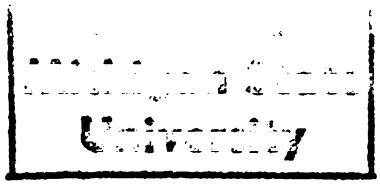

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THE EFFECT OF APPLE CULTIVAR, HARVEST MATURITY AND STORAGE DURATION ON THE CARBON DIOXIDE EVOLUTION RESPONSE OF FRUIT DAMAGED BY BRUISING

presented by

MICHAEL LEE PARKER

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Major professor

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THE EFFECT OF APPLE CULTIVAR, HARVEST MATURITY AND STORAGE DURATION ON THE CARBON DIOXIDE EVOLUTION RESPONSE OF FRUIT DAMAGED BY BRUISING

By

Michael Lee Parker

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ABSTRACT

THE EFFECT OF APPLE CULTIVAR, HARVEST MATURITY AND STORAGE DURATION ON THE CARBON DIOXIDE EVOLUTION RESPONSE OF FRUIT DAMAGED BY BRUISING

By

Michael Lee Parker

Carbon dioxide evolution was examined as a possible measure for determining relatively small quantities of bruise damage to apples. Experiments were conducted to determine the effect of apple cultivar, harvest maturity and storage duration on the carbon dioxide damage response of the fruit to varying degrees of damage.

Application of impact bruise damage ranging from slight to severe to 'Macspur', 'Empire' and 'Rome Beauty' apple fruit resulted in highly significant increases in carbon dioxide evolution over non-damaged fruit. This carbon dioxide response significantly increased with increased degrees of damage; however, it was not affected by other factors observed. The damage treatments also resulted in highly significant increases in the percentage of total fruit tissue damage as assessed by visual means. The amounts were significantly affected by cultivar, time of harvest and storage duration.

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The help of James Brown and Carol (Zurawski) Westerhof was very much appreciated in setting up the research and in data collection.

And most of all I would like to express my gratitude to God and my family and close friends. For the love, patience, wisdom and endurance I received I will always be grateful.

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INTRODUCTION

Damage to produce during handling, transportation and marketing results in an estimated 9-17 percent loss of produce at a value of 1.0-2.0 billion dollars annually (32). Damage to apples destined for fresh market apples approximates a loss of 13.4 million dollars with up to a 5 percent loss in fruit volume (32). Unpublished data of Schoorl (37) shows a 1,000-mile truck transport with six handling operations to bruise 10-24 percent of the apples. It has been shown that apples destined for processing lose approximately 2.8 percent total weight due to the bruising that occurs through all operations after harvest (46). Mechanical injury during handling is the major cause of produce damage (10,37) which results in bruising for apples (12).

Bruising occurs in all operations and has an accumulative effect on a commodity (23), increasing the amount of damage at each operation. Citrus fruit in Japan became more severely bruised as it passed through sorting equipment along a packinghouse line (3). Similarily, apples moving through marketing channels in New York City resulted in progressively increased bruising (10). The accumulation of bruising adversely affects the quality of fruit and even slight, barely visible bruising may result in considerable reduction in quality as the fruit is much more susceptible to decay as with sweet cherries (26). Improving the protective quality of the packages in which the product is marketed can reduce damage. A practical method for determining and comparing the protective

quality of packages would be useful in selecting a superior package. A suitable method to detect bruising must be fast, accurate and objective.

Research with a wide range of fruit has indicated that an increase in carbon dioxide evolution of fruit following damage offers possibilities as a damage indicator. Carbon dioxide is a natural product evolved from the fruit and is easily and accurately measured with current technology (18,22).

The objective of this research was to determine the reliability of carbon dioxide as a damage indicator for relatively small quantities of bruising and to determine how this damage response is affected by cultivar, fruit maturity and storage duration.

LITERATURE REVIEW

Bruising is a term applied to injury, distortion or bursting of cells resulting from a physical force applied to fruit tissues. In apples, browning of the tissue is a consequence of cell sap oxidation (11,27,40) which is mediated by concentrations of chlorogenic acids and flavanol in the fruit tissue (16). The amount of bruised apple tissue is highly correlated to the energy absorbed by the tissue during the applied force (11). Physical injury to apples by slowly applied compression forces results in more bruise damage to the tissue than a rapid impact of the same energy level; for a given quantity of bruising, an impact force must be twice as great in order to yield the same amount of damaged tissue as a compression force (11,27).

Much fruit bruising could be eliminated or greatly reduced by improving the protective qualities of the packages in which fruit are handled and marketed. Klein (18) has proposed that apple package performance could be based on carbon dioxide evolution of the fruit as a damage indicator. There are many factors which may affect bruising and the physiological response of the fruit to bruising that need to be understood before this method can be developed, particularily fruit cultivar and maturity and the length of time the fruit has been stored (27,38,40,46). Apple fruit maturity significantly affects the degree of bruising since fruit maturation results in softening of tissue and greater susceptibility to damage (46). Apple tissue becomes less elastic so that the energy absorption through temporary cell deformation is decreased and the cells absorb less energy before becoming permanently damaged

(27). It has been demonstrated that bruise diameter and depth increase with advancing maturity of the fruit (14,38,46). The same is likely true for cherries since the most mature fruit yielded higher weight losses than less mature fruit following physical injury (26). Tomato fruit at the breaker stage of maturity with red color initially evident were found to be four times more readily bruised than fruit at the mature green stage and eight times more readily bruised than at the immature stage (23). Also, the degree of bruising in peaches is related to maturity, with the most mature fruit receiving the most damage (43).

The extent of bruising and the degree and rate of browning of the damaged tissue varies by cultivar of apple (14,16,40,41,46). Schoorl and Holt (40) found 'Jonathan', 'Delicious' and 'Granny Smith' apples after 5 months of storage to have highly significant differences in the volume of bruised tissue resulting from a standard applied force. At this time 'Jonathan' was the most severly bruised followed by 'Delicious' and 'Granny Smith', respectively. Wennergren and Lee (46) reported varietal differences in the bruising of 'Golden Delicious', 'York' and 'Stayman' fruit. Bruising, measured as percent of bruised flesh showed 'York' fruit to be the most resistant to bruising followed by Golden Delicious' and then 'Stayman'. Hyde and Ingle (14) report the order of increased resistance of apples to bruising as 'Stayman', 'McIntosh', 'Delicious', 'Rome', 'Golden Delicious' and 'Jonathan'.

Duration of the storage period also affects the susceptibility of fruit to bruise damage. Schoorl and Holt (40) reported that the longer the fruit are in storage prior to bruising, the greater the

amount of bruised tissue results when a given force is applied. Fruit ripening and deterioration increase with aging and is accompanied by decreases in flesh firmness during the storage period (2). On the other hand Hyde and Ingle (14) showed no increase in the degree of bruising for 6 cultivars of apples stored at 2°C for a maximum of nine weeks, but rather a decrease in bruising for fruit stored for longer storage periods.

Visual assessment of mechanical damage to fruit to determine the extent and source of injury in the handling chain is time consuming and generally impractical for evaluating large quantities of fruit. Various methods for detection of the actual occurance of damage have been considered. An artificial potato containing sensors and transmitters to measure and record impacts and other damaging forces was developed for analyzing handling systems (31). Another type of device was used for 'Red Delicious' and 'McIntosh' apples (7), whereby computer image analysis of bruising was made as the fruit passed a detector in a packinghouse line. The extent of damage in apples can also be measured by decreases in the electrical resistance of fruit tissue when cell sap is released upon rupture of the cell walls and membranes (8).

A major criterion for predicting the amount of fruit damage that may occur in a package is the amount of energy absorbed by the package and its contents (11,13,37,38). Holt and Schoorl (11), found a high correlation between the quantity of bruised tissue and the amount of energy absorbed by the apple. One method predicts the percentage of bruised fruit in various packages for both apples and pears (38). The percentage of fruit damage in packages is predicted

by an equation based on a relationship of fruit cultivar, drop height and number of drops (37,38). Prediction of where the most fruit damage is likely to occur in multilayered packages (39) and the types of packages that should provide the most protection for the fruit have also been reported (37). Holt et. al.(13,41) recently proposed the analysis of packaging and handling systems as determined by the energy absorbed by a package which could be used for predicting the amount of bruising the fruit receives. This method relates the bruise volume to the energy absorbed by the tissue, considering bruise resistance of the fruit, the equation of the motion of the drop surface and the mass and rebound height of the package (41).

Various methods have been used to bruise fruit for experimental purposes. Robitaille and Janick (34) dropped a 190 gram stainless steel weight, through a guidance tube unto an apple resting in a cork ring. Schoorl and Holt (11,40) bruised apple halves firmly fixed in place by use of a specially designed device which used a spring loaded projectile to strike the fruit. Hyde and Ingle (14) used a free swinging weight, much like a pendulum, to bruise fruit. Greenham (8) used a thumb tip to apply pressure bruises to apples. Other methods employed are drops of varying heights and numbers, Instron type equipment and various devices to damage fruit for experimental purposes. No standard or generally accepted method has been used.

The physiological responses of fruit to physical injury has been suggested by many as a possible means for quantifying damage for many fruit. Cutting of cantaloupe doubled the carbon

dioxide(CO_2) evolution with a ten fold increase in ethylene(C_2H_4) evolution (24). Avocados increased in respiration, as measured by CO_2 evolution and oxygen(O_2) uptake upon shaking (1). The dropping of cranberries caused an increase in CO_2 evolution with a greater number of drops yielding higher increases in CO_2 evolution (22). The bruising of tart cherries produced an increased CO_2 evolution (33). Vibration forces applied to sweet cherries yielded an increase in CO_2 evolution, but variable results in C_2H_4 evolution (26). Tomatoes exposed to vibration, impacts and dropping had increased rates of CO_2 evolution (20,21,29) and tomatoes damaged by cutting or dropping had increases in C_2H_4 evolution (20,25). Citrus fruit yield a marked increase in CO_2 evolution following physical injury. Damage as a result of dropping, rolling, compression and vibration increased the CO_2 evolution of the fruit with greater levels of damage (3,6,15,17,44,45). Ethylene increases in citrus were variable, with 'Satsuma' mandarin exhibiting no increase in evolution of C_2H_4 after dropping (15), whereas 'Marsh' grapefruit showed intermediate increases in C_2H_4 as a result of dropping (45).

Injury to apples at the preclimacteric stage of fruit development, prior to the production of endogenous C_2H_4, caused an increase in C_2H_4 evolution, whereas damage to fruit at a later stage of maturation in which endogenous C_2H_4 was being produced resulted in a decrease in C_2H_4 evolution by the fruit (18,19,34). This variability would make the use of C_2H_4 as an indicator of damage in apples dependent upon a knowledge of the specific stage of maturity of the fruit. Studies involving numerous cultivars of apple indicate consistent increased CO_2 output responses as a result of

dropping (7,18,42). Apples in which the tissue is cut also exhibited higher levels of CO_2 evolution (9,30). The extent of the CO_2 response in apples is not the same for all cultivars, for example 'Winter Banana' apples exhibited an increased CO_2 evolution following damage from dropping, whereas 'Rome Beauty' apples had no significant increase in CO_2 evolution due to damage (42).

MATERIALS AND METHODS

Three cultivars of apples were selected to provide a range in fruit bruising characteristics. 'Macspur', a strain of 'McIntosh', an early fall harvested apple with flesh that softens fairly rapidly after harvest and fruit with a normal storage life expectancy of three to six months, depending on the method of storage. 'Empire' was selected as representative of a cultivar harvested midseason which retains good flesh firmness for four to seven months in cold storage. A late season harvested apple which retains its flesh firmness during long term storage, up to nine months, was represented by 'Rome Beauty'('Rome'). The 'Macspur' fruit was harvested from trees with M7 rootstocks, 'Empire' from trees on M26 with an interstem, and the 'Rome' from trees with either MM111 or M2 rootstocks. 'Macspur' and 'Empire' were harvested from trees approximately 9 years old and the 'Rome' were harvested from trees approximately 20 years old. All were grown in experimental plots at the Michigan State University Graham Station.

Fruit of each cultivar were harvested at three harvest maturities, one week before the estimated optimum harvest date for long-term storage, at optimum and one week after optimum for each cultivar for each of the three storage durations. The optimum harvest date was based on predicted harvest dates recommended by Michigan State University for the cultivars of 'McIntosh', 'Jonathan' and 'Delicious' calculated from bloom data and temperature conditions following bloom for 30 days, internal ethylene production of the individual fruit and the accumulation of ethylene from ten fruit sealed in a container as developed by

Dilley (5).

For each cultivar at each harvest date approximately 500 fruit of uniform size were carefully hand harvested and placed in foam tray packs. Trays were randomly placed in corrugated tray pack boxes to minimize tree and handling variation. The boxes were lined with a polyethylene film to prevent excessive moisture loss, yet loosely closed to provide sufficient aeration to allow normal respiration during storage. The fruit were stored in air at approximately 1°C for 100 or 199 days after harvest. The fruit for the 0-day storage treatment were not refrigerated.

Impact damage to the fruit was applied using the pendulum shown in Fig. 1. The arm of the pendulum was constructed with 1/2-inch steel rod and the striking surface of the pendulum constructed with a piece of 3-inch angle iron covered with teflon sheeting to prevent abrasion of the cuticle and epidermis of the fruit. The pendulum was designed to strike the fruit at the base of the pendulum arc before reaching the pendulum stop. The fruit was transferred by the force of the pendulum into a padded catching container to prevent further damage to the fruit. The pendulum provided a standard force that could be delivered to any selected location on the fruit, for this experiment on the cheek of the apple. Since the amount of tissue bruised by impact varies with different tissues of the apple (4), it was important to place the bruise in approximately the same location each time. The pendulum also offered a means to reproduce an impact force and to vary the impact force in the increments desired. With the pendulum, a bruise was applied to one side of the fruit without damaging any other part of the fruit. Five damage

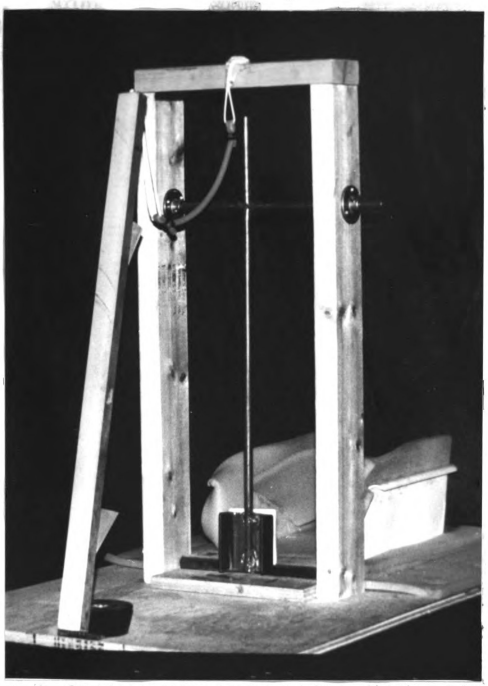
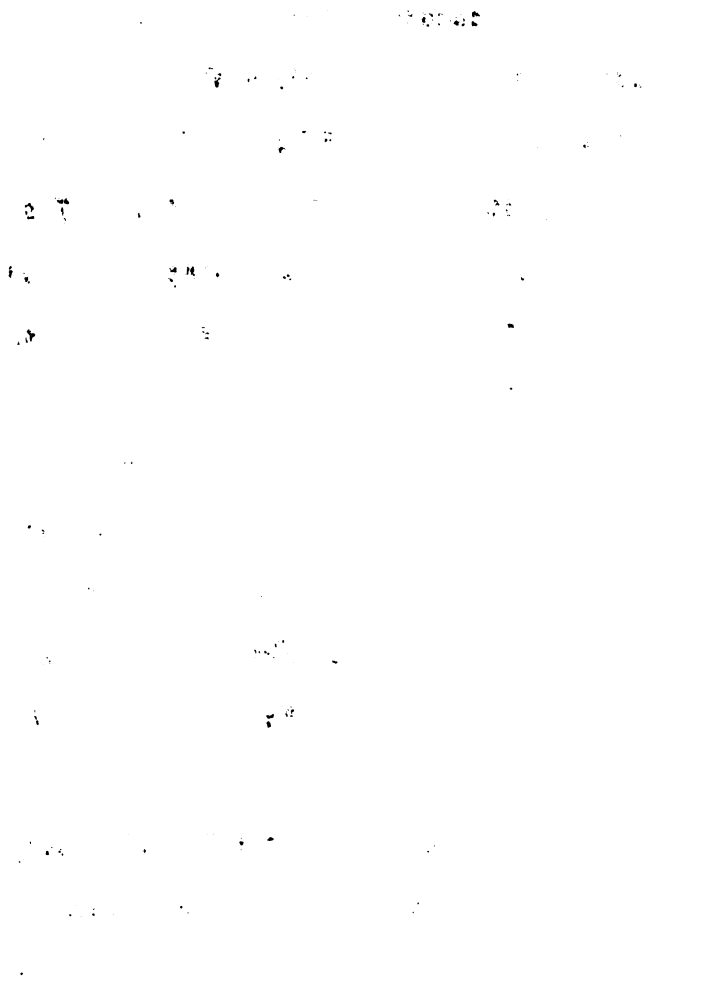


Figure 1. Pendulum apparatus constructed for application of force to produce bruising to apple fruit. A fruit is placed on the platform and the pendulum released to strike the fruit, which is deflected into the padded catching container.



per fruit, 2 small bruises per fruit on opposite sides, 4 small bruises per fruit equally spaced around the equator of the fruit and 1 large bruise approximately equivalent to 3 small bruises in respect to quantity of damaged tissue. The small bruises were applied with the pendulum swinging through an arc of 40.5° with an arm radius of 52 cm which produced a bruise approximately equivalent to dropping the fruit 15 cm onto a hard surface. The one large bruise was applied with the pendulum swinging through an arc of 90° at an arm radius of 52 cm which yielded a bruise approximately equivalent to a drop of 50 cm.

A factorial experimental design consisting of 4 factors completely randomized was selected with the factors analysed being cultivar, harvest date, storage period and damage level. An experimental run was made at each of the 3 storage periods using 3 cultivars, 3 harvest dates and 5 damage levels. There were 5 replications of each damage level.

Approximately 100 fruit were removed from cold storage the day before the start of each experimental run except for the 0-day storage period which was made the day after harvest. This time period allowed the fruit to equilibrate to the laboratory temperature of approximately $21^{\circ}C$ to ensure uniform bruising results (27,36). Ten fruit were randomly selected for measurement of internal ethylene, flesh firmness, and relative starch content using the procedure outlined by Saltveit (35).

Three fruit, free of visible defects, were randomly placed in each of 25 plastic airtight freezer containers of 2.5 liter

capacity. Each fruit sample was weighed in the containers and the containers were randomly assigned to treatments the day before starting an experiment. Following the bruising treatment the containers were closed with snap-on airtight lids fitted with serum caps to facilitate sampling of the internal atmosphere with syringes. The headspace atmosphere of the containers was analyzed for CO_2 content after closure at hourly intervals up to six hours. The procedure was modified after the initial run of 'Macspur' at the 0-day storage period in order to obtain greater accuracy in measuring the CO_2 response. Instead of immediately treating the fruit at the start of the experimental run, a pretreatment analysis of the CO_2 evolution of each sample of fruit was made hourly for four hours to provide a base level of CO_2 evolution for each sample. The containers were then opened and completely aerated prior to the damage treatment of the fruit. After all treatments were applied the containers were resealed and the atmosphere of the containers analyzed hourly as in the original method.

The CO_2 content of the container atmosphere was determined by removing a 1 ml sample with a syringe for injection into a Carle 8700 (804-B) gas chromatograph with silica gel molecular sieve columns in parallel with a differential thermal conductivity detector, using helium as the carrier gas. The gas chromatograph output was recorded with a Hewlett-Packard 3390A integrator which was calibrated to give readings as percentage of CO_2.

After the 6-hour reading the sealed containers remained sealed on the laboratory bench for approximately 24 hours to allow the brown discoloration of the bruised tissue to occur (16,41). Upon

opening, the browned tissue and skin was excised from each apple and weighed. This was done by cutting the skin around the perimeter of the visible bruise on the surface of the fruit. Then all of the brown tissue and skin was removed by using a small scoop to remove only the brown tissue.

The CO_2 evolution data was calculated in units of ml/kg hr, taking into account fruit weight, headspace of the containers and the length of time the containers were sealed. The data were analyzed for CO_2 evolution for each hour and for the net response as determined by subtracting the posttreatment CO_2 hourly readings of each sample from the pretreatment readings where the latter data were obtained. The weights of bruised tissue were expressed as percent of total fruit weight for evaluation by analysis of variance. A correlation of CO_2 evolution and percent of fruit weight bruised for the entire experiment was determined for the fourth and fifth hour post-treatment. Correlations were also made with modifications in the data for CO_2 evolution and percent of fruit weight bruised. A correlation of the entire experiment, omitting the data for the control, to eliminate zeros in the data, was determined for the data at the fourth and fifth hour post-treatment. The correlation between the increase in CO_2 from pre-treatment over post-treatment CO_2 mean percent bruised tissue weight per treatment was determined. Several correlations between CO_2 and percent of bruised tissue for individual cultivars, maturities and storage durations were determined also.

RESULTS

The physical and physiological characteristics of the fruit at the beginning of each experimental run are presented in Tables 1,2 and 3. Fruit internal ethylene values increased with the later harvest dates for each cultivar, internal ethylene values also increased as the fruit were stored from 0 days to 199 days. Flesh firmness values decreased markedly after 100 days of storage (Table 2). The fruit stored for 199 days (Table 3) had similar characteristics to those stored for 100 days. 'Macspur' fruit had the least flesh firmness, whereas 'Rome' fruit were usually the most firm throughout the experiment. Starch content decreased for 'Macspur' and 'Empire' at the second and third harvests and upon storage for 100 days (Table 1) and 199 days (Table 3). The starch rating of 'Rome' fruit at the earliest harvest (Table 1) was high, indicating a low starch content. The subsequent changes due to later harvests and longer storage periods, therefore, were small.

Since hourly readings were similar, singular hourly CO_2 data for main effects and interactions were utilized. Two methods were evaluated to determine the most suitable time and method of data collection. The first method utilized the rate of total CO_2 evolved at 1 to 6 hours after the fruit treatment, whereas, the second method utilized the difference in rate of CO_2 before treatment and the rate of CO_2 after the damage treatment at 1 to 4 hours. The mean square values and their level of significance as determined by analysis of variance are presented in Tables 4 and 5. The occurance of highly significant mean square values were similar for the two methods. The selected hour and method of data

Table 1. The internal ethylene, flesh firmness and starch rating of 10 apples at one day following harvest (0-day storage duration).

Harvest	Harvest Date	Cultivar	Internal Ethylene (ppm)			Mean Flesh Firmness (kg)	Mean Starch Rating
			0.5	Less than 1.0	1.0 and greater		
	(1983)		(no. of fruit)				
1	9-12	Macspur	10	0	0	7.9	2.7
1	10-03	Empire	8	1	1	8.1	2.0
1	10-12	Rome	9	0	1	9.1	7.7
2	9-19	Macspur	9	0	1	7.1	3.9
2	10-10	Empire	6	2	2	7.9	3.7
2	10-19	Rome	1	0	9	8.6	7.9
3	9-26	Macspur	0	0	10	6.7	7.0
3	10-17	Empire	8	0	2	7.5	5.3
3	10-26	Rome	10	0	0	8.6	8.6

Table 2. The internal ethylene, flesh firmness and starch rating of 10 apples at 100 days following harvest (100-day storage duration).

Harvest	Harvest Date	Cultivar	Internal Ethylene (ppm)			Mean Flesh Firmness (kg)	Mean Starch Rating
			0.5	Less than 1.0	1.0 and greater		
	(1983)		(no. of fruit)				
1	9-12	Macspur	0	0	10	3.3	8.2
1	10-03	Empire	0	0	10	5.1	9.0
1	10-12	Rome	0	1	9	4.7	8.8
2	9-19	Macspur	1	0	9	3.7	8.6
2	10-10	Empire	0	0	10	4.1	3.5
2	10-19	Rome	0	0	10	4.8	8.6
3	9-26	Macspur	0	0	10	4.0	9.0
3	10-17	Empire	0	0	10	4.6	8.5
3	10-26	Rome	0	0	10	4.8	8.8

Table 3. The internal ethylene, flesh firmness and starch rating of 10 apples at 199 days following harvest (199-day storage duration).

Harvest	Harvest Date	Cultivar	Internal Ethylene (ppm)			Mean Flesh Firmness (kg)	Mean Starch Rating
			0.5	Less than 1.0	1.0 and greater		
	(1983)		(no. of fruit)				
1	9-12	Macspur	0	0	10	4.3	9.0
1	10-03	Empire	0	0	10	4.7	9.0
1	10-12	Rome	0	0	10	5.2	9.0
2	9-19	Macspur	0	0	10	3.6	10.0
2	10-10	Empire	0	0	10	3.7	9.0
2	10-19	Rome	0	0	10	5.5	9.0
3	9-26	Macspur	0	0	10	3.4	10.0
3	10-17	Empire	0	0	10	3.9	9.0
3	10-26	Rome	0	0	10	5.4	9.0

Table 4. Analysis of variance mean square values for rate of carbon dioxide evolution of apples determined at one to six hours post-treatment.

Source of Variation	df	Mean Square Values					
		1 Hour	2 Hour	3 Hour	4 Hour	5 Hour	6 Hour
Cultivar	2	599.9**	620.3**	613.9**	643.1**	645.0**	403.8**
Harvest	2	3939.0**	3159.6**	2109.9**	1571.2**	1345.6**	1031.4**
Storage Duration	2	10024.7**	6552.4**	5770.3**	4118.5**	3950.1**	3073.4**
Damage Treatment	4	36.5**	48.4**	42.6**	33.8**	45.6**	29.5**
Cultivar x Harvest	4	80.3**	42.1**	67.7**	31.4**	45.6**	19.5**
Cultivar x Storage Duration	4	191.4**	200.7**	147.7**	91.3**	85.2**	100.1**
Harvest x Storage Duration	4	509.7**	347.4**	230.1**	204.4**	173.1**	142.4**
Cultivar x Damage Treatment	8	8.1	4.5	3.2	2.4	1.6	2.8
Harvest x Damage Treatment	8	9.4	1.9	2.8	1.2	1.6	1.2
Storage Duration x Damage Treatment	8	7.7	11.0*	5.6	5.7	3.8	3.9
Cultivar x Harvest x Storage Duration	8	211.1**	119.3**	125.3**	75.2**	87.7**	77.2**
Cultivar x Harvest x Damage Treatment	16	9.5	2.7	1.4	0.9	1.1	1.4
Cultivar x Storage Duration x Damage Treatment	16	9.4	3.2	2.2	3.6	2.5	2.2
Harvest x Storage Duration x Damage Treatment	16	9.0	1.9	1.5	1.5	1.1	1.4
Error	570	9.5	4.5	3.3	2.9	2.3	2.5

* Indicates significance at the .05 level of probability and ** indicates significance at the .01 level of probability as determined by the F test.

Table 5. Analysis of variance mean square values for rate of carbon dioxide evolution of apples determined at one to four hours post-treatment rates minus pre-treatment rates. The data for 'Macspur' at the 0-day storage duration are not included.

Source	df	Mean Square Values			
		1 Hour	2 Hour	3 Hour	4 Hour
Cultivar	2	129.3**	81.6**	19.4**	127.2**
Harvest	2	154.0**	530.9**	271.5**	61.5**
Storage Duration	2	1088.9**	345.9**	355.7**	53.0**
Damage Treatment	4	31.7**	41.9**	49.1**	31.8**
Cultivar x Harvest	4	57.8**	23.2**	46.3**	44.6**
Cultivar x Storage Duration	3(1)[a]	141.5**	47.8**	25.7**	9.4**
Harvest x Storage Duration	4	485.9**	74.3**	19.9**	32.6**
Cultivar x Damage Treatment	8	11.0	2.5	4.2	1.4
Harvest x Damage Treatment	8	17.8	7.6	2.7	2.6
Storage Duration x Damage Treatment	8	10.8	6.5	2.8	5.4
Cultivar x Harvest x Storage Duration	6(2)[a]	30.9	36.9**	38.1**	13.7**
Cultivar x Harvest x Damage Treatment	16	13.5	5.0	4.3	2.0
Cultivar x Storage Duration x Damage Treatment	12(4)[a]	22.9	6.4	2.9	1.9
Harvest x Storage Duration x Damage Treatment	16	13.2	5.9	2.8	1.7
Error	500(72)[a]	16.1	4.5	3.1	2.2

[a] Values in parentheses are the number of missing values for data not collected.

** Indicates significance at the .01 level of probability as determined by the F test.

collection must detect a clear separation of the damage treatments to facilitate analysis of the data and the method must be easily executed. The method employing post-treatment data only, therefore was selected for use since the complete set of data was available and this method was much quicker as pretreatment readings were not needed. Data collected at the fifth hour were selected for use because the F test(mean square value divided by error term) was greatest at the fifth hour than at the others due to a small error value and high mean square value. Furthermore, all main effects of cultivar, damage treatment, time of harvest and storage duration were highly significant at all hourly observations (Table 4).

The CO_2 response due to damage treatment decreased in rate of evolution and was consistent for all damage treatments throughout (Fig. 2). By the fifth hour there were significant differences(.05 level of probability) in CO_2 evolution rates between all damage treatments except one and two small bruises per fruit and two small bruises and one large bruise per fruit (Table 6). Four small bruises per fruit had the highest rate of CO_2 evolution with the differences being highly significant from all other damage treatments, including one large bruise per fruit. The latter was the second highest in rate of CO_2 evolution.

The only significant interaction for CO_2 evolution involving damage treatment was for the second hour reading. This interaction of damage treatment X storage duration (Table 4) was significant at the .05 level of probability.

Figure 2. The effect of damage to apples of all cultivars, storage durations and harvests on the rate of CO_2 evolution measured at one to six hours following application of the damage.

Figure 2.

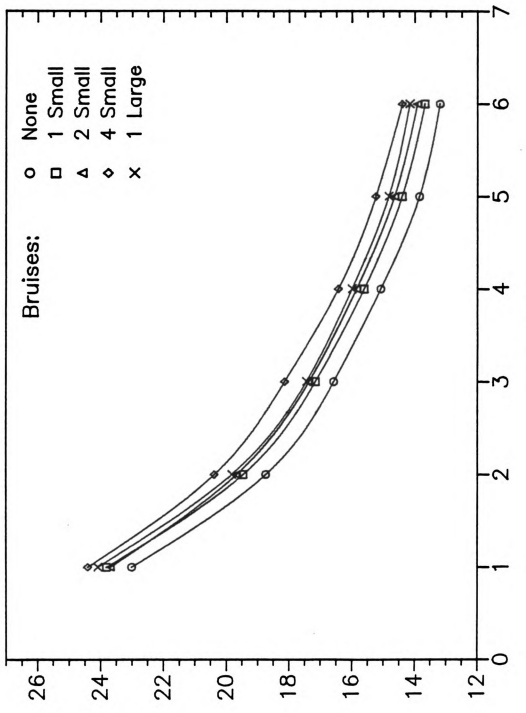

Bruises:
None
1 Small
2 Small
4 Small
1 Large
CO_2 (ml/kg•hr)
HOURS
26
24
22
20
18
16
14
12
0
1
2
3
4
5
6
7

Table 6: The rate of CO_2 evolution of apples at the fifth hour following damage treatment, for all cultivars, storage durations and harvest maturities.

Damage Treatment	CO Evolution (ml/kg hr)
0 Small Bruises/Fruit	13.85 a[z]
1 Small Bruise/Fruit	14.40 b
2 Small Bruises/Fruit	14.64 bc
4 Small Bruises/Fruit	15.24 d
1 Large Bruise/Fruit	14.83 c

LSD at .01 = 0.34

[z] Letters signify difference by LSD at .01 level of probability.

The mean square values for the amount of tissue damage are presented in Table 7. This analysis excluded the non-bruise treatment because of the absence of damaged tissue, and therefore zero values. All main effects were highly significant. As shown in Table 8, highly significant differences occured between amounts of bruised tissue for all damage treatments. As true for CO_2 evolution, the greatest tissue damage occured for the treatment of four small bruises per fruit. However, the second most damaging treatment as measured by bruised tissue was one large bruise. This was unlike the CO_2 response (Table 6) in which the rate of CO_2 evolution for one large bruise was similar to that of two small bruises per fruit.

Table 7. Analysis of variance mean square values for percent of bruised tissue based on the total weight of the fruit. The control treatment data (zero values) were excluded in the analysis.

Source	df	Mean Square Values
Cultivar	2	2.3**
Harvest	2	4.0**
Storage Duration	2	41.9**
Damage Treatment	3	174.0**
Cultivar x Harvest	4	0.5**
Cultivar x Storage Duration	4	4.5**
Harvest x Storage Duration	4	0.4**
Cultivar x Damage Treatment	6	0.2*
Harvest x Damage Treatment	6	0.2*
Storage Duration x Damage Treatment	6	2.7**
Cultivar x Harvest x Storage Duration	8	1.1**
Cultivar x Harvest x Damage Treatment	12	0.06
Cultivar x Storage Duration x Damage Treatment	12	0.3**
Harvest x Storage Duration x Damage Treatment	12	0.1
Error	454(2)[a]	0.08

[a] The value in parentheses is the number of missing values.

* Indicates significance at the .05 level of probability and ** indicates significance at the .01 level of probability as determined by the F test.

Table 8. Mean percentages of excised bruised tissue according to damage treatment.

Treatment	Percent ot total fruit weight bruised	
1 Small Bruise/Fruit	0.88	a[z]
2 Small Bruises/Fruit	1.73	b
4 Small Bruises/Fruit	3.40	d
1 Large Bruise/Fruit	2.88	c

LSD at .01 = 0.09

[z] Letters indicate highly significant differences at the .01 level of probability.

Various correlation relationships of CO_2 evolution and percent of bruised tissue were found to account for no more than 13 percent of the variance, see Table 9. Correlations of all the data at the fourth and fifth hours post-treatment account for 7.5 and 8.0 percent of the variance, respectively. Correlations in which the control values (0 values) were removed at the fifth hour account for 11.6 percent of the variance. A correlation of the 'Empire' fruit at the fourth hour post-treatment accounted for 13.0 percent of the variance, this correlation accounted for the most variance of all the correlations (Table 9).

Table 9. Correlation values between carbon dioxide evolution rates and percentage of bruised tissue at different hours of observation. Data for all cultivars, harvests and storage durations were included along with several of the individual interactions.

Cultivar, Harvest and Storage Duration of Fruit in Correlation	df	R^2 Value
4 hr Post-treatment All Data	666	.077
4 hr Index (Pre/Post) All Data	94	.096
4 hr Post-treatment All Macspur 1st Harvest 1st Storage Duration	21	.055
4 hr Post-treatment All Empire	221	.134
4 hr Post-treatment All Empire 2nd Harvest All Storage Durations	72	.082
4 hr Post-treatment All Empire 2nd Harvest 2nd Storage Duration	22	.016
5 hr Post-treatment All Data	669	.082
5 hr Post-treatment All Data w/o Control	534	.118

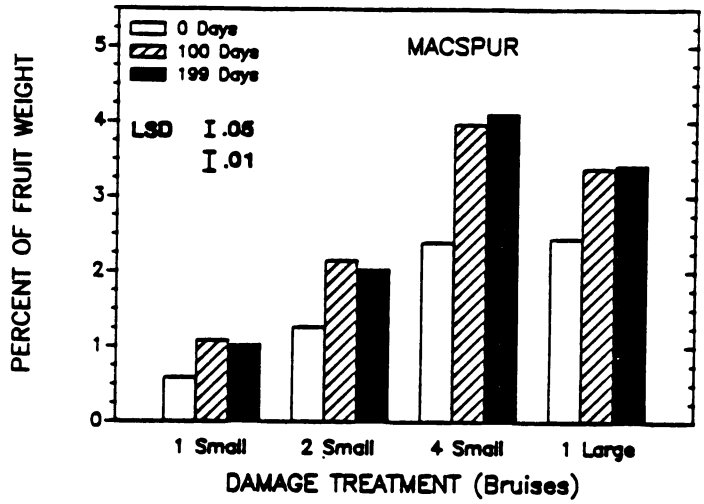
FACTORS AFFECTING TISSUE DAMAGE

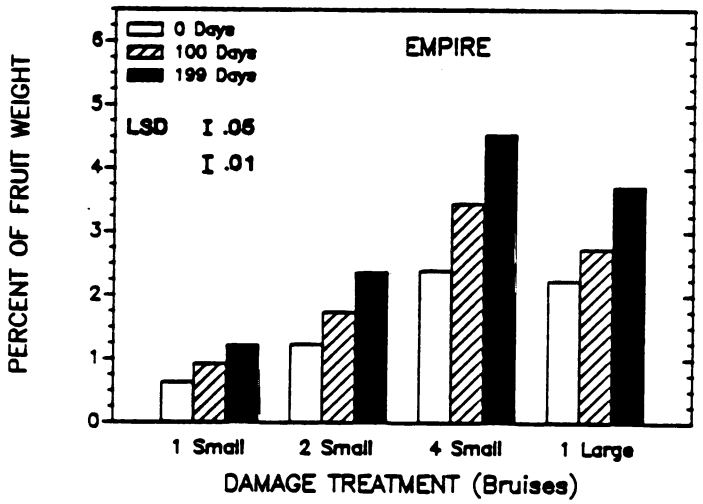
There was a highly significant three way interaction of tissue damage as determined by brown discoloration involving cultivar, storage duration, and damage treatment,see Table 7. This three way interaction is illustrated by the histograms of Figs. 3,4 and 5.

Each histogram presented in Figure 3 is for a cultivar relative to the damage treatments applied following each storage duration. For 'Macspur' there were highly significant differences at each damage treatment between fruit stored 0 days and those stored for 100 and 199 days, with no significant difference between the damage response of fruit stored for 100 and 199 days. 'Empire' fruit had highly significant differences between all three storage durations at all damage treatments, with the least damage at 0 days and the most at 199 days. 'Rome' fruit responded similar to 'Macspur' in that there were highly significant differences in the damage response between fruit stored 0 days and those held for 100 and 199 days, but not between fruit stored for 100 and 199 days. As true for 'Empire', the least damage to 'Macspur' and 'Rome ' occured at 0 days of storage.

The separate histograms of Figure 4 are for the damage treatments. The application of 1 small bruise per fruit, upper left, resulted in no significant difference between cultivars for both the 0 and 100-day storage durations. The only significant difference occured at the 199-day storage duration; it was between the two cultivars of 'Empire' and 'Rome'. Two small bruises per fruit, upper right histogram, resulted in significant differences

Figure 3. The effect of damage treatment and storage duration on the percent of bruised tissue for three apple cultivars.


MACSPUR
0 Days
100 Days
199 Days
LSD I .05
I .01
PERCENT OF FRUIT WEIGHT
0
1
2
3
4
5
1 Small
2 Small
4 Small
1 Large
DAMAGE TREATMENT (Bruises)


EMPIRE
0 Days
100 Days
199 Days
LSD I .05
I .01
PERCENT OF FRUIT WEIGHT
0
1
2
3
4
5
6
1 Small
2 Small
4 Small
1 Large
DAMAGE TREATMENT (Bruises)

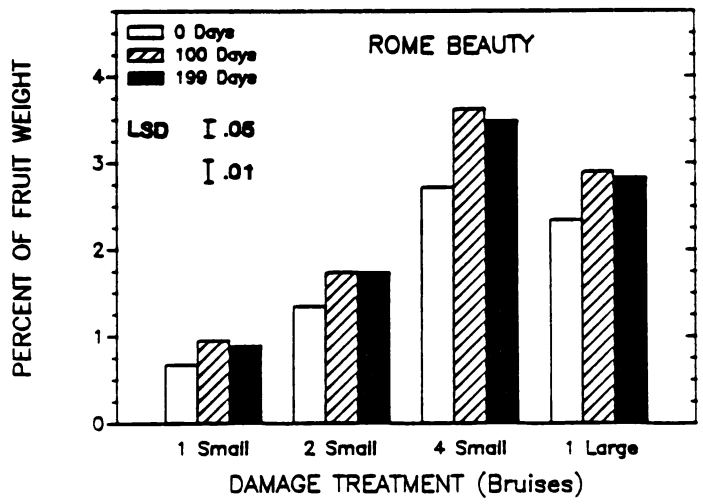

ROME BEAUTY
0 Days
100 Days
199 Days
LSD I .05
I .01
PERCENT OF FRUIT WEIGHT
0
1
2
3
4
1 Small
2 Small
4 Small
1 Large
DAMAGE TREATMENT (Bruises)

Figure 3.

Figure 4. The effect of cultivar and storage duration on the percent of bruised tissue for the levels of damage applied to apples.

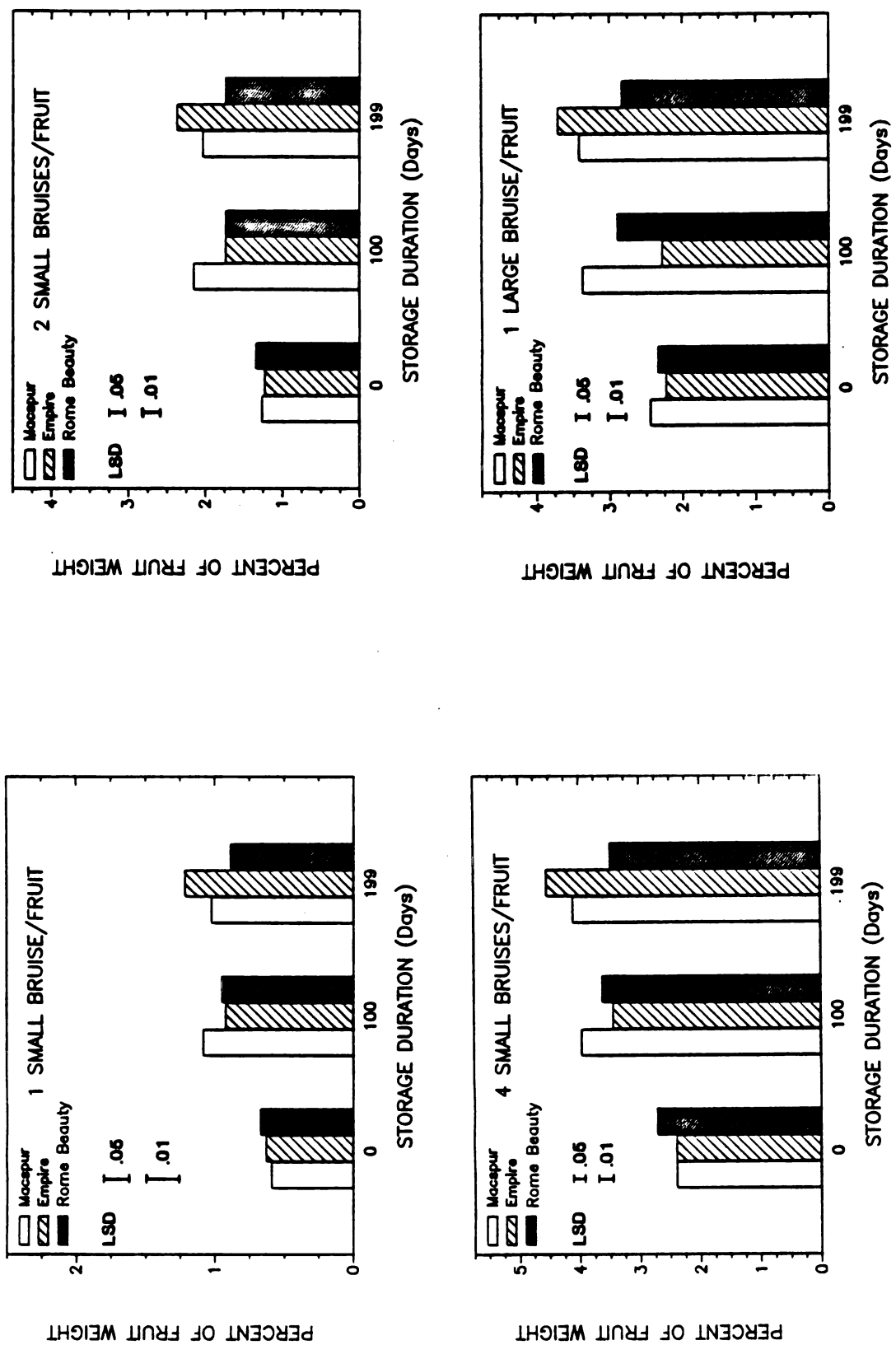

1 SMALL BRUISE/FRUIT
2 SMALL BRUISES/FRUIT
1 LARGE BRUISE/FRUIT
4 SMALL BRUISES/FRUIT
Macspur
Empire
Rome Beauty
LSD .05
.01
PERCENT OF FRUIT WEIGHT
STORAGE DURATION (Days)
0
100
199

Figure 4.

Figure 5. The effect of damage treatment and apple cultivar on the percent of bruised tissue for three storage durations.

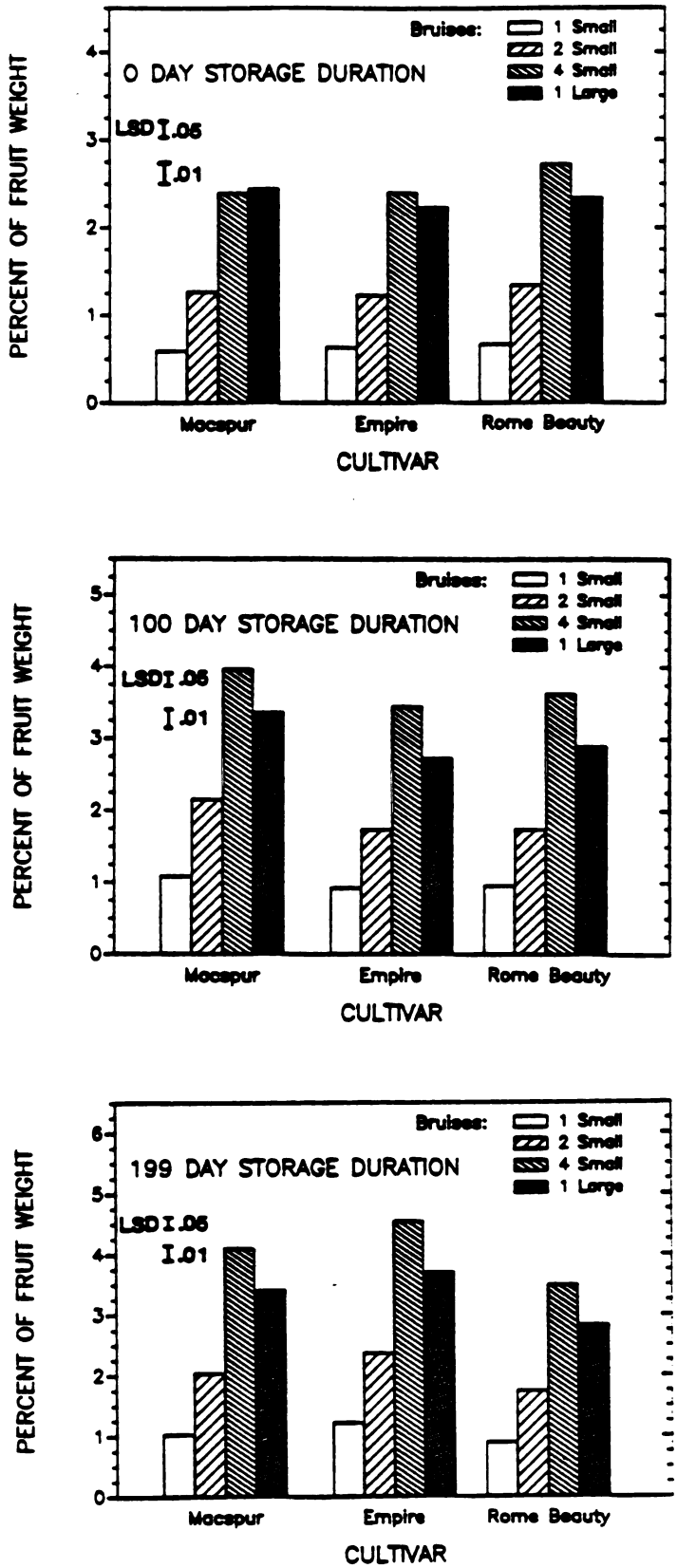

Bruises:
1 Small
2 Small
4 Small
1 Large
0 DAY STORAGE DURATION
LSD I.05
I.01
PERCENT OF FRUIT WEIGHT
Macspur
Empire
Rome Beauty
CULTIVAR
100 DAY STORAGE DURATION
199 DAY STORAGE DURATION

Figure 5.

between all three cultivars at the 0-day storage duration. After the 100-day storage duration 'Macspur' had highly significantly greater amounts of bruised tissue both 'Empire' and 'Rome'. For the 199-day storage duration, there were highly significant differences between all three cultivars with 'Empire' receiving the most damage followed by 'Macspur' and 'Rome'. For four small bruises per fruit, lower left histogram, no significant differences occured between 'Macspur' and 'Empire' at the 0-day storage duration. There were highly significant differences between the response of 'Macspur' and 'Empire' to that of 'Rome', with 'Rome' being the most severely bruised. At the 100-day storage duration there were highly significant differences between the response of 'Macspur' and that of 'Empire' and 'Rome', with 'Macspur' being the most severely bruised with 'Empire' and 'Rome' responding similarily. Fruit stored for 199 days had highly significant differences between the response of all three cultivars with 'Empire' being the most severely bruised followed by 'Macspur' and 'Rome', respectively. For one large bruise per fruit, shown in the lower right histogram, there were significant differences at the 0-day storage duration only between the response of 'Macspur' and 'Empire', with 'Macspur' and 'Rome' being bruised similarily, above that for 'Empire'. For the 100-day storage duration there were highly significant differences between all three cultivars, with 'Macspur being the most severely bruised followed by 'Rome' and 'Empire', respectively. There were highly significant differences between the response of all cultivars for the 199-day storage duration with 'Empire' being

the most severely damaged followed by 'Macspur' and 'Rome', respectively.

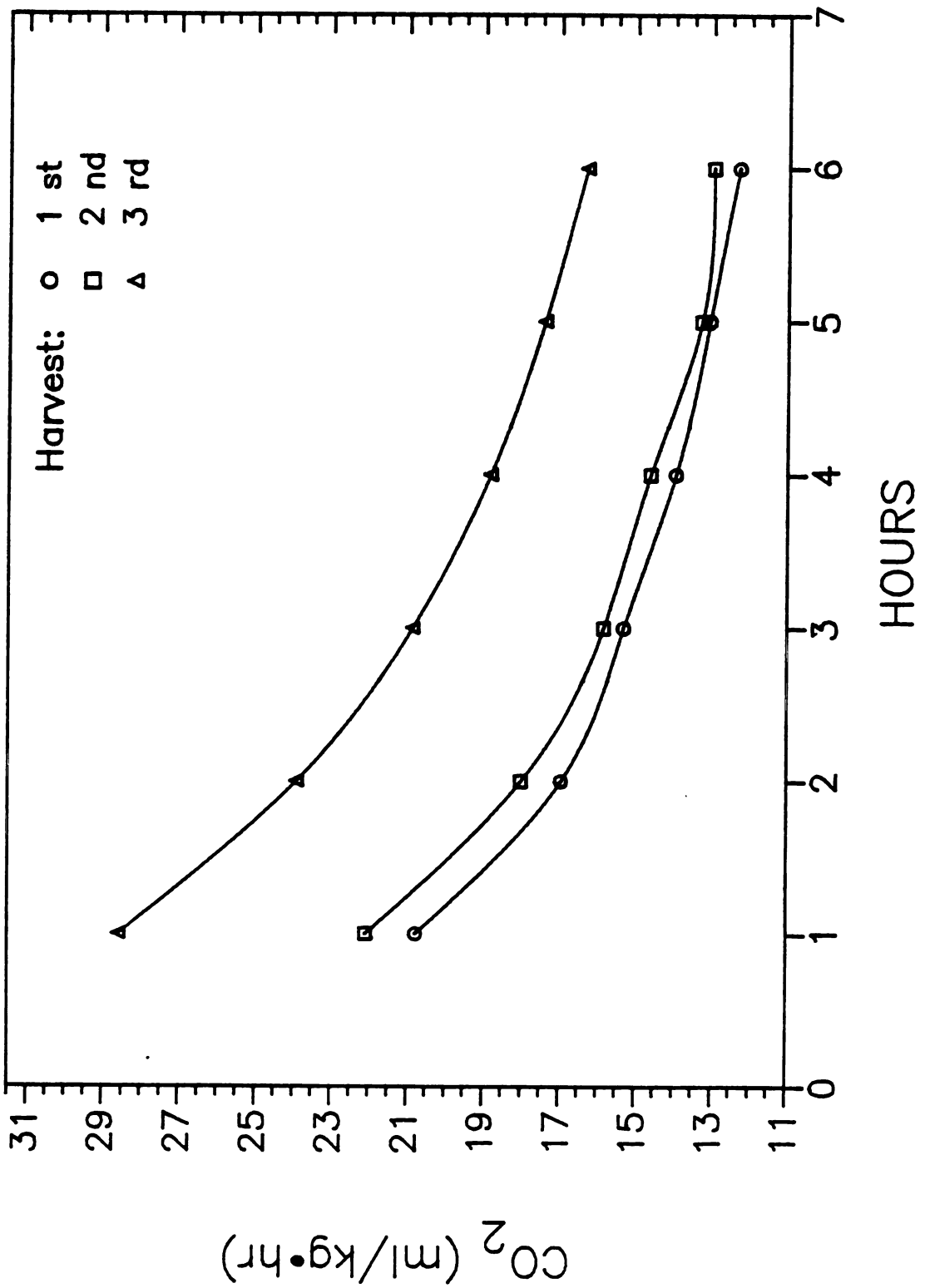
The effects of damage and cultivar on the amount of bruised tissue for the storage durations are illustrated in Fig. 5. At the time of harvest (0-day storage duration) there were highly significant differences for 'Macspur' and 'Empire' between all damage treatments except four small bruises per fruit and one large bruise per fruit. For 'Rome' fruit there were highly significant differences between all damage treatments with four small bruises being the most severe treatment followed by one large bruise, two small bruises, and one small bruise, respectively. After storage for 100 and 199 days, all cultivars had highly significant differences between all damage treatments. Four small bruises per fruit was the most severe followed by one large bruise, two small bruises and one small bruise, respectively.

FACTORS AFFECTING CARBON DIOXIDE EVOLUTION

The CO_2 evolution of the fruit over time for the main effect of harvest date is shown in Fig. 6. As characteristic of the curves for damage treatment (Fig. 2), there was a decline in the rate of CO_2 evolution with the passage of time at which the CO_2 was analyzed. Fruit harvested one week after optimum maturity (third harvest) had the highest rate of CO_2 evolution, being approximately 1.3 times greater than the other two harvests at all times of measurement (Fig. 6). Fruit harvested at optimum maturity (harvest two) had the next highest rates of CO_2 evolution followed closely by fruit harvested one week earlier. The difference between fruit of

Figure 6. The effect of time of harvest of apples for all cultivars, storage durations and damage treatments on the rate of CO_2 evolution at one to six hours following the application of the damage.

Figure 6.


Harvest: o 1 st
□ 2 nd
△ 3 rd
CO_2 (ml/kg•hr)
HOURS
31
29
27
25
23
21
19
17
15
13
11
0
1
2
3
4
5
6
7

harvest three and harvest one or harvest two was highly significant, whereas the differences between harvest one and harvest two were highly significant at all readings except the fifth hour.

The effect of storage duration on the rates of CO_2 evolution for all cultivars, harvests and damage treatments is depicted in Fig. 7. Fruit stored for 199 days had the highest rates of CO_2 evolution followed by fruit stored for 100 days. The lowest rates of CO_2 evolution were for fruit treated at harvest (0-day storage duration). The differences between all three storage durations were highly significant at all readings. The rates for fruit stored for 100 days were approximately seventy percent of those for fruit stored for 199 days; whereas at 0 days the rates were about sixty percent of those for fruit stored for 199 days.

The reponse of the cultivars, including all harvest dates, storage durations and damage treatments, is illustrated in Fig. 8. 'Empire' had the highest rate of CO_2 evolution followed by 'Macspur', then 'Rome'. There were highly significant differences in the rate of CO_2 evolution between all three cultivars. All curves followed the decreasing pattern with time of sampling as observed in Figs. 6 and 7.

There was no significant interaction involving damage treatment on CO_2 evolution at any time of sampling (Table 4). All two way and three way interactions involving harvest date, storage duration and/or cultivar were highly significant (Table 4). The three way interaction of harvest date x storage duration x cultivar was highly significant as well, with the trends graphically shown in Figs. 9,10, and 11.

Figure 7. The effect of storage duration of apples for all cultivars, damage treatments and harvest on the rate of CO_2 evolution measured at one to six hours following application of the damage.

Figure 7.

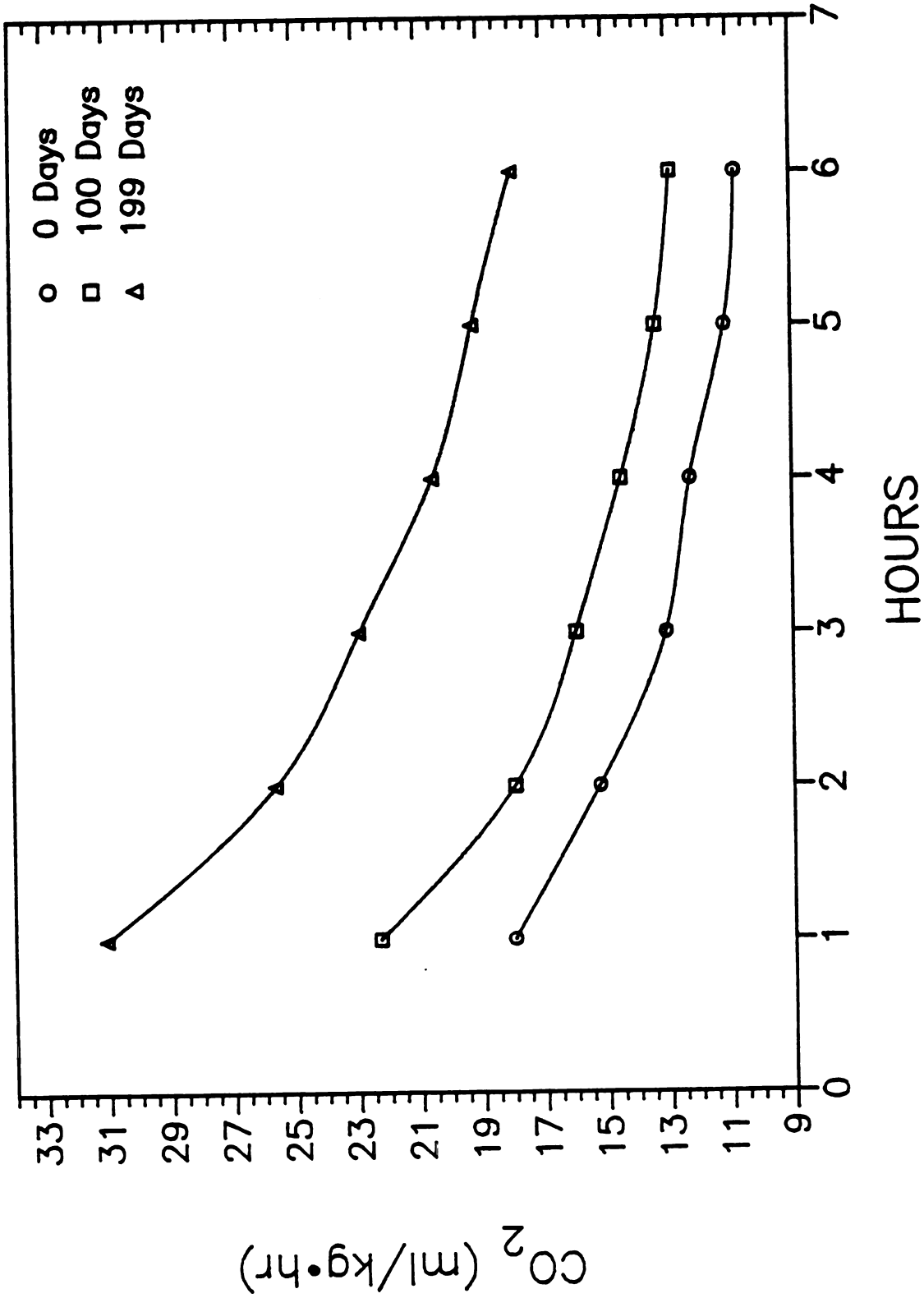

0 Days
100 Days
199 Days
HOURS
CO_2 (ml/kg•hr)
33
31
29
27
25
23
21
19
17
15
13
11
9
0
1
2
3
4
5
6
7

Figure 8. The effect of cultivar of apples for all damage treatments, storage durations and harvests on the rate of CO_2 evolution measured at one to six hours following the application of damage.

Figure 8.

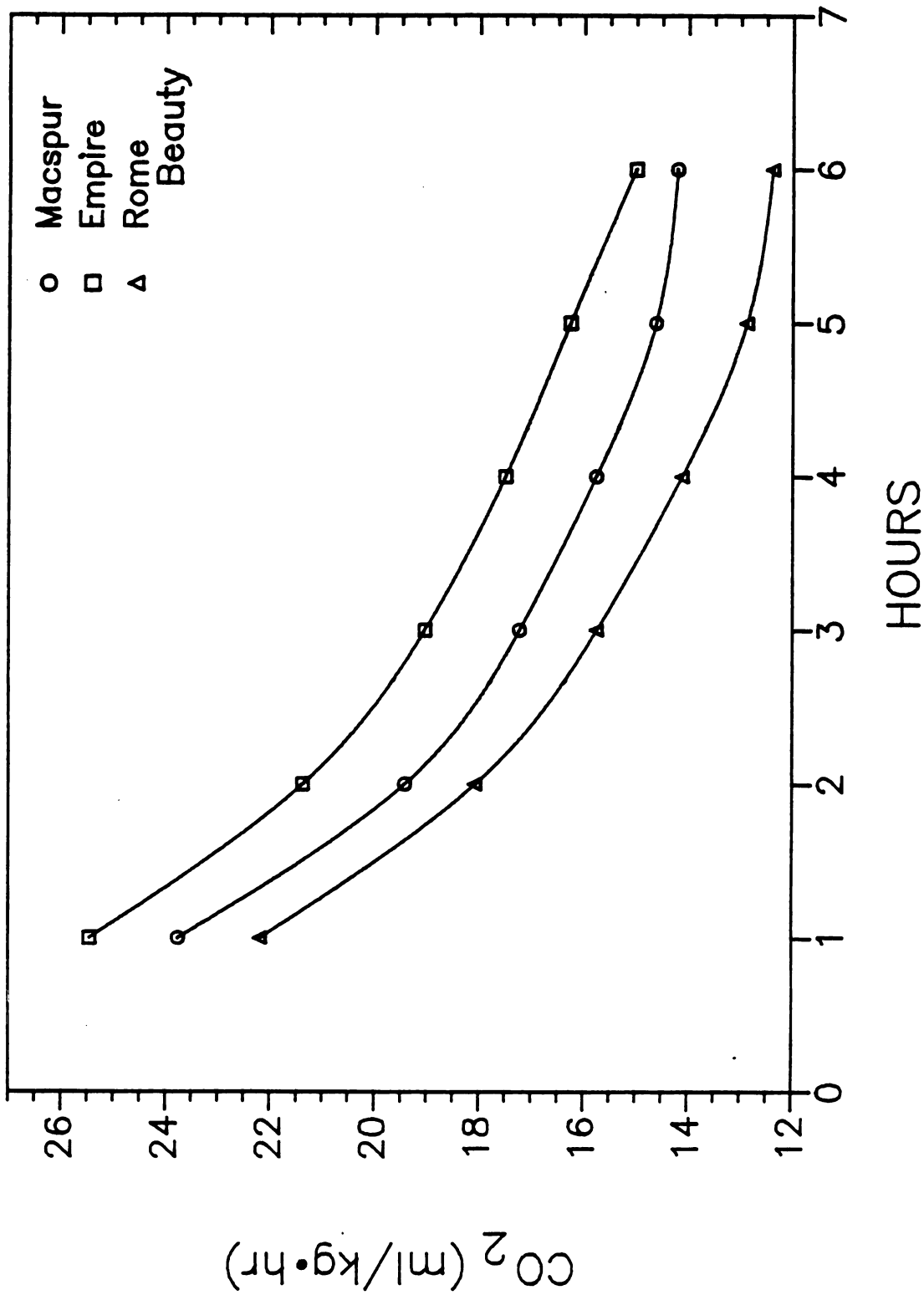

Macspur
Empire
Rome Beauty
HOURS
CO_2 (ml/kg•hr)
0
1
2
3
4
5
6
7
12
14
16
18
20
22
24
26

Figure 9. The effect of apple cultivar and time of harvest on the rate of CO_2 evolution measured at the fifth hour following the damage treatment of apples at three storage durations.

Figure 9.

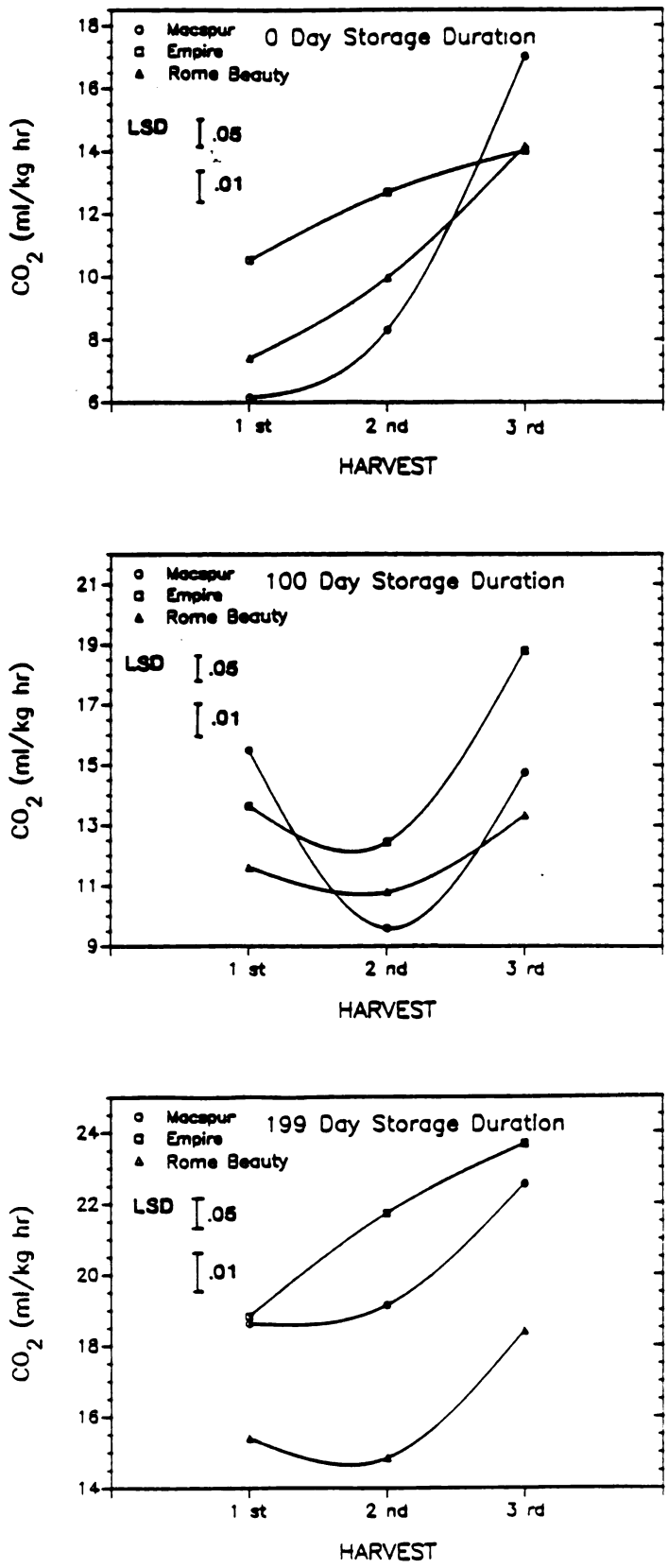

Macapur
Empire
Rome Beauty
0 Day Storage Duration
LSD .05
.01
CO_2 (ml/kg hr)
18
16
14
12
10
8
6
1 st
2 nd
3 rd
HARVEST
Macapur
Empire
Rome Beauty
100 Day Storage Duration
LSD .05
.01
CO_2 (ml/kg hr)
21
19
17
15
13
11
9
1 st
2 nd
3 rd
HARVEST
Macapur
Empire
Rome Beauty
199 Day Storage Duration
LSD .05
.01
CO_2 (ml/kg hr)
24
22
20
18
16
14
1 st
2 nd
3 rd
HARVEST

Figure 10. The effect of time of harvest and storage duration on the rate of CO_2 evolution measured at the fifth hour following the damage treatment of apple cultivars.

Figure 10.

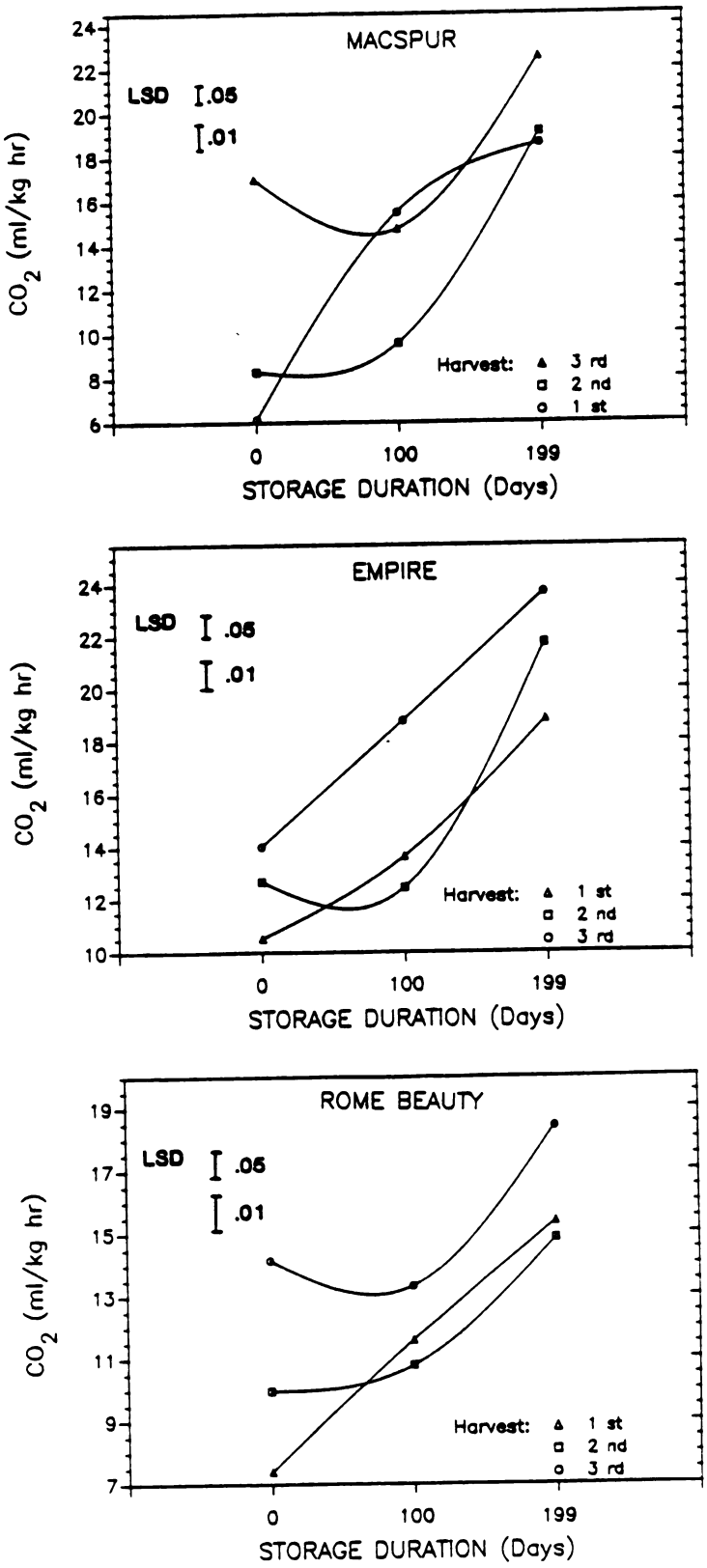
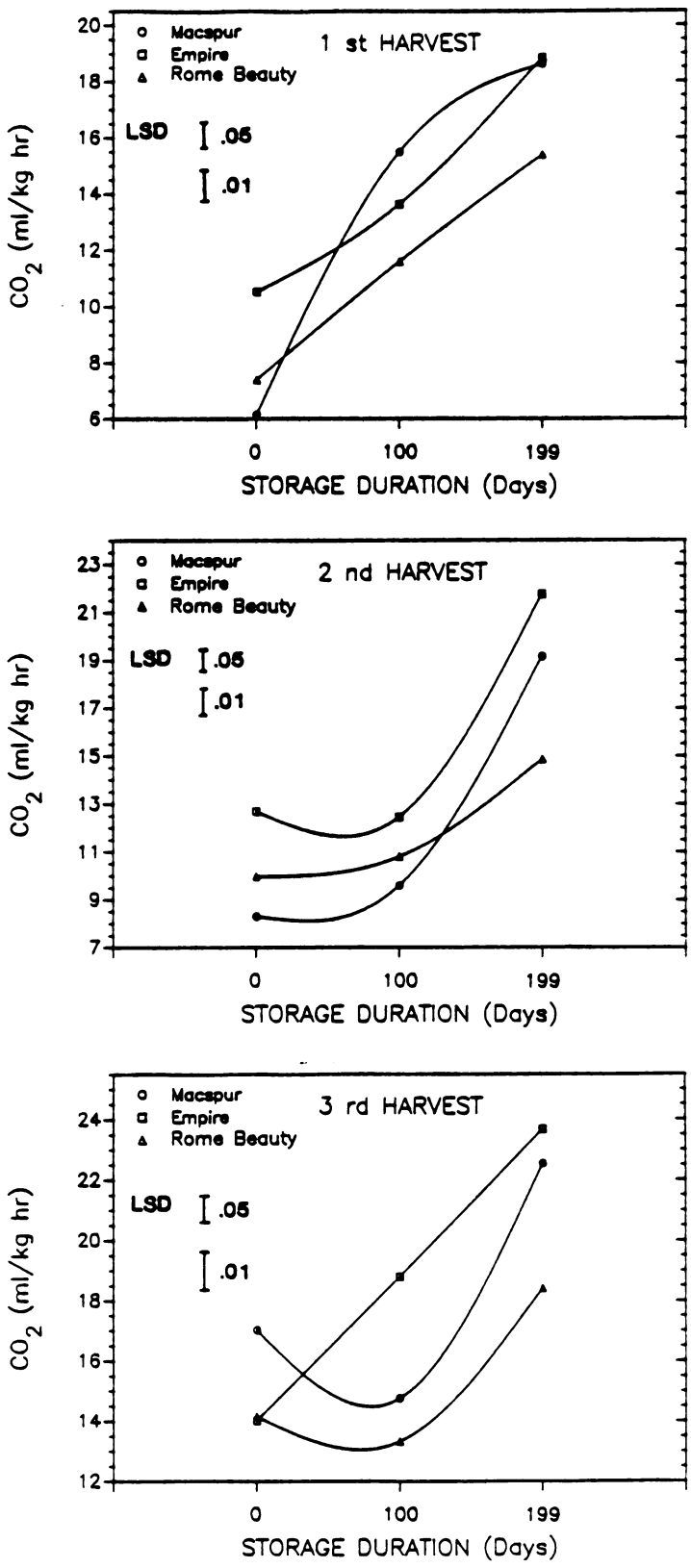

MACSPUR
LSD .05
.01
CO_2 (ml/kg hr)
Harvest: 3 rd
2 nd
1 st
STORAGE DURATION (Days)
EMPIRE
LSD .05
.01
CO_2 (ml/kg hr)
Harvest: 1 st
2 nd
3 rd
STORAGE DURATION (Days)
ROME BEAUTY
LSD .05
.01
CO_2 (ml/kg hr)
Harvest: 1 st
2 nd
3 rd
STORAGE DURATION (Days)

Figure 11. The effect of cultivar and storage duration on the rate of CO_2 evolution measured at the fifth hour following the damage treatment for time of harvest.

Figure 11.


Macspur
Empire
Rome Beauty
1 st HARVEST
LSD .05
.01
CO_2 (ml/kg hr)
20
18
16
14
12
10
8
6
0
100
199
STORAGE DURATION (Days)
Macspur
Empire
Rome Beauty
2 nd HARVEST
LSD .05
.01
CO_2 (ml/kg hr)
23
21
19
17
15
13
11
9
7
0
100
199
STORAGE DURATION (Days)
Macspur
Empire
Rome Beauty
3 rd HARVEST
LSD .05
.01
CO_2 (ml/kg hr)
24
22
20
18
16
14
12
0
100
199
STORAGE DURATION (Days)

All cultivars of the 0-day storage duration had increasing rates of CO_2 evolution with each succeeding harvest (Fig. 9). 'Empire' fruit had the highest rate of CO_2 evolution for harvests one and two, yet the lowest rates at harvest three, similar to that of 'Rome'. 'Macspur fruit of the first and second harvests had the lowest rates of CO_2 evolution at the 0-day storage duration, with a highly significant increase at harvest three to the highest rate of CO_2 evolution. 'Rome' of harvests one and two had intermediate levels of CO_2 evolution between that of 'Empire' and 'Macspur' with fruit of harvest three being similar to that of 'Empire' after a highly significant increase.

After 100 days of storage(Fig. 9), all three cultivars had a decrease in the rate of CO_2 evolution at the second harvest. 'Macspur' had the highest rates of CO_2 evolution of all three cultivars at the first harvest. A significant decrease to the lowest rate of all cultivars occured at the second harvest with a highly significant increase at the third harvest which was intermediate in rate between the other two cultivars. 'Empire' fruit had an intermediate rate of CO_2 evolution at the first harvest with a significant decrease at the second harvest, but this level was the highest of all three cultivars at the second harvest. With 'Empire' there was a highly significant increase in the rate of CO_2 evolution at the third harvest to the highest rate of all fruit of the 100-day storage duration. 'Rome' fruit had the lowest rate of all three cultivars at the first harvest with a non-significant decrease, at the .05 level of probability, at the second harvest. A

slight but significant increase was seen at the third harvest, this rate was the lowest of all three cultivars at the third harvest.

All three cultivars after 199 days of storage had similar patterns of CO_2 evolution relative to sequence of harvest (Fig. 9). 'Empire' and 'Macspur' of harvest one had similar rates of CO_2 evolution. For fruit of the second and third harvests, 'Empire' had the highest rates of CO_2 evolution, 'Macspur' was intermediate and 'Rome' was lowest. 'Rome' fruit of the first and second harvests had similar rates of CO_2 evolution, but there was a significant increase for harvest three. All rates of CO_2 evolution from 'Rome' were highly significant lower than the levels of CO_2 evolution of 'Macspur' and 'Empire' from all three harvests.

The graph for each cultivar in Fig. 10 illustrates the effects of harvest date and storage duration on CO_2 evolution. 'Macspur' fruit of harvest three had the highest levels of CO_2 evolution for all three storage durations. There was a decrease at 100 days for harvest three to a level similar to harvest one and then a large increase for fruit stored 199 days, as was true for all harvests. Harvest two fruit had intermediate levels of CO_2 at the 0-day storage duration with the lowest rates of all three cultivars at the 100-day storage duration after a small significant increase in CO_2 evolution. There were highly significant increases for harvest two fruit with successive storage durations to the 199-day storage duration at a level similar to that of harvest one. Harvest one fruit had the lowest rates of CO_2 evolution at the 0-day storage duration. With a highly significant increase at the 100-day storage duration to a level equivalent to harvest three fruit. Then there

was highly significant increase at the 199-day storage duration which was similar to harvest two, still significantly below the harvest three fruit.

'Empire' fruit of harvest three (Fig. 10) had significantly higher rates of CO_2 than fruit of harvests one and two. The rate increased significantly with each increase in storage duration. Harvest one fruit also increased with each increase in storage durations with the lowest rates of the three harvests at the 0 and 199-day storage duration and between harvest two and harvest three at the 100-day storage duration. Harvest two fruit had levels of CO_2 evolution intermediate between harvest one and three at the 0 and 199-day storage durations. 'Empire' from harvest two had the lowest rate at the 100-day storage duration with no increase in the rate of CO_2 evolution between the 0 and 100-day storage durations.

Harvest three of the 'Rome' fruit (Fig. 10-bottom) had the highest rates of CO_2 evolution for all harvest dates with similar rates at the 0 and 100-day storage durations with a large increase at the 199-day storage duration. Harvest one fruit had an increase in the rate of CO_2 evolution with each successive storage duration, with the lowest rates of all three cultivars at the 0-day storage duration. Intermediate levels of CO_2 evolution occured at the 100 and 199-day storage durations were similar to those of harvest two. Harvest two fruit had intermediate levels of CO_2 evolution at the 0-day storage duration with no significant increase after the 0-day storage duration and a highly significant increase after the 100-day storage duration.

The effect of storage duration on the cultivar response of

fruit from different harvests are presented in Fig. 11. For harvest one fruit there was an increase in CO_2 evolution for all three cultivars at each successive storage duration. 'Empire' fruit had the highest rate of CO_2 evolution at the 0-day storage duration, it was intermediate at the 100-day storage duration, and similar to 'Macspur' at the 199-day storage duration. The 'Rome' fruit had an intermediate rate of CO_2 evolution at the 0-day storage duration, and the lowest of the 3 cultivars at the 100 and 199-day storage durations. 'Macspur' fruit, although with the lowest rate of CO_2 evolution at the 0-day storage duration, increased greatly to the highest at the 100-day storage duration.

The CO_2 response of harvest two fruit (Fig. 11) of all cultivars was similar for fruit stored for 0 and 100 days. 'Empire' fruit had the highest rate of CO_2 evolution following all three storage periods with no significant change between the 0 and 100-day storage durations with a highly significant between the 100 and the 199-day storage durations. 'Rome' fruit had intermediate levels of CO_2 evolution for the 0 and 100-day storage durations, with no significant change between the two. The increase at the 199-day storage was highly significant, but this fruit had the lowest rate of CO_2 evolution of all three cultivars at the 199-day storage duration. 'Macspur' fruit had the lowest rates of CO_2 evolution for the 0 and 100-day storage durations, with a highly significant increase between the two but smaller in magnitude than other increases for the cultivars of that harvest. A much higher rate for 'Macspur' occured at the 199-day storage duration than at the 100-day reading. It was intermediate in rate of CO_2 evolution at

the 199-day storage duration.

The lower graph in Fig. 11 depicts a varied response of the three cultivars from harvest three. 'Macspur' had the highest rate of CO_2 at the 0-day storage duration, it decreased to an intermediate level at the 100-day storage duration and remained intermediate in spite of a highly significant increase at the 199-day storage duration. 'Empire' and 'Rome' fruit had similar rates of CO_2 evolution at the 0-day storage duration, but significantly below that of 'Macspur'. 'Empire' fruit significantly increased in CO_2 output at both the 100 and 199-day storage durations to the highest rate of all three cultivars. 'Rome', on the other hand, did not significantly change at the 100-day storage duration and showed a highly significant increase at the 199-day storage duration. 'Rome' had the lowest rates of CO_2 evolution of all three cultivars at the 100 and 199-day storage durations.

Fruit of harvest three generally had the highest rates of CO_2 evolution for all three cultivars. The harvest one fruit had intermediate levels of CO_2 evolution at the 0 and 199-day storage durations and the lowest rate when sampled after 100 days of storage. Harvest one fruit were lowest in CO_2 evolution at the 0-day storage duration, intermediate at the 100-day storage duration, and variable at the 199-day storage duration.

DISCUSSION

The similar and significant increases in CO_2 evolution and percentage of bruised tissue that resulted from the damage treatments substantiate the findings of Klein (18) for a CO_2 damage response of apples. The relatively small amounts of bruising applied which yielded measurable increases in the rate of CO_2 evolution indicate that the CO_2 damage response is suitable for determining small differences in the protective qualities of packages used for apple handling. One small bruise, approximately equivalent to dropping a fruit from a height of 15 cm yielded a significant increase in CO_2 evolution. One large bruise, approximately equivalent to dropping fruit from a height of 50 cm, yielded a lower increase in CO_2 evolution than four small bruises. Drop heights of 50 cm are not likely to occur during normal handling practices, whereas, numerous small drops of 15 cm may occur.

Neal and Hulme (30) and Klein (18) concluded that the increased CO_2 evolution was due to physical damage resulting from the decarboxylation of malic acid released from cells upon rupture of the cellular membrane, as a result of the physical stress. Marks and Varner (21) reported this same process to be the source of the increased CO_2 damage response in tomatoes. The almost complete absence of significant interactions of damage treatment with cultivar, harvest time and/or storage duration on CO_2 evolution suggests that there was a consistent damage response, so that regardless of variations in other fruit characteristics, there is a

consistent measurable increase in CO_2. Previous studies (Dewey, Klein and Parker-Unpublished data) have shown that this consistent damage response occurs for numerous cultivars and for apples of various maturities and storage backgrounds. The CO_2 damage response was found by Klein (18) to occur even in apple fruit harvested five weeks after ontogeny.

The significant three way interaction involving cultivar, storage duration and damage treatment on the amount of bruised tissue substantiates the findings of Hyde and Ingle(14) that changes in fruit characteristics are of effect on the amount of damage. It is widely accepted that apple fruit cultivars, times of harvest and storage durations have variable maturation, ripening and senescent responses, all of which result in variable responses in susceptability to bruising (38,40,42,46). All three cultivars responded similarly at harvest (Fig. 5), but the effect of fruit maturation and softening was observed at the 100-day storage duration with 'Macspur' being bruised to an amount highly significant above that of 'Empire' and 'Rome' for all treatments except 1 small bruise per fruit. Yet after 199 days of storage, 'Empire' was more severly bruised than 'Macspur' and 'Rome'. This was likely due to the earlier onset of senescence for 'Macspur' since barely 75 sound fruit were available from the approximately 200 harvested for this experimental run. It is unlikely that the 'Macspur' used at this time were representative of the fruit at harvest in that they were probably the hardiest and firmest fruit of that harvest.

As would be expected, the other interactions showed that the

fruit receiving the most severe damage treatments had the greatest amount of bruised tissue. 'Rome' fruit had the least amount of bruised tissue probably as a result of greater inherent flesh firmness at harvest and thereafter.

The significant interaction of harvest x cultivar x storage duration on CO_2 evolution was a likely result of fruit maturation, ripening and senescence. The increased rates of CO_2 evolution between harvest one and harvest three are accountable in that fruit from harvest three would be well into the climacteric rise so as to be producing and evolving large quantities of CO_2 at the time of harvest. Harvest two fruit should be lower in rates of CO_2 evolution if picked at the optimum time prior to the initiation of ripening. Harvest one fruit would have a low rate of CO_2 because the fruit had not entered the climacteric phase of respiration associated with maturation and the coinciding increase in CO_2 and C_2H_4 evolution. Observing the 'Macspur' maturity response, which was the most consistent, (Table 1) it is likely that the fruit at harvest one were picked before the climacteric rise, whereas harvest two was just before the climacteric rise and harvest three fruit were already in the climacteric rise.

'Rome Beauty', a long-term storage cultivar, had the lowest rates of CO_2 evolution, probably because of the lower rates of respiration characteristic for this cultivar with maturation and ripening (39). The main effect of cultivars on the rate of CO_2 tended to be misleading showing 'Empire' significantly above 'Macspur' in terms of rate of CO_2 evolution at all observations. According to the three way interaction, 'Macspur' and 'Empire'

fluctuated with similar rates of CO_2 evolution. Possibly the similarity in rates of CO_2 evolution for these two cultivars is related to the inherent genetic characteristics of the fruit because 'Empire' was developed from a cross of 'McIntosh' and 'Red Delicious'.

The causes for fruit stored for the longest duration to have the highest rates of CO_2, with the fruit stored for 100 days intermediate, are probably related to the maturity and ripening response of the fruit. During cold storage the low temperature decreases the rate of respiration considerably and the fruit matures at a slower rate. But when the fruit are brought to room temperature the respiration rate increases drastically. Fruit stored for 199 days with the highest rate of CO_2 evolution was most likely a maturity response of the fruit at the end of ripening and entering the senescent stage of maturity.

CO_2 evolution differences due to the respiration response to temperature are probably minimal since all experiments were conducted under similar temperature conditions. The temperatures observed for each experimental run by means of a maximum-minimum thermometer showed fluctuations no greater than $3^{\circ}C$ for the 24 hr period. Since the fruit was harvested and transported under variable conditions of temperature and were placed in cold storage at different times, these factors would tend to increase experimental variation.

The decrease in the rate of CO_2 evolution of the fruit during the time period in which the CO_2 response was measured is a typical response of the fruit due to handling. The maximum concentration of

CO_2 in the static system used was observed to be 3.2 percent at the sixth hour, with a range in the containers from 0.13 to 0.94 at the first hour and 0.43 to 3.2 at the sixth hour. It is unlikely that at the fifth hour, which was selected as the most suitable time, that feed back inhibition of CO_2 was a factor on respiration. Oxygen concentrations did not drop below 19 percent within the containers sealed for 24 hours (data omitted). It is more likely that the decrease was due to a decrease of the fruit handling response rather than to an inhibition of respiration by low oxygen or high carbon dioxide in the containers.

The lack of meaningful correlations between CO_2 response and the amount of damaged tissue was not unexpected due to the complex interactions observed. There were numerous highly significant interactions of damage treatment on the amount of damaged tissue which did not occur for the CO_2 response. The subjective selection process required for excising damaged tissue might result in considerable judgmental error in making a distinction between damaged and nondamaged tissue, which is based primarily on the discoloration of damaged tissue as a result of the oxidative browning reaction. With maturation and ripening the ensuing decrease in flesh firmness could result in numerous undamaged cells being masked by the discoloration of a relatively small amount of damaged cells. The studies of Klein (18) that show the CO_2 response to result from only ruptured cells would be a more reliable measure of actual damage and truly an objective measure of this damage.

Some changes in methods may decrease the variation observed in this study. This could best be corrected by increasing the number

of fruit per sample, which was not feasible for this research because of the numerous factors studied. The use of more apples per sample would decrease the effect of natural fruit variations.

SUMMARY AND CONCLUSIONS

Apple fruit varying widely in stages of maturation and ripeness as a result of cultivar, time of harvest and storage duration were subjected to degrees of physical damge by bruising ranging from slight to severe. Each factor was of significant influence upon the amount of damaged tissue and the increase in carbon dioxide evolution brought about by the damage treatment. The two measures of damage, however, were not correlated, probably because of the differing influence of these factors on the two methods of damage assessment. Four of the six interactions involving damage treatment were significant for the amount of damaged tissue, whereas, none of the six were significant for carbon dioxide evolution.

The significant differences between all damage levels ranging from no bruising to four small bruises to one large bruise indicate that the carbon dioxide response was a reliable measure of damage, regardless of the cultivar, time of harvest and storage duration of the fruit. It is suggested that the carbon dioxide response is a more suitable method of assessing damage than the amount of damaged tissue as determined by visual examination.

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