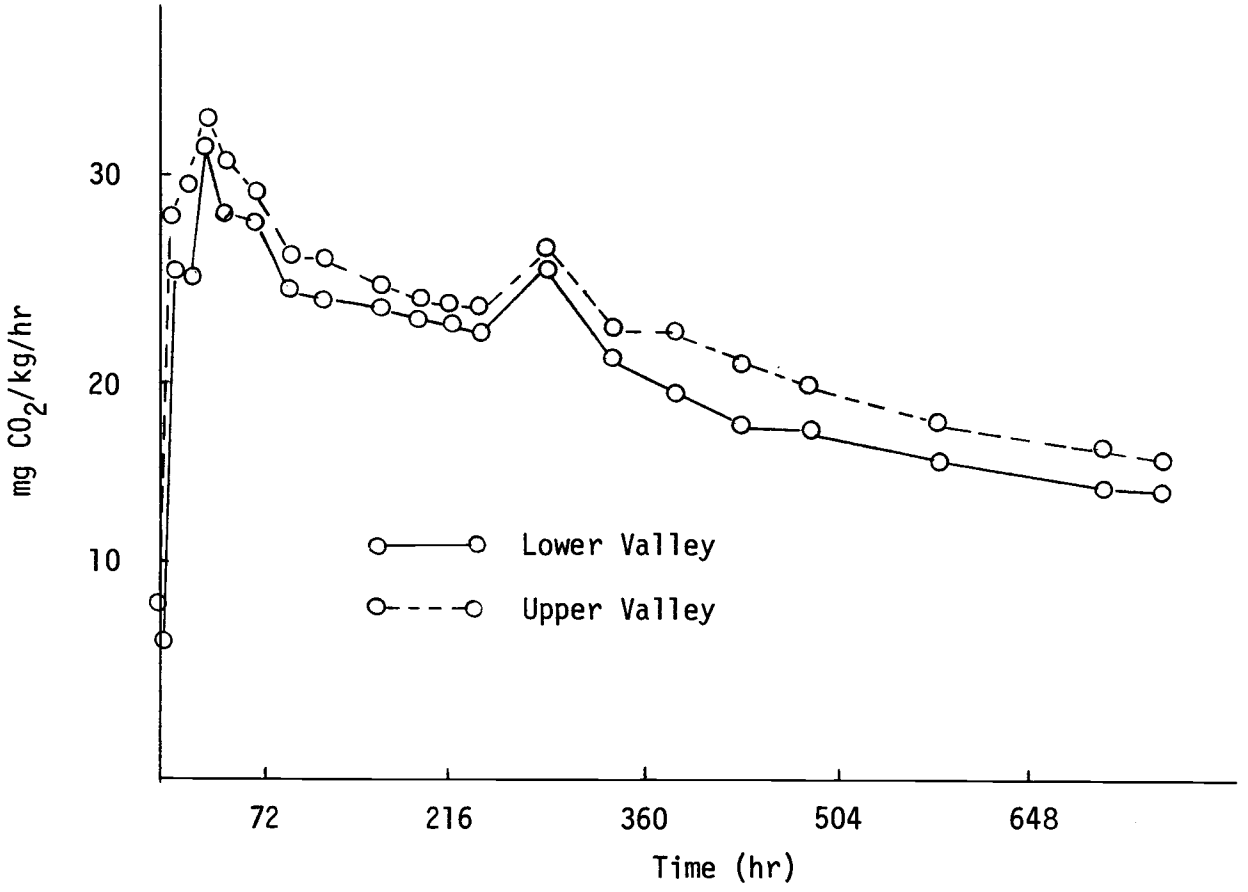


Figure 8. Respiration rates during ripening of Red Delicious apples from the Hood River Valley. All 2,4-D rates are combined.

Table 4. The effect of 2,4-D on respiration rate during ripening of Red Delicious apples from the lower Hood River Valley. Values are expressed in mg CO_2/kg/hr.

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
0	6.15	6.59	6.37	6.80	7.17
12	26.03	28.09	28.18	23.75	27.62
24	26.17	28.65	24.52	23.75	28.62
36	30.26	29.64	29.56	30.33	32.16
48	28.61	28.92	28.89	29.72	31.06
72	30.97	28.33	26.64	29.67	30.25
96	25.90	25.50	24.58	25.85	25.69
120	24.90	25.00	25.10	23.96	25.52
168	23.13	24.77	25.46	22.54	26.47
192	23.10	22.85	25.02	23.53	23.63
216	23.10	22.82	25.02	23.86	23.33
240	22.50	22.25	24.49	22.98	23.08
288	26.44	25.36	27.12	27.28	27.32
336	21.83	21.41	23.43	20.99	22.35
384	19.62	19.16	20.72	20.59	21.31
432	19.83	19.65	20.40	19.00	20.35
480	20.57	17.76	20.35	18.94	19.47
576	14.82	16.20	17.79	16.18	17.72
696	15.56	15.48	16.60	14.97	16.23
744	15.15	15.42	16.19	14.75	16.05

(1) Tabulated values and means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Table 5. The effect of 2,4-D on respiration rate during ripening of Red Delicious apples from the upper Hood River Valley. Values are expressed as n mg CO_2/kg/hr.

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
0	6.78	8.04	7.12	7.22	7.94
12	30.05	30.54	26.84	31.88	26.85
24	31.87	32.31	30.67	28.88	30.48
36	35.15	36.40	34.26	32.58	33.10
48	32.58	32.31	31.69	29.81	31.94
72	32.46	29.19	29.66	30.61	30.51
96	27.74	28.55	28.08	29.52	28.21
120	27.52	25.71	26.70	25.09	25.80
168	28.19	28.29	28.21	26.66	26.52
192	26.35	26.17	26.51	23.80	25.00
216	26.03	24.70	25.10	23.25	23.88
240	25.53	25.04	25.16	23.52	23.95
288	27.83	27.35	27.31	25.98	26.22
336	23.99	23.77	23.58	21.82	23.18
384	24.66	23.08	23.48	23.23	24.21
432	22.40	22.49	21.43	21.10	21.91
480	21.57	19.92	21.05	19.57	20.27
576	19.65	19.28	18.23	18.19	17.75
696	18.21	16.82	16.40	16.98	16.06
744	17.80	17.00	16.27	16.52	15.66

(1) Tabulated values and means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Effects of 2,4-D on Ethylene Production in Red Delicious Apples During Ripening Period

While the respiration experiment on Red Delicious apples was in progress, ethylene samples were withdrawn from the continuous air exhaust system and chromatographed. Ethylene evolved by apples from all treated and untreated plots at both locations remained below detectable limits (1.2×10^{-2} η moles/kg/hr) during the first 24 hr. Ethylene was detectable in all samples withdrawn at the 36-hr sampling period.

Fruit samples from all trees which had been treated at 0, 2.24, and 4.48 kg/ha of 2,4-D produced ethylene during the ripening process at approximately the same rate of increase (Figure 9, Tables 6, 7). The amount of ethylene produced by Red Delicious apples taken from all treated plots at each location increased from 2.196 η moles/kg/hr at the 36-hr sampling to a maximum of 3.2 η moles/kg/hr after 264 hr.

During comparable sampling periods, the concentration of ethylene in samples from the upper valley apples was higher than in samples from lower valley apples. However, when graphed (Figure 9), the two locations were nearly parallel. After maximum ethylene production was attained at 264 hr, apples from both locations showed a steady but gradual decrease in ethylene production from 3.2 η moles/kg/hr to 2.5 η moles/kg/hr at 528 hr when the experiment was terminated.

Use of the t test indicated a statistically significant difference between apples at the lower and upper locations in the

quantities of ethylene produced. Ethylene production data then was analyzed for each location (Appendix Tables 7, 8, 12). There were no significant differences in ethylene production within a specific measuring period in the upper valley apples when the treatment rates were varied (Appendix Table 8). However, the lower valley apples showed a significant difference in ethylene production at the 5% level after 48 hr and again at 120 hr (Appendix Table 7).

In buffer strip plot samples only, a difference (LSD_{05}) in ethylene concentration at 48 hr existed among 0, 2.24 kg/ha, and the 4.48 kg/ha rates (Appendix Table 7). The difference in production which existed at the sampling of time interval 120 hr was only between the 4.48 kg/ha strip-strip treatment and the check plot apples (Appendix Table 7). There were no significant differences at any other sampling periods. Since the aforementioned differences occurred only in fruit harvested from buffer strip plots or in fruit harvested from trees at the 2.24 kg/ha rate of 2,4-D instead of the 4.48 kg/ha rate, the values do not appear to be meaningful. This is further substantiated by the fact that data from all other sampling periods were within the expected confidence limits (LSD_{05}).

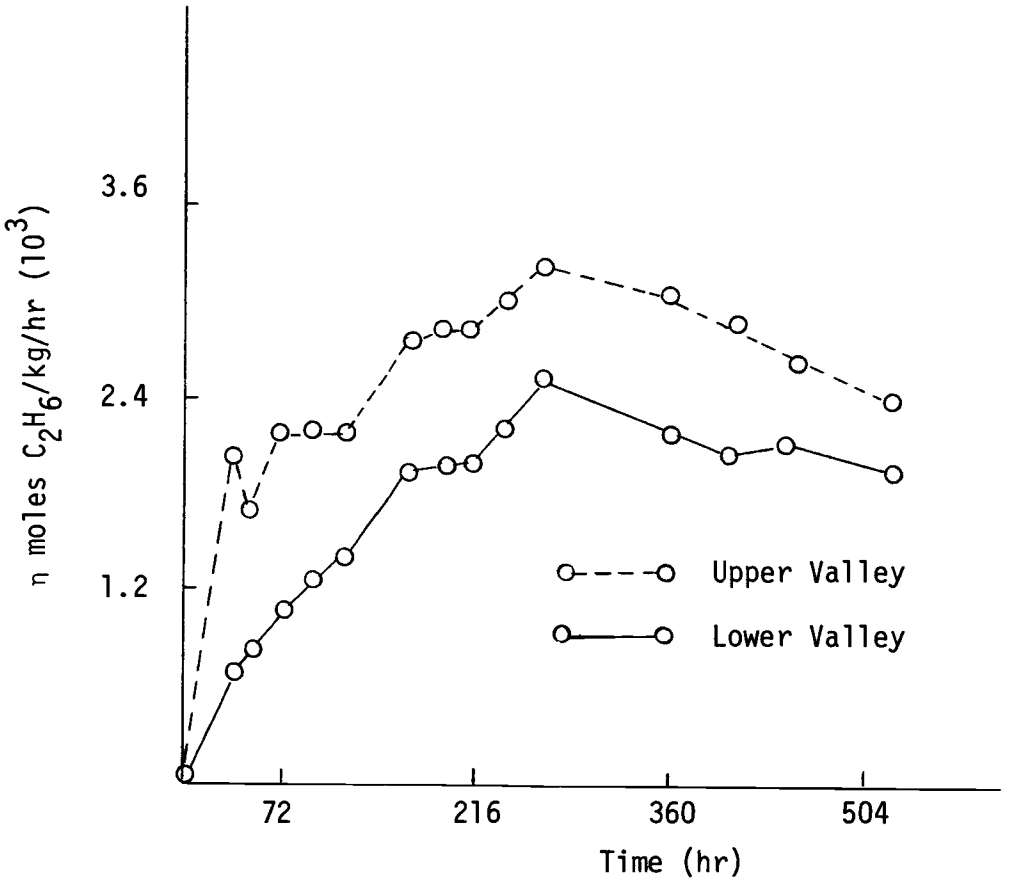


Figure 9. Ethylene production during ripening of Red Delicious apples from the Hood River Valley. All 2,4-D rates are combined.

Table 6. The effects of 2,4-D on ethylene production during ripening of Red Delicious apples from the lower Hood River Valley. Values are expressed as n moles C_2H_4/kg/hr (10^3)

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
36	.888	.804	.730	.912	.888
48	.972	.720	.624	.816	.840
72	1.140	1.044	.900	1.116	1.164
96	1.072	1.260	1.700	1.272	1.428
120	1.308	1.512	1.320	1.272	1.596
144					
168	1.752	1.920	2.015	1.884	2.028
192	2.069	2.040	1.824	1.572	1.920
216	1.956	2.004	2.040	2.076	1.944
240	2.016	2.316	2.256	2.076	2.376
264	2.388	2.496	2.676	2.460	2.628
360	1.980	2.136	2.244	2.145	3.216
408	1.812	2.124	2.184	2.016	2.328
456	1.956	2.184	2.148	2.064	2.544
528	1.728	1.950	2.304	1.644	2.196

(1) Tabulated values are means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

Table 7. The effects of 2,4-D on ethylene production during ripening of Red Delicious apples from the upper Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Sampling Time (hr)	Rate (kg/ha)				
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS
36	2.196	2.232	1.980	1.896	1.812
48	1.692	1.680	1.704	1.476	1.632
72	2.004	2.244	2.148	2.268	2.124
96	2.028	2.544	2.268	2.124	2.088
120	2.136	2.376	2.148	2.112	2.088
144					
168	2.532	3.204	2.712	2.616	2.652
192	2.604	3.288	2.964	2.628	2.676
216	2.700	3.108	2.856	2.856	2.772
240	2.844	3.264	3.000	2.940	2.988
264	3.192	3.660	3.252	3.108	2.952
360	2.892	3.204	2.976	2.928	3.204
408	3.000	2.892	3.024	2.736	2.808
456	2.796	2.820	2.400	2.700	2.448
528	2.496	2.544	2.244	2.412	2.412

(1) Tabulated values are means of 2 replications.

(2) TT, 2,4-D application including suckers and tree trunks.

(3) SS, 2,4-D application to buffer strip.

2,4-D Residues in Soil

In order to determine how closely the actual quantities of 2,4-D applied approximated the intended application rates, soil samples from all plots were collected, extracted according to the procedures outlined in the Materials and Methods section, and analyzed on a Varian gas liquid chromatograph. Based on an average efficiency of 85-89% 2,4-D recovery from soil samples, the theoretical and actual rates applied were within 2% (Figure 10, Table 8). The 2,4-D disappearance from the soil was very rapid; approximately 75% had been lost after 14 days (Figure 11) from the 2.24 kg/ha plots, and 60% was lost from the 4.48 kg/ha plots. After an additional 7 days, 97% had been lost from all test plots. These values are in close agreement with work done by Audus (1) and Torstensson (76).

These trials were established during July when temperatures usually reach 32 C during the day. Fields in the upper valley where the plots were located, had had previous applications of 2,4-D at 2.24 kg/ha each of the four preceding years; however, those orchards in the lower valley had never been treated with 2,4-D. In these trials, orchards without prior herbicide history lost 2,4-D as rapidly as those orchards with a known 2,4-D history. No detectable residues (0.02 ppm level of sensitivity) were found after 28 days.

2,4-D Residue in Fruit

Pre-application analyses for 2,4-D residues were made from both the Bartlett pears and Red Delicious apples, as well as analyses of fruit at weekly intervals following application. No detectable

levels of 2,4-D down to the 0.02 ppm level of sensitivity were found in any fruit samples analyzed.

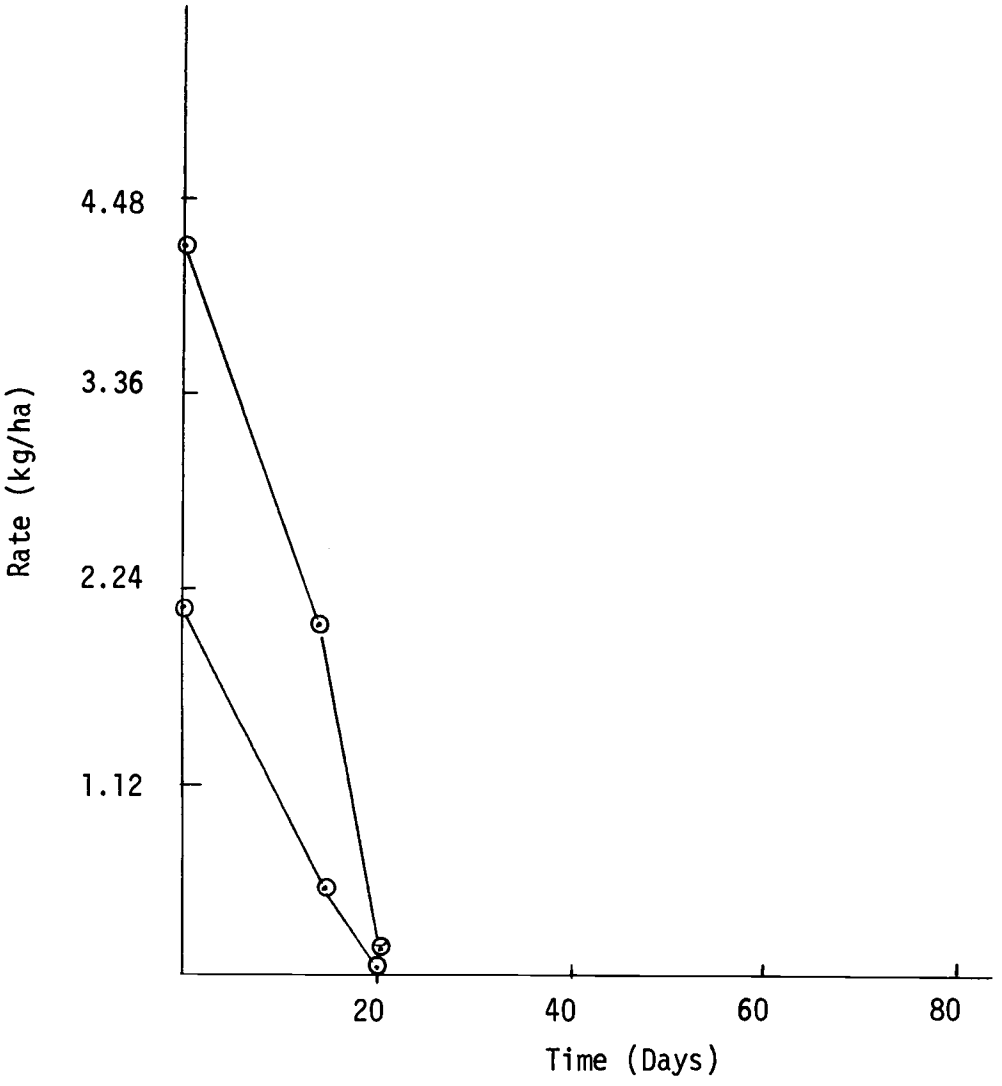


Figure 10. Disappearance of 2,4-D from orchards in the Hood River Valley, initial application, July 29, 1971.

Table 8. The disappearance of 2,4-D from orchards in the Hood River Valley.

Rate (kg/ha)	Upper Valley R1	R2	R3	Ave	Lower Valley R1	R2	R3	Ave
July 29, 1971								
0	N.D.*	N.D.	N.D.		N.D.	N.D.	N.D.	
2.24 TT	2.03	1.99	2.14	2.05	2.11	-	1.98	2.05
2.24 SS	2.05	1.99	2.17	2.07	2.08	2.23	-	2.16
4.48 TT	4.04	4.22	3.93	4.06	4.20	3.99	4.17	4.10
4.48 SS	4.51	4.33	4.04	4.29	4.20	3.98	4.63	4.27
August 13, 1971								
0	N.D.	N.D.	N.D.		N.D.	N.D.	N.D.	
2.24 TT	.56	.43	.56	.52	.47	.60	.56	.54
2.24 SS	.54	.59	.48	.54	.60	-	.67	.64
4.48 TT	1.77	2.16	2.06	2.00	1.95	1.99	1.74	1.89
4.48 SS	2.13	1.79	1.97	1.96	1.99	1.95	2.11	2.02
August 20, 1971								
0	N.D.	N.D.	N.D.		N.D.	N.D.	N.D.	
2.24 TT	.07	.03	.06	.05	.07	.07	.07	.07
2.24 SS	.06	.07	.13	.09	.08	-	-	.08
4.48 TT	.11	.16	.13	.13	.20	.20	.12	.17
4.48 SS	.13	.07	-	.10	.27	.22	.27	.25

*Not detectable (.02 ppm level of sensitivity)

August 27, 1971 - N.D.
September 3, 1971 - N.D.
September 24, 1971 - N.D.
October 15, 1971 - N.D.

GENERAL DISCUSSION AND CONCLUSIONS

Applications of 2,4-D amine were made to Red Delicious apple and Bartlett pear orchards without apparent injury to the trees. These results are in contradiction to reports by Benson (7, 8). Further, no detectable levels of 2,4-D (2 ppm level of sensitivity) were found in samples when residue analyses were conducted on fruit at various stages of maturity.

Fruit respiration and ethylene production experiments were conducted and showed that 2,4-D treatments in the orchards did not alter either the respiration rates or ethylene production of apples or of pears.

The cause for injury in Washington orchards which Benson previously reported is not clear. Except in sand or gravel soils, 2,4-D is not readily leached and is rapidly degraded by microorganisms in warm soils (3, 32, 35, 36, 76). Results of residue analyses in this study are in harmony with research previously reported. Therefore, an appreciable amount of 2,4-D reaching the root zone of the apple and pear trees would not be expected. If, indeed, 2,4-D were leached into the root zone, it does not readily move acropetally in trees (21), and its presence would not likely cause injury to the trees.

During the course of this research project, blanket applications of 2,4-D were made in orchards so that the suckers and tree trunks would be sprayed. Applications also were made, leaving a 1-m buffer strip on each side of the tree row, thereby reducing to

a minimum the opportunity for chemical contact with the trees. Even when the tree trunks were contacted by the spray, no evidence of chemical effect on the trees was noted.

It would appear, then, that the injury to orchards reported by Benson may have resulted from spray drift or volatility to the foliage. Spray drift generally results when applications are made under windy conditions or when volatile formulations of 2,4-D are used. The source of contamination may have been drift from 2,4-D applications to wheat fields in the locality.

Results of experiments reported in this thesis clearly demonstrate that when 2,4-D is properly applied to Red Delicious apple and Bartlett pear orchards, it is a safe and efficient means for controlling broadleaf weeds in orchards. Volatile formulations, including all forms of 2,4-D ester, should be avoided. Precautions should be taken to avoid spray drift onto foliage of the fruit trees.

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APPENDICES

APPENDIX A

SAMPLE CALCULATIONS

I. Ethylene

A sample calculation is performed on ethylene standard samples for the construction of a standard curve.

(1) Combining Boyle's law and Charles' law, $\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$, the temperature and volume for this ethylene experiment was corrected as follows:

$$V_2 = \frac{P_1V_1T_2}{T_1P_2} = \frac{(760\ mm(22.4L)(293K)}{(273K)(745\ mm)} = 24.52\text{-L}$$

(2) 1 ml ethylene = $\frac{1\ ml}{24,520\ ml} = 4.08 \times 10^{-5}$ moles = 40,800 η moles

(3) Add 0.991 ml of ethylene to 999 ml flask for use as a standard.

(4) 1 ml of the diluted standard = 40.80 η moles

Volume Injected (ml)	η moles Injected	Attenuation	Peak Height	Attenuation x Peak Height
0.25	10.20	10	63 64.5 62 63.5 60	632
0.50	20.40	20	56 56 57 59.5 57	1134
1.0	40.80	40	46 46 46 46 46	1840

(5) Add 15.2 ml ethylene in 152 ml flask; 1 ml of the standard = 4080 η moles

0.025	102	80	63 62 63 62 60.5	4968
0.05	204	160	50 51 52.5 51 51	8166
0.10	408	320	52.5 54 53.5 52.5 53	16983

(6) Attenuation x peak height for 408 η moles = 16983

(7) Attenuation x peak height for 1 η mole = $\frac{16983}{408}$ = 41.6

(8) η moles/injection = attenuation x peak height x $\frac{1}{41.6}$

(9) η mole/injection = attenuation x peak height x 0.02404

Derivation of the factor 0.02404 was conveniently used to make all calculations.

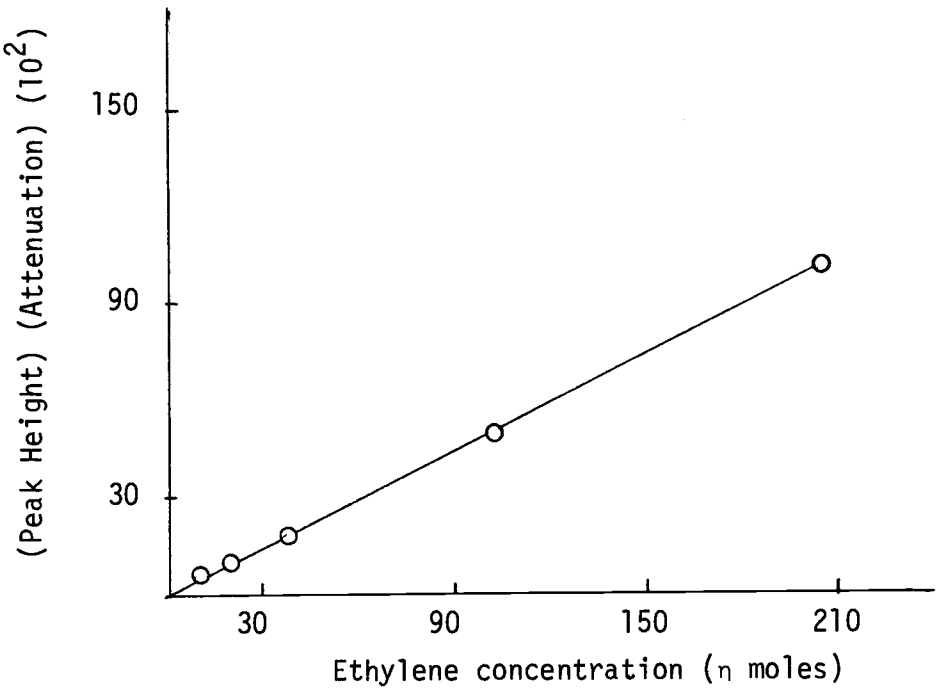

Figure 1. Standard curve for determining ethylene concentration.

II. CO_2

(1) Combining Boyle's law and Charles' law,

$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$; the temperature and volume for the CO_2 experiment was corrected as follows:

$$V_2 = \frac{P_1V_1T_2}{T_1P_2} = \frac{(760\ mm)(22.4\text{-}L)(293K)}{(273K)(745\ mm)} = 24.52\text{-}L$$

(2) A CO_2 sample with a concentration of 3,000 ppm would contain:

$$\frac{3 \times 10^3\ ml\ CO_2}{1 \times 10^6\ ml\ CO_2} = \frac{x}{1\text{-}L}$$

$$x = 3\ ml\ CO_2/L$$

(3) Air flow for experiment was 12-L/hr, therefore:

$$(3\ ml\ CO_2/L)(12\text{-}L/hr) = 36\ ml\ CO_2/hr$$

(4) On a mole basis:

$$\frac{36\ ml/hr}{22{,}520\ ml} \times 44{,}000\ mg = 70.74\ mg/hr$$

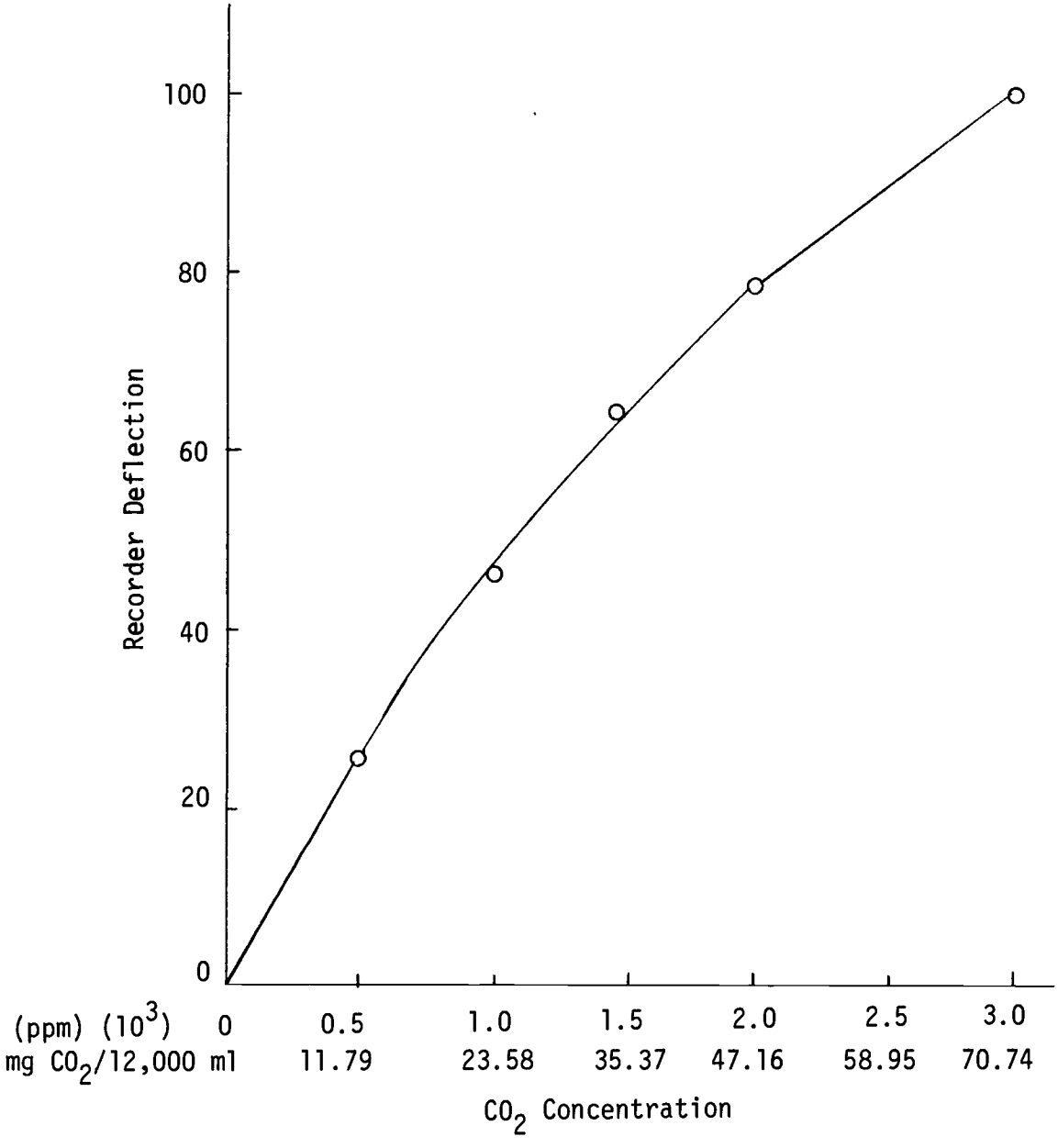

Figure 2. Standard curve for the determination of CO_2 concentration.

Table 1. Beckman infrared spectrophotometer scale deflections (expressed in mg CO_2/12-L)

Def.	mg CO_2/12-L	Def.	mg CO_2/12-L	Def.	mg CO_2/12-L
1	.28	34	15.92	67	36.90
2	.70	35	16.51	68	37.73
3	1.18	36	17.10	69	38.55
4	1.77	37	15.57	70	39.38
5	2.2	38	18.04	71	40.20
6	2.64	39	18.75	72	41.03
7	2.95	40	19.45	73	41.85
8	3.54	41	20.04	74	42.68
9	4.01	42	20.63	75	43.62
10	4.36	43	21.22	76	44.45
11	4.83	44	21.81	77	45.39
12	5.31	45	22.40	78	46.22
13	5.78	46	22.99	79	47.16
14	6.20	47	23.58	80	48.06
15	6.72	48	24.17	81	49.00
16	7.19	49	24.76	82	49.99
17	7.55	50	25.35	83	50.98
18	8.02	51	25.94	84	51.99
19	8.49	52	26.65	85	53.06
20	8.96	53	27.35	86	53.93
21	9.43	54	27.99	87	54.23
22	9.90	55	28.58	88	56.00
23	10.38	56	29.24	89	57.06
24	10.85	57	29.95	90	58.24
25	11.32	58	30.65	91	59.30
26	11.79	59	31.24	92	60.25
27	12.26	60	31.83	93	61.43
28	12.73	61	32.54	94	62.72
29	13.20	62	33.13	95	63.95
30	13.68	63	33.88	96	65.13
31	14.15	64	34.66	97	66.31
32	14.74	65	35.37	98	67.79
33	15.33	66	36.08	99	69.25
				100	70.74

APPENDIX B

STATISTICAL ANALYSIS

III. 2,4-D Residue Analysis:

(1) Inject a standard which contains 10 η gm/μl

Volume Injected	Peak Height (Scale Unit)	η gm/Injection	η gm/Scale Unit
3 μl	62	30	.48
3 μl	62	30	.48
3 μl	62	30	.48
2 μl	42	20	.48

(2) Sample weight = 50 gm

(3) Dilution 1:20 ml

(4) μl injected = 5

(5) Peak height = 70 scale units

(6) η gm injected = (70 scale units)(.48 η gm/scale unit)

$$= 33.60 \text{ η gm}$$

(7) $\text{η gm/μl} = \frac{33.60 \text{ η gm}}{5 \text{ μl}} = 6.72 \text{ η gm/μl}$

(8) (η gm)(dilution ml) = (6.72)(20) = 135.49 η gm

(9) $\text{ppm} = \frac{135.49 \text{ η gm}}{50 \text{ gm sample}} = 2.71 \text{ ppm}$

(10) 1.15 ppm derived from the weight of a 15.25 cm plow sole weighing 2 x 10^6 pounds; a 5-cm section would equal 1.5 ppm for a 1.12 kg/ha rate.

(11) $\#/A = \frac{\text{ppm}}{1.5 \text{ ppm}}$

Table 2. The effect of 2,4-D on respiration of Bartlett pears from the upper and lower Hood River Valley. Values are expressed as mg CO_2/kg/hr.

Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
0	9.860	9.745	9.145	10.695	9.423	1.205	1.610
12	34.808	36.740	36.773	38.393	36.398	0.991	3.863
24	32.480	35.615	34.280	36.353	33.843	1.651	3.563
36	42.035	42.933	45.495	49.248	44.400	1.982	6.012
48	44.323	45.093	47.878	47.915	46.313	0.593	6.333
72	49.583	51.448	52.875	55.265	51.445	0.894	6.727
96	58.293	60.020	60.258	60.350	57.770	0.210	7.977
120	59.443	58.043	64.150	62.763	62.820	1.918	5.605
144	54.330	56.123	57.845	59.368	59.080	0.658	7.868
156	52.123	50.728	51.960	53.575	51.948	0.168	7.453
180	47.770	46.750	48.840	49.960	48.965	0.317	6.581
204	44.118	43.398	45.453	45.570	46.280	0.293	6.535
228	37.893	38.428	38.590	40.260	30.095	0.311	4.844
252	35.530	35.403	35.423	37.368	36.415	0.231	5.384

(1) Tabulated values are means of 4 replications

(2) *Significant F value at P = .05; F(4,5) = 5.19

Table 3. The effects of 2,4-D on ethylene production during ripening in Bartlett pears from the lower Hood River Valley. Values are expressed as n moles C_2H_4/kg/hr (10^3)

Time (hr)	Rates of 2,4-D Applied (kg/ha)							
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%	LSD 1%
0								
24	2.124	2.052	2.28	2.244	2.544	0.100	2.196	
48	3.708	3.540	3.888	3.372	4.128	0.387	1.728	
72	4.296	4.044	4.728	3.888	3.780	0.963	1.392	
84	4.608	4.404	3.924	3.912	4.248	0.377	1.800	
96	5.124	5.244	7.332	4.788	4.824	21.903**	0.828	1.296
120	5.652	6.072	5.628	5.424	5.868	1.530	0.732	
144	5.160	5.448	5.604	5.352	6.000	0.552	1.548	
156	4.308	5.376	5.136	4.788	5.460	0.855	1.836	
168	3.600	3.996	3.828	3.696	3.960	0.094	2.100	
192	3.048	3.240	3.516	2.964	3.672	0.656	1.344	
216	1.992	2.100	2.040	2.220	2.064	0.102	0.96	

(1) Tabulated values are means of 2 replications

(2) *Significant F value at P = ,05; F(4,5) = 5.19

(3)**Significant F value at P = .01; F(4,5) = 11.39

Table 4. The effects of 2,4-D on ethylene production during ripening in Bartlett pears from the upper Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
0							
24	1.600	1.068	2.220	2.676	1.632	1.833	1.656
48	2.750	2.652	3.192	3.756	3.264	2.802	1.600
84	3.888	3.468	4.416	4.620	3.600	0.764	2.880
96	4.260	4.464	5.304	6.060	4.920	1.095	2.508
120	5.064	6.048	5.760	6.864	5.988	0.895	2.484
144	4.584	6.096	5.232	6.084	5.748	1.682	1.800
156	4.548	4.896	6.708	5.784	5.868	2.251	1.884
168	4.632	5.088	6.648	5.496	5.100	3.139	1.572
192	2.736	4.248	4.308	3.684	4.152*	7.277*	0.888
216	2.136	2.280	2.844	2.376	3.288*	5.513*	0.756

(1) Tabulated values are means of 2 replications

(2)*Significant F value at P = .05; F(4,5) = 5.19

Table 5. The effect of 2,4-D on respiration rate during ripening of Red Delicious apples from the lower Hood River Valley. Values are expressed in mg CO_2/kg/hr.

Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
0	6.15	6.59	6.37	6.80	7.17	1.356	1.702
12	26.03	28.09	28.18	23.75	27.62	.504	2.689
24	26.17	28.65	24.52	23.75	28.62	1.569	3.298
36	30.26	29.64	29.56	30.33	32.16	1.189	5.694
48	28.61	28.92	28.89	29.72	31.06	.749	4.893
72	30.97	28.33	26.64	29.67	30.25	.234	9.416
96	25.90	25.50	24.58	25.85	25.69	.187	5.718
120	24.90	25.00	25.10	23.96	25.52	.206	7.574
168	23.13	24.77	25.46	22.54	26.47	.223	6.927
192	23.10	22.85	25.02	23.53	23.63	.582	5.491
216	23.10	22.82	25.02	23.86	23.33	.469	5.71
240	22.50	22.25	24.49	22.98	23.08	.804	10.531
288	26.44	25.36	27.12	27.28	27.32	.246	5.830
336	21.83	21.41	23.43	20.99	22.35	.620	3.959
384	19.62	19.16	20.72	20.59	21.31	.195	5.562
432	19.83	19.65	20.40	19.00	20.35	.223	4.642
480	20.57	17.76	20.35	18.94	19.47	.409	4.664
576	14.82	16.20	17.79	16.18	17.72	.483	4.876
696	15.56	15.48	16.60	14.97	16.23	.313	5.220
744	15.15	15.42	16.19	14.75	16.05	.525	4.124

(1) Tabulated values are means of 2 replications

(2) *Significant F value at P = .05; F(4,5) = 5.19

Table 6. The effect of 2,4-D on respiration rate during ripening of Red Delicious apples from the upper Hood River Valley. Values are expressed as mg CO_2/kg/hr.

Sampling Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
0	6.78	8.04	7.12	7.22	7.94	.546	4.731
12	30.05	30.54	26.84	31.88	26.85	.804	7.630
24	31.87	32.31	30.67	28.88	30.48	.658	5.783
36	35.15	36.40	34.26	32.58	33.10	.708	4.452
48	32.58	32.31	31.69	29.81	31.94	.565	4.830
72	32.46	29.19	29.66	30.61	30.51	.585	8.812
96	27.74	28.55	28.08	29.52	28.21	.301	3.941
120	27.52	25.71	26.70	25.09	25.80	.388	4.623
168	28.19	28.29	28.21	26.66	26.52	1.479	3.796
192	26.35	26.17	26.51	23.80	25.00	2.323	2.862
216	26.03	24.70	25.10	23.25	23.88	.479	2.679
240	25.53	25.04	25.16	23.52	23.95	.482	2.716
288	27.83	27.35	27.31	25.98	26.22	.736	2.727
336	23.99	23.77	23.58	21.82	23.18	2.394	2.455
384	24.66	23.08	23.48	23.23	24.21	1.201	2.836
432	22.40	22.49	21.43	21.10	21.91	.789	1.323
480	21.57	19.92	21.05	19.57	20.27	.284	7.764
576	19.65	19.28	18.23	18.19	17.75	.800	1.247
696	18.21	16.82	16.40	16.98	16.06	.632	1.251
744	17.80	17.00	16.27	16.52	15.66	.467	2.637

(1) Tabulated values are means of 2 replications

(2)*Significant F value at P = .05; F(4,5) = 5.19

Table 7. The effects of 2,4-D on ethylene production during ripening of Red Delicious apples from the lower Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
36	.888	.804	.730	.912	.888	.553	360
48	.972	.720	.624	.816	.840	5.737*	204
72	1.140	1.044	.900	1.116	1.164	1.939	275
96	1.072	1.260	1.700	1.272	1.428	1.713	336
120	1.308	1.512	1.320	1.272	1.596	5.577*	228
144							
168	1.752	1.920	2.015	1.884	2.028	1.145	384
192	2.069	2.040	1.824	1.572	1.920	4.729	180
216	1.956	2.004	2.040	2.076	1.944	0.818	240
240	2.016	2.316	2.256	2.076	2.376	0.905	588
264	2.388	2.496	2.676	2.460	2.628	2.515	270
360	1.980	2.136	2.244	2.145	3.216	0.602	600
408	1.812	2.124	2.184	2.016	2.328	4.028	348
456	1.956	2.184	2.148	2.064	2.544	2.212	540
528	1.728	1.950	2.304	1.644	2.196	10.458*	324

(1) Tabulated values are means of 2 replications

(2)*Significant F value at P = .05; F(4,5) = 5.19

Table 8. The effects of 2,4-D on ethylene production during ripening of Red Delicious apples from the upper Hood River Valley. Values are expressed as η moles C_2H_4/kg/hr (10^3)

Time (hr)	Rates of 2,4-D Applied (kg/ha)						
	0	2.24 TT	2.24 SS	4.48 TT	4.48 SS	F	LSD 5%
36	2.196	2.232	1.980	1.896	1.812	0.876	708
48	1.692	1.680	1.704	1.476	1.632	0.446	516
72	2.004	2.244	2.148	2.268	2.124	0.158	972
96	2.028	2.544	2.268	2.124	2.088	2.158	516
120	2.136	2.376	2.148	2.112	2.088	0.414	672
144							
168	2.532	3.204	2.712	2.616	2.652	1.697	744
192	2.604	3.288	2.964	2.628	2.676	0.673	1.296
216	2.700	3.108	2.856	2.856	2.772	0.448	840
240	2.844	3.264	3.000	2.940	2.988	0.516	780
264	3.192	3.660	3.252	3.108	2.952	3.267	528
360	2.892	3.204	2.976	2.928	3.204	0.204	1.236
408	3.000	2.892	3.024	2.736	2.808	0.154	1.128
456	2.796	2.820	2.400	2.700	2.448	1.012	708
528	2.496	2.544	2.244	2.412	2.412	0.287	780

(1) Tabulated values are means of 2 replications

(2)*Significant F value at P = .05; F(4,5) = 5.19

Table 9. ANOVA 3 - Three factor analysis of variance for the respiration rate of Bartlett pears during ripening.

Problem Code: Pear 2 - Respiration

Source	DF	SS	MS	F
LO	1	133.8191	133.8191	8.102
TR	4	476.7432	119.1858	7.216
TI	13	47594.1111	3661.0855	221.648
LO x TR	4	284.7691	71.1923	4.310
LO x TI	13	169.2997	13.0231	.788
TR x TI	52	515.3790	9.9111	.600
LO x TR x TI	52	732.4035	14.0847	.853
Error	140	2312.4595	16.5176	
TOTAL	279	52218.984131		

Means

LO

(1)	(2)
45.7293	44.3466

TR

(1)	(2)	(3)	(4)
43.5775	43.8762	44.9436	47.2123
(5)			
45.5802			

TI

(1)	(3)	(3)	(4)
9.8265	37.0100	34.4215	45.4985
(5)	(6)	(7)	(8)
46.8815	52.5955	60.4750	62.7885
(9)	(10)	(11)	(12)
57.3735	53.4735	49.4320	45.0350
(13)	(14)		
39.0685	36.6520		

LO x TR

(1 ,1)	(1 ,2)	(1 ,3)	(1 ,4)
45.5857	44.7996	45.0889	46.2850
(1 ,5)			
46.8871			
(2 ,1)	(2 ,2)	(2 ,3)	(2 ,4)
41.5693	42.9529	44.7982	48.1396
(2 ,5)			
44.2732			

Table 10. ANOVA 3 - Three factor analysis of variance for ethylene production of Bartlett pears during ripening.

Problem Code: Pear 12H - Ethylene Production

Source	DF	SS	MS	F
LO	1	.0128	.0128	3.216
TR	4	.0925	.0231	5.793
TI	10	2.3390	.2339	58.620
LO x TR	4	.0897	.0224	5.622
LO x TI	10	.1645	.0165	4.124
TR x TI	40	.1169	.0029	.732
LO x TR x TI	40	.0912	.0023	.571
Error	110	.4389	.0040	
TOTAL	219	3.345491		

Means

LO

(1)	(2)
.3613	.3460

TR

(1)	(2)	(3)	(4)
.3174	.3523	.3804	.3571
(5)			
.3611			

TI

(1)	(2)	(3)	(4)
.1752	.2905	.3258	.3409
(5)	(6)	(7)	(8)
.4244	.5107	.4697	.4509
(9)	(10)	(11)	
.3992	.2994	.2035	

LO x TR

(1 ,1)	(1 ,2)	(1 ,3)	(1 ,4)
.2997	.3499	.4091	.3913
(1 ,5)			
.3564			
(2 ,1)	(2 ,2)	(2 ,3)	(2 ,4)
.3350	.3547	.3517	.3229
(2 ,5)			
.3657			

Table 11. ANOVA 3 - Three factor analysis of variance for the respiration rate of Red Delicious apples during ripening.

Problem Code: Apple 2 - Respiration

Source	DF	SS	MS	F
LO	1	433.8056	433.8056	113.918
TR	4	89.9589	22.4897	5.906
TI	19	13423.1255	706.4803	185.522
LO X TR	4	77.0566	19.2641	5.059
LO X TI	19	131.6961	6.9314	1.820
TR X TI	76	154.8121	2.0370	.535
LO X TR X TI	76	146.0524	1.9217	.505
Error	200	761.6125	3.8081	
TOTAL	399	15218.119675		

Means

LO

(1)	(2)
24.4855	22.4027

TR

(1)	(2)	(3)	(4)
23.8454	23.6501	23.5927	22.5124
(5)			
23.6196			

TI

(1)	(2)	(3)	(4)
7.0380	27.9795	28.5895	32.3330
(5)	(6)	(7)	(8)
30.2580	29.4255	26.7265	25.4670
(9)	(10)	(11)	(12)
26.0080	24.7425	24.2175	23.7200
(13)	(14)	(15)	(16)
26.8175	22.5260	22.0000	20.9485
(17)	(18)	(19)	(20)
19.9220	17.6735	16.3320	16.1565

LO X TR

(1,1)	(1,2)	(1,3)	(1,4)
25.4467	24.8482	24.3597	23.8080
(1,5)			
23.9645			
(2,1)	(2,2)	(2,3)	(2,4)
22.2440	22.4520	22.8258	21.2167
(2,5)			
23.2747			

Table 12. ANOVA 3 - Three factor analysis of variance for the respiration rate of Red Delicious Red apples during ripening.

Problem Code: Apple 1 - Ethylene Production

Source	DF	SS	MS	F
LO	1	.3187	.3187	818.702
TR	4	.0050	.0012	3.183
TI	16	.5359	.0335	86.031
LO x TR	4	.0064	.0016	4.132
LO x TI	16	.0346	.0022	5.553
TR x TI	64	.0200	.0003	.802
LO x TR x TI	64	.0219	.0003	.877
Error	170	.0662	.0004	
TOTAL	339	1.008566		

Means

LO

(1)	(2)
.1443	.2056

TR

(1)	(2)	(3)	(4)
.1688	.1804	.1752	.1734

(5)
.1769

TI

(1)	(2)	(3)	(4)
.1231	.1033	.1361	.1453
(5)	(6)	(7)	(8)
.1503	.1950	.1985	.2021
(9)	(10)	(11)	(12)
.2157	.2411	.2200	.2081
(13)	(14)	(15)	(16)
.2112	.1833	.1770	.1348

(17)
.1290

LO x TR

(1,1)	(1,2)	(1,3)	(1,4)
.1377	.1424	.1469	.1425

(1,5)
.1522

(2,1)	(2,2)	(2,3)	(2,4)
.2000	.2184	.2035	.2043

(2,5)
.2016