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PHYSIOLOGICAL CAUSES FOR CHANGES IN CARBON DIOXIDE AND ETHYLENE PRODUCTION BY BRUISED APPLE FRUIT TISSUES

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PHYSIOLOGICAL CAUSES FOR CHANGES IN CARBON DIOXIDE AND ETHYLENE PRODUCTION BY BRUISED APPLE FRUIT TISSUES

By

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ABSTRACT

PHYSIOLOGICAL CAUSES FOR CHANGES IN CARBON DIOXIDE AND ETHYLENE PRODUCTION BY BRUISED APPLE FRUIT TISSUES

By

Joshua D. Klein

Apples (Malus domestica Borkh., cv. 'Empire') dropped onto a hard, smooth surface so as to receive an impact bruise midway between stem and calyx rapidly evolved CO_2 in proportion to drop height and number. The response occurred for fruits ranging in maturity from the preclimacteric stage of endogenous enthylene production to the postclimacteric stage. Enhanced CO_2 production, which continued for 12 to 24 hours after damage, may serve as a good index for damage that occurs to fruit during handling and transport.

Neither aerobic nor anaerobic respiratory increases were responsible for the enhanced CO_2 output. Rather, the increased CO_2 production was due to decarboxylation of vacuolar malic acid by cytoplasmic malic enzyme in the 0.5 cm of cortical tissues below the fruit surface at the bruise site, as shown by a decrease in titratable acidity of extracts from bruised tissues compared to nonbruised beginning 1 to 3 hours post-impact. Further evidence for the enzymatic

nature of the CO_2 response is that the optimal temperature range for simultaneously increased CO_2 production and decreased malate concentration in bruised tissues was 20 to 30°C.

Bruising of preclimacteric fruit caused an increase in ethylene evolution, whereas bruising of fruits that had begun to ripen caused a decrease in ethylene evolution. Concentrations of 1-aminocyclopropane-1-carboxylic acid (ACC) were similar in bruised and nonbruised tissues during the initial 24 hours after impact-bruising, but by 48 hours the ACC in bruised tissues had declined to 60% of the levels in nonbruised tissues. Although ethylene production by both bruised and nonbruised tissues increased with temperature increase from 0 to 30°C, the relative decrease in ethylene evolution due to bruising remained consistent at 50%. This was also true at 40°C despite the overall marked decrease in ethylene production by both bruised and nonbruised tissues.

The similar ability of bruised and nonbruised apple tissues to convert exogenously supplied ACC to ethylene together with the consistent percentage decrease in ethylene production by bruised tissues at 0 to 40°C, indicates that the decrease in ethylene production upon bruising is due to physical destruction of the cells, rather than to a physiological reaction as found for the CO_2 response.

To Adina, of course.

רבות בנות עשו חיל

ואת עליית על כלנה.

משלי לא:כט

Many have done valiantly,

But you exceed them all.

Proverbs 31:29

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Dr. Miklos Faust of the USDA Fruit Lab, Beltsville, MD, first taught me that horticultural research is not only its own reward, but can be fun, too. In the 11 years since then (and certainly before), my parents and grandparents have always supported my academic and other pursuits, and for this I am profoundly grateful.

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INTRODUCTION

Mechanical damage occurring during the handling, storage, and shipping of fresh produce is a major cause of postharvest losses. The damage is time-consuming and difficult to detect and quantify, since each fruit must be individually inspected. Frequently, the damage is not immediately apparent, so that numerous examinations may be required as the produce moves through marketing channels.

A nondestructive system to detect and quantify damage to large quantities of produce would be invaluable in designing or comparing shipping and marketing containers that would afford maximum protection from postharvest injury. Basic requirements for such a system would include ease, reproducibility, and rapidity of measurements. The system should also eliminate the need for visual inspection of produce, since it is a subjective and lengthy process that requires the package to be opened and each item inside to be examined for evidence of injury. Since many crops respond to injury by changes in carbon dioxide or ethylene production, it is possible that atmospheric composition changes within a sealed container of produce may provide a useful, rapid, and accurate index of produce damage.

Since little is known about the effect of physical damage on physiological functions of apple fruit tissue, the purpose of the research reported here was (1) to examine the effects of fruit maturity

and physical injury on ethylene and CO_2 production by bruised apples and to determine if there is a correlation between degree of injury and amount of volatile evolution and (2) to determine the physiological bases for increased CO_2 production, and to a lesser extent for the ethylene response by bruised apple fruits.

LITERATURE REVIEW

Introduction

Postharvest losses of fresh produce at the consumer level range from 10 to 30%, mostly due to mechanical damage (20). Researchers attempting to quantify and develop indices of injuries that occur as crops move from growers to consumers have measured energy absorbed by test packages (22,23,45,46,47), number of bruises (35), and such physiological parameters as percent soluble solids, titratable acidity, and respiratory activity (26,37,39). Many studies have focused on the relationship between increased CO_2 production by fruits and mechanical damage caused by impact[1] (6,11,14,25,26,28,29,42,50), compression (1,30,31), or vibration (37,38,39) during harvesting, handling, and transport. Crops showing damage response include apple[1] (10, 48), avocado (6,56), banana (34), cantaloupe (36), cherry (30,31,37,41), cranberry (32), grapefruit (14,50), lemon (14), orange (11,14,25,26), and tomato (1,29,38,39). In all cases, an increased output of CO_2 from damaged fruits compared to nondamaged fruits usually occurred immediately following injury and declined to basal (control) levels within 2 to 7 days. Increases or decreases in ethylene production also occur in response to injury (2,17,28,32,42).

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[1]Massey, jr., L.M. 1982. NE-103 Annual report (Jan. 1 to Dec. 31, 1982) Postharvest physiology of fruits. 13 p.

Nature of the CO_2 response by injured fruits

Although many of the reports of increased CO_2 production in response to injury refer to enhancement of respiratory activity, there has been limited research on the actual effect of damage on oxygen uptake to verify it as a respiration response. Avocados that were shaken or dropped were advanced by as much as 10 days in respiratory climacteric as measured by O_2 uptake, compared to undamaged fruit (6). There was an immediate increase in respiration upon damage, followed by a decrease, with a subsequent strong increase in O_2 uptake leading to the early climacteric rise. However, the response was erratic, was not consistently observed, and was not compared with CO_2 production. Other researchers (5,56) found no advance in the onset of either the CO_2 or the ethylene climacteric at 20°C for avocados wounded by removal of a plug of pulp. Damaged fruit held at 14°C, however, showed a two-day advance in onset of both CO_2 and ethylene climacteric (56).

Burg and Thimann (10) noted increased O_2 uptake as well as enhanced CO_2 production in cut plugs and slices of apple fruits. Robitaille and Janick (42), however, pointed out that O_2 uptake should decrease rather than increase in destroyed apple tissue cells, and suggested therefore that increased CO_2 production by bruised apples was not a result of normal respiration.

Carbon dioxide output by bruised Satsuma mandarin orange fruits increased preferentially over O_2 uptake during the first 5 hours after injury (25). The respiratory quotient (RQ = CO_2 output

rate/O_2 uptake rate of tissue) during this period changed from a basal level of 1.26 to 1.99, indicating enhanced CO_2 production. In the next 4 hours, however, O_2 uptake was markedly stimulated and the RQ fell to 1.00. This fall reflected both a decrease in the enhanced CO_2 output and an increase in O_2 uptake. CO_2 output still exceeded basal levels 9 hours after bruising.

The respiratory quotient in this case (25) does not seem to reflect actual mitochondrial respiratory activity, since changes in CO_2 output and O_2 uptake were not temporally linked. Further evidence that the O_2 uptake and CO_2 output were not related is that similar increases in CO_2 production were noted in bruised Satsumas held under either aerobic or anaerobic conditions (25). In addition, although bruised tomatoes emitted more CO_2 than nonbruised fruit at comparable stages of maturity (1), their mitochondria phosphorylated at equal rates and had similar Q_{O_2} (N).

When respiratory activity was measured on individual tart cherry fruits before and after bruising O_2 consumption due to bruising rose 50%, while CO_2 production increased 126% (41). The RQ increased from a basal level of 1.8 to 2.47 after bruising. Since respiratory quotients that approximate unity are generally considered to occur in systems that utilize carbohydrates as a substrate, while RQs less than 1.0 derive from systems utilizing lipids and RQs greater than 1.0 indicate acid substrates (19), the source of the 'extra' CO_2 in damaged tissues in the above-cited studies (25,41) was likely acidic. Further evidence for an acid substrate comes from studies of bruised tomatoes (29,39) and Satsuma mandarins (26), in which acids

decreased in bruised fruits compared to nonbruised, while soluble solids remained constant and CO_2 production increased.

CO_2 evolution and malate decarboxylation in fruits

Marks and Varner (31) suggested that the additional CO_2 produced following bruising of fruit is due to successive decarboxylation of malate and pyruvate. When they labelled the organic acid fraction of 'Royal Anne' cherries by exposing the fruits to $^{14}CO_2$, they found more than 95% of the total radioactivity in malic acid. Subsequent paper chromatographic and autoradiographic comparisons of extracts of bruised and nonbruised fruit indicated that malate was the only radioactive constituent to disappear more rapidly from the bruised fruit than from the controls.

Neal and Hulme (40) demonstrated that the ability of apple peel discs to decarboxylate malate increased as fruits matured (the 'malate effect'). They found that malate loss was precisely balanced by increased production of CO_2 and acetaldehyde, in a ratio of 2:1:1 of CO_2 produced:malate utilized:acetaldehyde formed. This is in agreement with the pathway:

$$\text{malate} \longrightarrow \text{pyruvate} + CO_2 \longrightarrow \text{acetaldehyde} + CO_2.$$

Dilley (13) found that the synthesis and specific activity of malic enzyme, which catalyzes malic acid decarboxylation, increased as apple fruits matured and proceeded through the climacteric rise in CO_2 evolution. Similar results were noted for pyruvate carboxylase (24), which catalyzes formation of acetaldehyde from pyruvic acid.

These changes may explain the observed loss of acidity in apples as they ripen (15), since the major acid in apple fruits is malate (49, 51).

Although malic enzyme seems to have an obligate requirement for oxygen (40), no increased O_2 uptake was noted during presumed enzyme activity when malate was added to peel discs from mature apples (18,40). In contrast, Fidler (15) found decreased titratable acidity of apples, presumably due to malic enzyme activity, under both aerobic and anaerobic conditions as fruit went through the climacteric. He also found a metabolic pathway for acetaldehyde metabolism which involved formation of both ethanol and CO_2 regardless of oxygen tension (16). Interestingly, Thomas (48) reported as early as 1931 an increased 'zymasis' in bruised applies that resulted in increased ethanol and acetaldehyde accumulation in the fruit. This accumulation was not caused by anaerobic fermentation at the bruise site, since it proceeded under conditions of 100% oxygen. 'Zymasis' thus seemingly corresponds to coupled malate/pyruvate decarboxylation.

Brusing of apple fruits leads to cell rupture (22), which in turn can result in mixing malic acid from the vacuole with cytoplasmic contents. Malic enzyme, which is associated with the cytoplasm rather than with the mitochondria (7), could then decarboxylate malic acid, resulting in an increase in CO_2 production by the damaged tissue. Formation of 'excess' CO_2 would decrease as the malate substrate is depleted by enzyme activity.

Enhanced CO_2 production by bruised tomatoes and oranges may be similar to that of apples. The titratable acidity of tomatoes (12,27,43) and oranges (44) decreased simultaneously with a decrease in malate concentration as the fruits ripen. The soluble fractions of these fruits also possess malic enzyme and pyruvate carboxylase, which increase in activity as fruits mature (7,21). The decreased acidity (26,38) and increased CO_2 production (25,26,29,37,38) noted in bruised tomatoes and citrus may thus be due to the linked activities of the above-mentioned enzymes, similar to the apparent case in apples.

Wound ethylene production in fruits

Various types of stress or damage can induce increased ethylene production by plants (2). The increased ethylene may, in turn, induce an early CO_2 or ethylene climacteric in wounded tissues (35,56), or may subsequently decline to basal levels (34). The ethylene-forming system involves the production of S-adenosylmethionine (SAM) from methionine. SAM, in turn, is converted enzymatically to 1-aminocyclopropane-1-carboxylic acid (ACC), with subsequent formation of ethylene from ACC (3,8). Increased ethylene production rates reported for cut tomato (9,55), orange albedo (55), and mung bean hypocotyl (55) tissues were due to enhanced ACC synthase activity and the consequently increased production of ACC and ethylene. The conversion of ACC to ethylene is inhibited by anaerobiosis (52), by excessively high or low temperatures (4,54), and by osmotic shock (4). These findings, plus discontinuities in Arrhenius plots for ethylene

production over the temperature range 0 to 30°C (4,33) suggest that membrane integrity is essential for the formation of ethylene from ACC.

Robitaille and Janick (42), upon observing that ethylene production decreased in apples bruised six weeks after harvest, hypothesized that cell disruption by bruising destroys the sites of ethylene synthesis. Lougheed and Franklin (28), however, noted increased internal ethylene concentrations in bruised preclimacteric apples. They proposed that bruising preclimacteric fruits induces ethylene production in immature, nonethylene-producing tissue, whereas damage to ripened fruit which are already forming ethylene endogenously destroys the ethylene productive tissue. Massey[1] harvested and bruised 'Idared' apples at intervals over a four-week period in the fall and found that internal ethylene concentrations of preclimacteric fruit increased proportionally to the number of impacts. The response was not evident once the fruit entered the climacteric rise in ethylene production. Burg and Thimann (10) reported no increase in ethylene evolution from cut postclimacteric apple tissues.

Bruising preclimacteric apples probably leads to a stimulation of ACC synthase activity, followed by increased ACC and ethylene concentrations in the fruit (55). In contrast to preclimacteric fruit, cell walls and membranes of fruit that have begun endogenous ethylene production and ripening are likely more susceptible to rupture upon bruising (22,46). This disruption of the site of ACC conversion to ethylene (4,33) would lead to an accumulation of ACC and an

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[1]Massey, NE-103 Annual report, 13 p.

inhibition, rather than a stimulation, of ethylene production in bruised tissues.

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Section I

Carbon dioxide and ethylene production by bruised 'Empire' apples

ABSTRACT

Apples (Malus domestica Borkh., cv. 'Empire') dropped onto a hard, smooth surface so as to receive an impact bruise midway between stem and calyx rapidly evolved CO_2 in proportion to the drop height and number. The increase in CO_2 evolution was not due to enhanced respiratory activity, since there was no concomitant increase in O_2 uptake by excised bruised tissues, nor was it due to localized anaerobic fermentaion at the bruise site. Rather, the increased CO_2 was due to decarboxylation of vacuolar malic acid by cytoplasmic malic enzyme in the 0.5 cm of cortical tissues below the fruit surface at the bruise site, as shown by a decrease in the titratable acidity of extracts from bruised tissues compared to controls. The CO_2 response occurred in fruits of all maturities. Bruising resulted in increased ethylene evolution from preclimacteric fruits, but caused a 50% decrease in ethylene evolution from fruits that had begun to ripen. Enhanced CO_2 evolution from bruised tissues is temperature-dependent, while the percentage decrease in ethylene is consistent over the temperature range of 0° to 40°C and is due to physical destruction of cells at the bruise site.

Keywords: apple--fruit damage--carbon dioxide--ethylene--malic acid

Introduction

Mechanical injury to fruit by physical forces causes changes in carbon dioxide (CO_2) and ethylene evolution by the fruit. Enhanced

CO_2 production (compared to basal control levels) resulted from impact damage to apple (8),[1] cranberry (21), grapefruit (11,35), orange (11), Satsuma mandarin (9,14,15), and tomato (18). Compression and vibration damage resulted in elevated CO_2 levels in treated cherries (19,20,23) and tomatoes (24,25). In all cases, CO_2 evolution rates returned to basal levels within 2 to 7 days following damage. Increasing the degree of damage by increasing the force of impact, number of impacts, force of compression, or time period and frequency of vibration resulted in proportionally enhanced CO_2 production in many fruits. Interest has focused on the measurement of CO_2 as an index of damage to fruit during harvesting, handling, and shipping.

The ethylene response of damaged fruit generally parallels that of CO_2 (2,12). Robitaille and Janick (25), however, observed a decrease in ethylene production by apples bruised 6 weeks after harvest, while Lougheed and Franklin (12) noted increase internal ethylene concentrations in bruised preclimacteric apples. To reconcile the differences in these observations, Lougheed and Franklin (12) proposed that bruising preclimacteric fruits induces ethylene production in non-ethylene-producing tissue, whereas damage to ripened fruits that are already producing ethylene destroys productive tissue. Internal ethylene concentrations of preclimacteric 'Idared' apples increased proportionally with the number of impacts, but the response

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[1]Massey, jr., L.M. 1982. NE-103 Annual Report (Jan 1 to Dec. 31, 1982). Postharvest physiology of fruits.

was not evident once the fruit entered the climacteric rise in ethylene production.[1] Yu et al. (37) have shown that wound ethylene may be due to enhanced activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, with consequently increased production of ACC and ethylene.

Although reports of enhanced CO_2 production following injury frequently refer to "enhanced respiratory activity," only a few studies have considered both O_2 uptake and CO_2 evolution in damaged fruit. CO_2 production by bruised Satsuma mandarins increased compared to O_2 uptake during the first 5 hours after injury (14). Subsequently, however, O_2 uptake was highly stimulated and there was a decrease in the enhanced CO_2 output. Oxygen consumption by bruised sweet cherries rose 50%, while CO_2 production increased 126% (27). In neither case were increases in both O_2 uptake and CO_2 output closely enough linked to indicate that the effect of bruising took place at the level of mitochondrial respiratory activity. Mitochondria isolated from bruised or unbruised tomatoes phosphorylated at equal rates and had similar Q_{O_2} (1).

Marks and Varner (19) found that labelled malic acid was the only radioactive constituent to disappear more rapidly from bruised cherries than from control fruit and concluded that the additional CO_2 produced following bruising is due to successive decarboxylation of malate and pyruvate. Similar mechanisms may explain the simultaneous increase in CO_2 production levels and decrease in titratable acidity noted in bruised tomatoes (18,24) and Satsuma mandarins (9,15).

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[1] Ibid.

The purpose of this investigation was to examine the effects of fruit maturity and physical damage by impact on ethylene and CO_2 production by bruised apple fruits, and to determine the physiological basis for enhanced CO_2 production by the damaged tissues.

Materials and methods

Fruit treatment. Apples (Malus domestica Borkh., cv. 'Empire'), selected for uniformity of size and freedom from defects, were carefully harvested from experimental plantings and used immediately or stored under refrigeration in air or controlled atmosphere conditions for later use. Fruit were equilibrated at 20°C for 12 to 18 hours before bruise treatment, which consisted of dropping individual fruit from a height of 1 m, except where otherwise indicated, onto a hard, smooth surface. Apples were dropped so as to receive an impact bruise approximately midway between the stem and calyx. Chalk dust applied to the impact surface identified the impact sites on fruit dropped from a height of 25 cm or less.

Compression bruises were administered only to apples harvested 21 days after full bloom by means of a plastic disc (75 mm diam x 3 mm thick) attached to the penetration head of an Effige penetrometer mounted on a modified drill press. A compression force of 6 kg was applied to each fruit for 10 seconds.

Individual mature fruits were placed in 500 ml respiration containers and either attached to a supply of ethylene-free air at ca. 25 ml/min flow rate or sealed for 1 hr to allow fruit emanations to accumulate in the headspace. In the latter case, sealed jars were

flushed with air between measurements before being resealed at the indicated times. In experiments with small immature fruits (fruitlets), two each were placed in 70 ml test tubes fitted with serum caps and constantly aerated at ca. 10 ml/min.

Tissue discs of bruised and unbruised tissue measuring 25 mm x 5 mm and weighing ca. 3 g were excised from a given apple with a cork borer, halved, and incubated at 20°C in 12 ml centifuge tubes sealed with serum caps. When CO_2 evolution from the tissue and malate concentration of the juice were determined for the same fruit, one-half of a disc was used for each analysis. Since each fruit provided its own control tissue, the effect of bruising on ethylene, CO_2, or malate concentrations in an individual fruit was expressed as a percent of control.

Gas and malic acid determination. Carbon dioxide concentrations were determined with a Carle 8700 (804-B) Basic gas chromatograph equipped with silica gel-molecular sieve columns mounted in parallel and a differential thermal conductivity detector, using He carrier gas at ca. 70°C. Acetaldehyde and ethanol headspace concentrations were determined with a BioGas model 12-110 gas chromatograph equipped with a Porapak-P column and a metal oxide semiconductor detector and using air carrier gas at ambient temperature. Ethylene was analyzed with a Varian Series 1700 gas chromatograph equipped with an activated alumina column and a flame ionization detector, and utilizing N_2 carrier gas at 70°C. Internal ethylene samples were withdrawn directly from the central cavity by inserting an 18 gauge x 4 cm needle through the fruit calyx according to the procedure of Saltveit (30).

Oxygen was determined with a Gilson differential respirometer, using manometric techniques (33). Tissues were placed in ca. 20 ml Warburg flasks containing 2.5 ml 0.05 M phosphate buffer (pH 4.5) at 20°C, with 5 N KOH and a paper wick in the center well as a CO_2 adsorbent. In some cases, malate (adjusted to pH 4.5 with KOH) was added to the buffer from the sidearm of the flask to yield a final concentration of 0.1 M malic acid. Flasks were allowed to equilibrate for 15 to 30 minutes prior to taking readings.

A 0.5 ml aliquot of expressed juice from tissue half-discs was added to 50 ml of distilled CO_2-free water for malic acid determinations (3). Diluted samples were titrated to pH 8.1 with 0.03 or 0.06 N NaOH and the results expressed as mg malic acid/100 ml extract (33, 36), after calculation as follows:

$$\text{mg malic acid/100 ml extract} = \frac{\text{ml NaOH} \times \text{N NaOH} \times 67 \times 100}{\text{ml sample}} \qquad (3).$$

Qualitative analysis of malic acid was done by paper chromatography (3). N-butanol (100 ml), water (100 ml), and formic acid (10.7 ml) were mixed in a separatory funnel with 15 ml of a 1% aqueous bromocresol green solution. After thorough shaking, the lower, aqueous layer was discarded and the upper layer placed in a chromatography developing tank. Aliquots (5 µl) of juice from bruised and unbruised tissues, as well as 0.3% (w/v) standard malate solution, were spotted onto Whatman No. 1 paper, which was then placed in the tank for approximately 6 hours. Upon removal from the tank, yellow spots corresponding to malate developed at an R_f of approximately 0.5.

Other experimental procedures. The effect of anaerobiosis on bruised and unbruised tissue discs was determined by twice evacuating tubes containing half-discs of tissue to 200 torr and restoring to ambient pressure conditions with N_2 or air. Anaerobiosis was also induced by vacuum-infiltrating unbruised tissues with a solution of 0.4 M mannitol (pH 4.0) in order to flood intercellular tissue airspaces. Headspace analyses of flooded tissues were statistically compared with untreated unbruised and bruised tissues of a given fruit for ethylene, CO_2, acetaldehyde, and ethanol concentrations.

The effect of the bruised tissue upon the CO_2 production by the whole fruit two hours after impact was determined by lightly covering the epidermis at the bruised area with a petrolatum/lanolin mix (1:1, v/v) or by excising the bruised tissue and covering the resultant cut surface with petrolatum/lanolin prior to sealing the fruit in containers. CO_2 evolution rates of unbruised fruit and fruit with intact bruises were compared with those of treated fruit.

The site of CO_2 production within bruised tissue was identified by taking a 12 mm diam. cork borer plug through the center of the bruised tissue and dividing it into 4 discs, each 5 mm thick. Similar plugs and discs were cut from the unbruised side of a given fruit 4 hours after bruising. Discs were sealed in tubes as described earlier and monitored for headspace accumulations of CO_2 and ethylene. Since the epidermis was retained on outermost discs, the rates of CO_2 evolution and O_2 uptake for epidermal tissues and the subjacent 0.5 cm of cortex were determined by a similar experiment in which the two tissues were cut as above and separated by a razor blade.

Fruit were equilibrated for 12-18 hours at 0°, 10°, 20°, 30°, and 40°C prior to determining the effect of temperature on CO_2 and ethylene evolved by bruised tissues. Following impact, fruit were returned to the appropriate temperature-controlled chamber for 2 hours prior to the removal of bruised and unbruised tissues for analyses of gas and malate concentrations.

Means were based on 4 to 6 replications in all experiments.

Results

The CO_2 evolution from mature whole apples dropped onto a hard surface was positively correlated to drop height at the 1% level of significance (Fig. 1). The number of drop impacts also affected CO_2 output (Table 1), with significant differences between fruit with 3 impacts and controls (0 impacts) for at least 24 hours after bruising. Between 3 and 12 hours after bruising, CO_2 production by fruits with 3 impacts was also significantly greater than that of fruit with 1 impact, which, in turn, exceeded controls during that time.

Fruitlets bruised by compression when harvested 21 days after full bloom responded immediately with a significant increase in CO_2 output (Fig. 2). This increase was not as marked when the fruits were rebruised 22.3 hours after the initial treatment. As with mature, impact-damaged fruit, fruitlets subjected to compression ultimately returned to CO_2 production rates similar to controls.

The increase in CO_2 production by excised tissues from damaged mature and ripening fruit was immediate and was invariably sustained

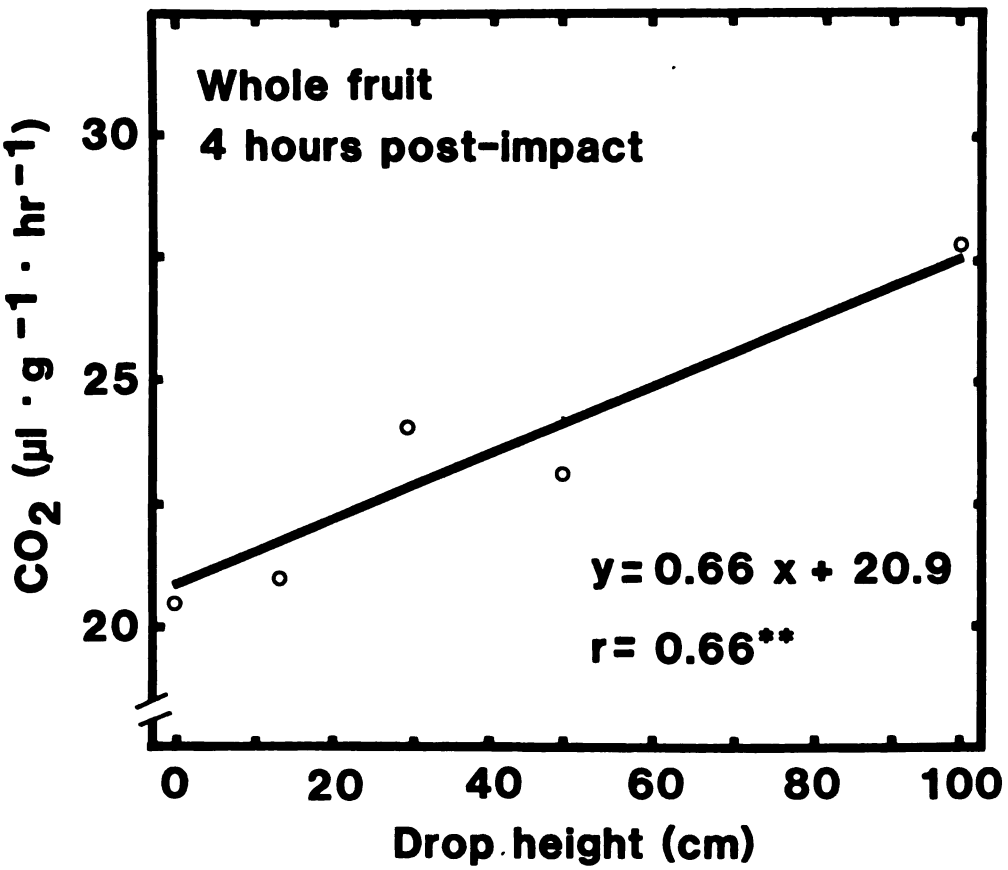


Figure 1.--Relationship between CO_2 production 4 hours post-impact and drop height of 'Empire' apples.

Table 1. Effect of 0, 1, and 3 drop-impacts from a height of 1 m on CO_2 evolution by 'Empire' apples.[z]

No. of Impacts	CO_2 evolution ($\mu l \cdot g^{-1} \cdot hr^{-1}$)					
	Hours post-impact					
	1	3	6	12	24	48
0	20a	20a	23a	22a	21a	22a
1	23ab	27b	28b	27ab	22ab	24a
3	26b	34c	34c	31c	26b	24a

[z]Means are from 2 pooled experiments, and are separated within columns by Duncan's multiple range test, 5% level.

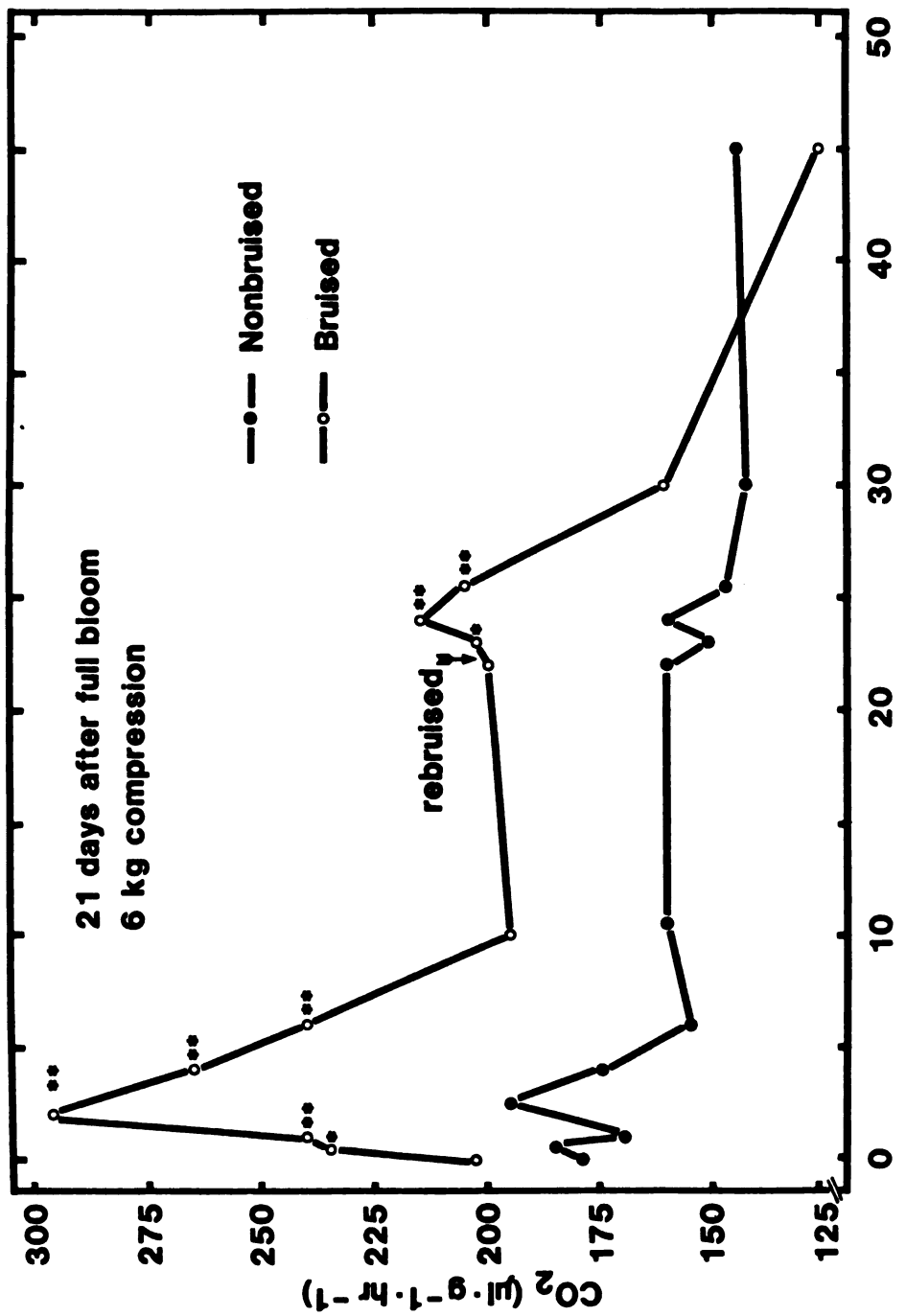


Figure 2. CO_2 production by 'Empire' apple fruits following harvest at 21 days after full bloom and bruising by 6 kg compression for 10 second. *,** --Differences between treatments at each time at 5% or 1% significance level respectively.

for at least 12 hours after impact, regardless of fruit age (Table 2). CO_2 production by bruised tissues remained higher than that of unbruised for at least 24 hours post-impact only in fruit that were preclimacteric or just at the climacteric rise in internal ethylene concentration.

Several experiments show that excess CO_2 output by bruised fruit came exclusively from the bruised tissue. The correlation between CO_2 evolution and height to impact in excised bruised tissue (Fig. 3) was similar to that of whole fruit as shown in Fig. 1, but the response was more pronounced and of greater statistical significance ($r = 0.66$, significant at 1% level for whole fruit; $r = 0.75$, significant at the 0.1% level for excised bruise). Damaged fruits in which the bruised tissue was excised or covered with petrolatum/lanolin initially produced significantly less CO_2 than fruit with intact bruises (Fig. 4). Fruit with excised or covered bruises produced CO_2 at the same rate as nonbruised fruit when measured 9 houurs post-impact. After 24 hours, however, fruits from all treatments were similar in CO_2 production.

Browning of apple cortex tissues due to bruising extended 13 to 15 mm below the fruit surface in fruit dropped 1 m. Figure 5 shows that the tissues at the fruit surface (and therefore nearest the point of impact) produced significantly more CO_2 than nonbruised tissues at 4 hours post impact. Deeper tissues showed no increases in CO_2 output. In a separate experiment, excised epidermal tissues from both bruised and unbruised sites on the apple 3 hours post impact produced 160 μl $CO_2 \cdot g^{-1} \cdot h^{-1}$. However, cortical tissues 0.5 cm below the epidermis

Table 2. Effect of fruit age on CO_2 production by tissues excised from impact-damaged 'Empire' apples[z]

Harvest	Days Cold Storage	Tissue	CO_2 ($\mu l \cdot g^{-1} \cdot hr^{-1}$)								
			Hours post-impact								
			1	3	6	12	24	48	72	96	120
9/12	0	Nonbruised	65	74	60	70	79	61	47	44	49
									ns	ns	ns
		Bruised	94	105	85	110	100	68	50	48	44
		% Nonbruised	143cd	141cd	141cd	157d	130bc	112ab	107a	109ab	91a
10/12	0	Nonbruised	65	--	71	52	59	63	65	--	--
		Bruised	83	--	82	72	83	57	58	--	--
		% Nonbruised	130c	--	114bc	136c	136c	92ab	87a		
10/18	15	Nonbruised	64	71	68	80	71	64	61	--	--
								ns	ns		
		Bruised	80	90	87	90	65	52	55	--	--
		% Nonbruised	125b	128b	126b	114b	92a	81a	92a	--	--
10/18	50	Nonbruised	67	68	67	64	64	62	63	64	--
						ns					
		Bruised	92	102	81	74	58	47	49	50	--
		% Nonbruised	129b	151c	122b	117b	92a	77a	78a	78a	--
10/18	79	Nonbruised	57	61	55	54	60	55	--	--	--
		Bruised	78	83	67	64	45	37	--	--	--
		% Nonbruised	140d	131c	122b	119b	75a	70a	--	--	--

[z]Mean separations within rows by Duncan's multiple range test, 5% level.

[y]Except where indicated ns, tissue differences within each sampling time and hour post-impact are significant at the 5% level.

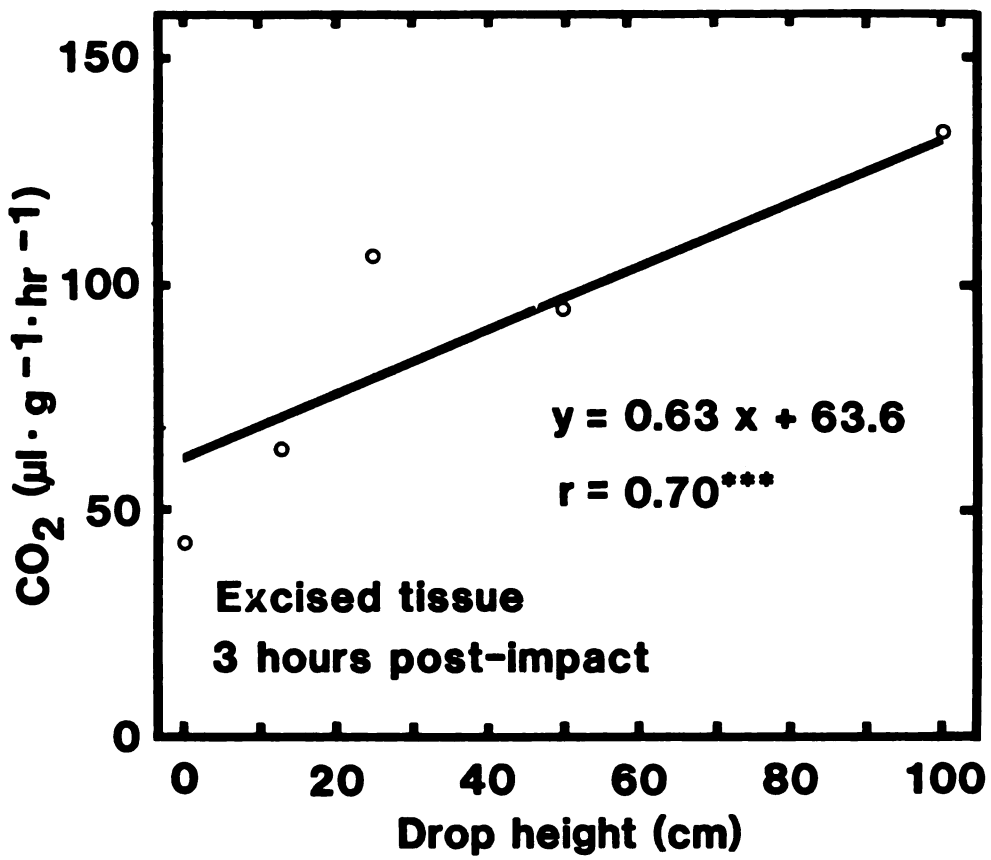


Figure 3. Relationship between CO_2 production by excised bruised tissue and drop height of 'Empire' apples 3 hours post-impact.

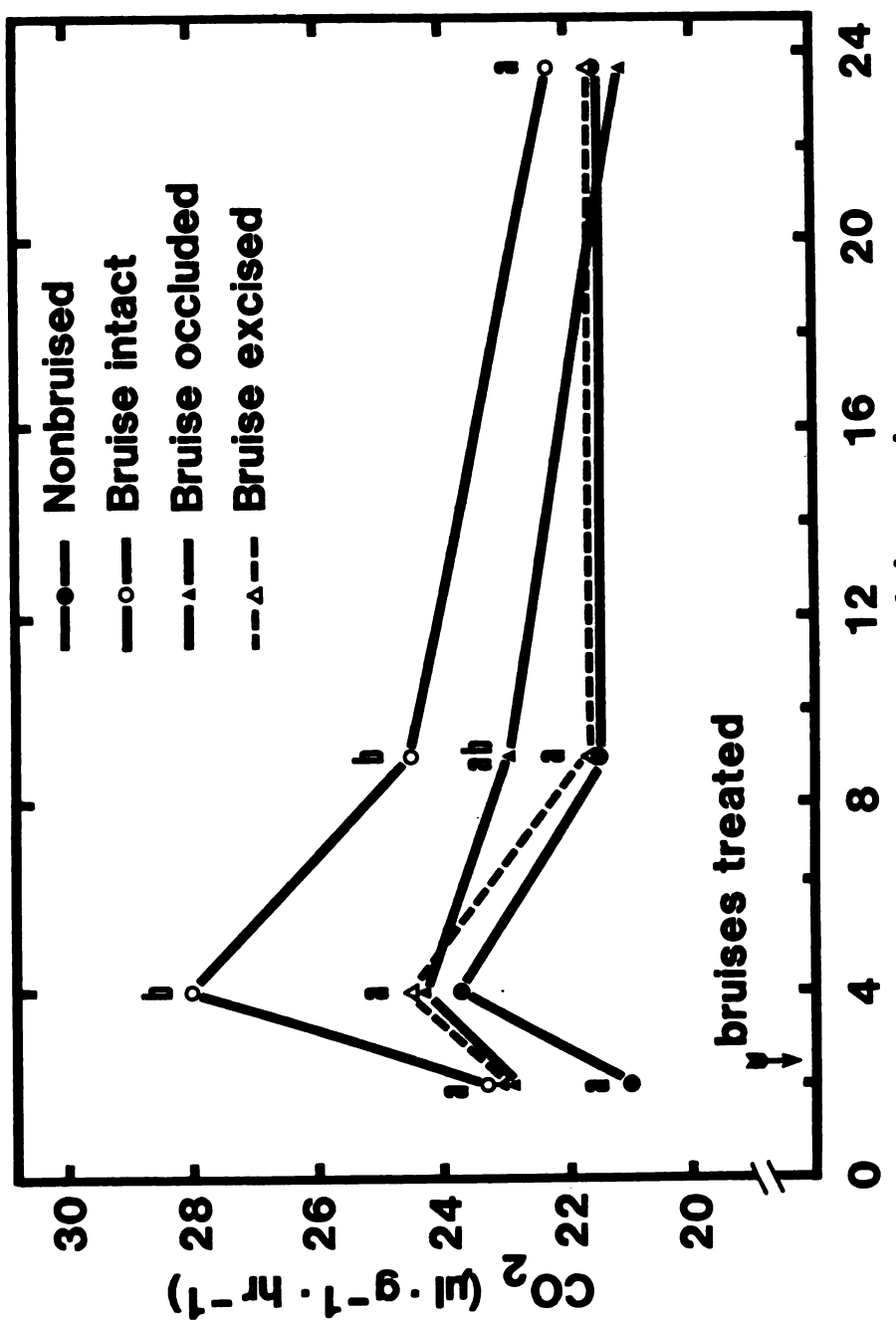


Figure 4. CO_2 production by 'Empire' apples bruised at 0 hours, with bruises excised or occluded 2.2 hours post-impact. Means separated at each time by Duncan's multiple range test, 5% level.

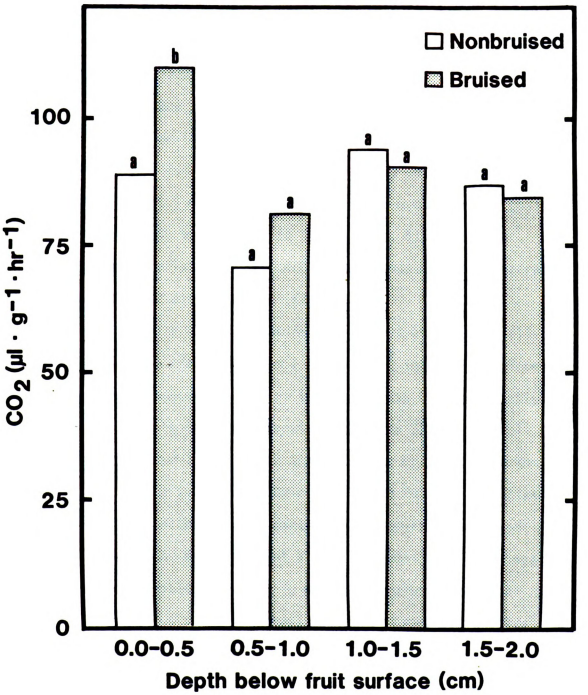


Figure 5. CO_2 production by bruised and nonbruised excised 'Empire' apple fruit tissues at successive intervals below fruit surface, 4 hours post-impact. Mean separations at each depth by Duncan's multiple range test, 5% level.

produced significantly more CO_2 at the bruise site (77 $\mu l \cdot g^{-1} \cdot hr^{-1}$) than at a control site (35 $\mu l \cdot g^{-1} \cdot hr^{-1}$).

Preliminary studies (16) showed that fruit which were preclimacteric in ethylene production (<0.1 $\mu l \cdot l^{-1}$ internal ethylene) had no consistent significant increases in external ethylene production or internal ethylene concentration due to bruising until approximately 24 hours after impact. After that time, ethylene evolution was positively correlated with drop height to impact (Fig. 6). A similar, although considerably less significant, response was measured separately for internal ethylene concentration (Fig. 7).

In studies with excised tissues, once fruit entered the climacteric rise in ethylene production (> 0.1 $\mu l \cdot l^{-1}$ internal ethylene), less ethylene was produced by bruised tissue than by controls (Table 3). Ethylene evolution from bruised tissues declined to 50% of control values by 6 hours after impact, with the exception of fruit from the Sept. 12 harvest. The value for ethylene evolution from bruised tissues of this harvest, expressed as a percentage of controls, fluctuated during the period after bruising, but always exceeded 60. There was occasionally considerable variation in ethylene evolution between fruits of the first two sampling dates at several times of sampling after impact. As a result, the overall mean ethylene values for control and bruise tissues in some instances do not correspond to the overall percentage increase or decrease in ethylene evolution due to damage, although the percentages were consistent within sampling times. The decrease in ethylene evolution was confined to the outermost section of tissue at the bruise site (Fig. 8).

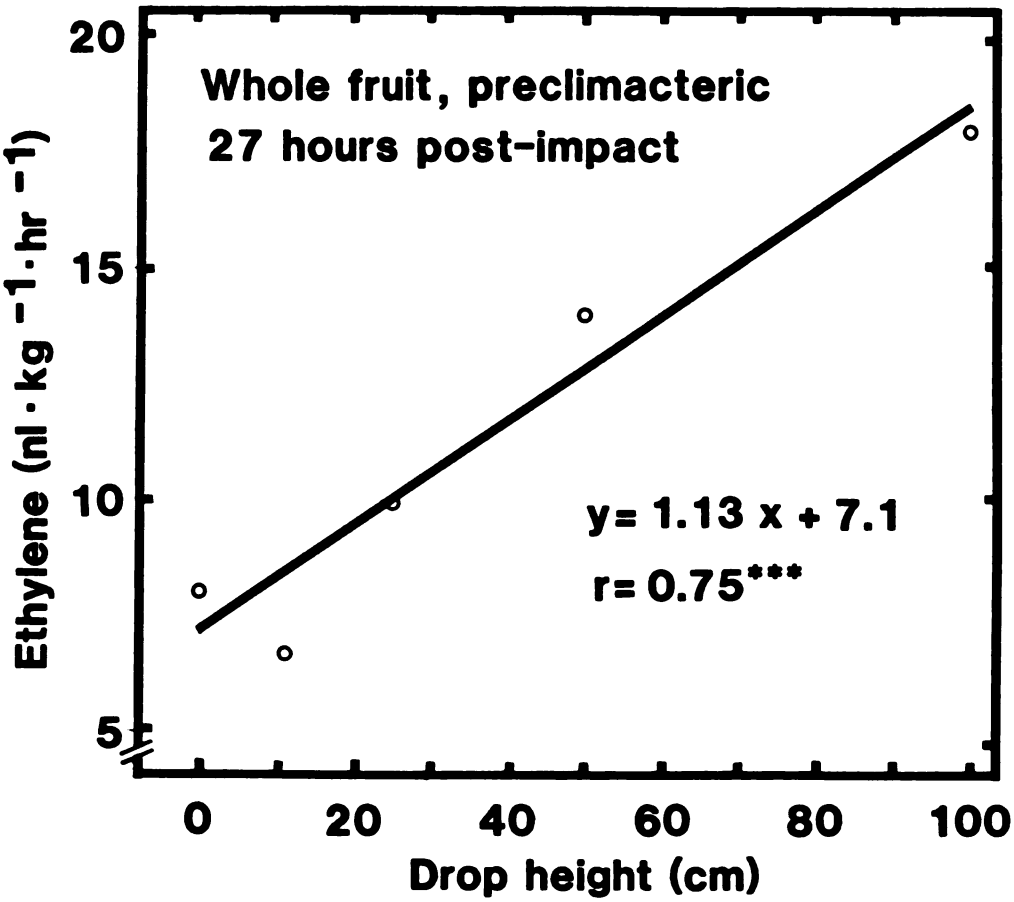


Figure 6. Relationship between ethylene evolution 27 hours post-impact and drop height of preclimacteric 'Empire' apples harvested August 31.

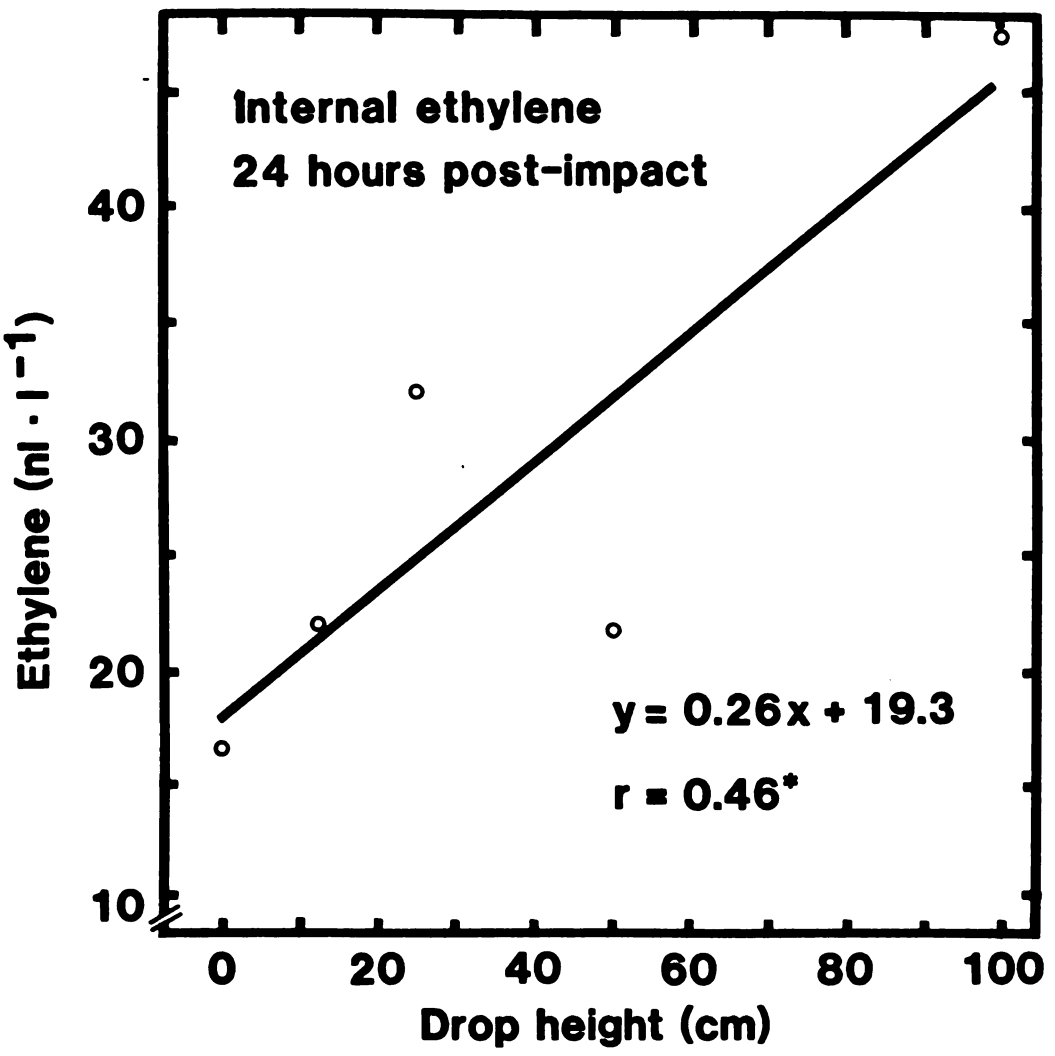


Figure 7. Relationship between internal ethylene concentration 24 hours post-impact and drop height of 'Empire' apples harvested August 26.

Table 3. Effect of fruit age on ethylene evolution from tissues excised from impact-damaged 'Empire' apples[z]

Harvest	Days Cold Storage	Initial Internal Ethylene ($\mu l \cdot l^{-1}$)	Tissue	Ethylene ($nl \cdot g^{-1} \cdot hr^{-1}$) Hours post-impact								
				1	3	6	12	24	48	72	96	120
9/12	0	0.04	Nonbruised	--	0.51	0.19	0.16	--	--	0.21	0.10	0.17
					ns					ns	ns	ns
			Bruised	--	0.35	0.30	0.16	--	--	0.15	0.15	0.13
			% Nonbruised	--	65a	176ab	115ab	--	--	93ab	214ab	85ab
10/12	0	0.20	Nonbruised	79.2	--	72.4	19.8	24.3	98.0	72.0	--	--
				ns								
			Bruised	34.3	--	29.0	5.4	2.8	24.0	17.5	--	--
			% Nonbruised	63c	--	39b	29ab	36ab	24a	26a	--	--
10/18	15	169	Nonbruised	73.0	25.0	75.3	42.8	70.0	88.9	78.7	--	--
			Bruised	32.1	13.5	27.1	23.0	16.1	20.8	22.7	--	--
			% Nonbruised	43c	52d	36bc	55d	23a	24a	29ab	--	--
10/18	50	203	Nonbruised	85.2	117.0	100.2	99.0	118.0	165.1	162.3	--	--
			Bruised	32.0	66.8	48.4	37.1	38.5	49.8	59.0	--	--
			% Nonbruised	49bc	58c	48bc	38ab	33a	32a	38ab	--	--
10/18	79	169	Nonbruised	115	141	74	100	91	97	--	--	--
			Bruised	65	72	35	40	29	30	--	--	--
			% Nonbruised	59c	52bc	48bc	43ab	35ab	32a	--	--	--

[z]% nonbruised means separated within rows by Duncan's multiple range test, 5% level.

[y]Except where indicated ns, tissue differences within each sampling time and within each hour post-impact are significant at the 5% level.

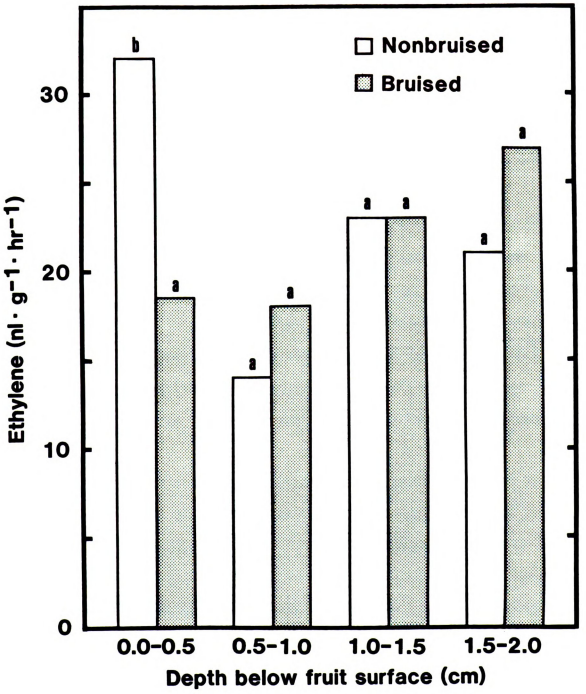


Figure 8. Ethylene evolution by bruised and nonbruised excised 'Empire' apple fruit tissues at successive intervals below fruit surface, 4 hours post-impact. Mean separations at each depth by Duncan's multiple range test, 5% level.

Oxygen uptake rates of bruised and unbruised tissues were similar when followed for 48 hours after impact (Fig. 9), with the only significant difference occurring at 10 hours post-impact. Addition of 0.1 M malate to the incubation medium had no effect on O_2 uptake (16).

Measurement of volatile evolution by excised tissues in air and in N_2 (Table 4) showed significantly decreased evolution of ethylene and CO_2 for both bruised and control tissues in N_2. Although ethylene evolution rates for damaged and non-damaged tissues were similar in N_2, a significantly greater amount of CO_2 was evolved by the bruised tissues in this atmosphere. Acetaldehyde evolution rates were significantly greater for bruised than nonbruised tissues only in N_2. Bruised tissues likewise produced significantly more ethanol than unbruised tissues in N_2. No ethanol was detected under aerobic conditions.

Unbruised tissue plugs, when vacuum-infiltrated with 0.4 M mannitol to serve as a possible model for impact-damaged tissues, produced ethylene and acetaldehyde in amounts equivalent to those produced by bruised tissues (Table 5). Tissues which had been infiltrated or bruised produced significantly less ethylene and significantly more acetaldehyde than untreated tissue. Bruised tissue produced significantly more CO_2 than nonbruised controls, with or without mannitol infiltration. Only infiltrated tissue produced ethanol.

The malic acid concentration in extracts taken periodically from bruised tissues decreased immediately and continually compared to

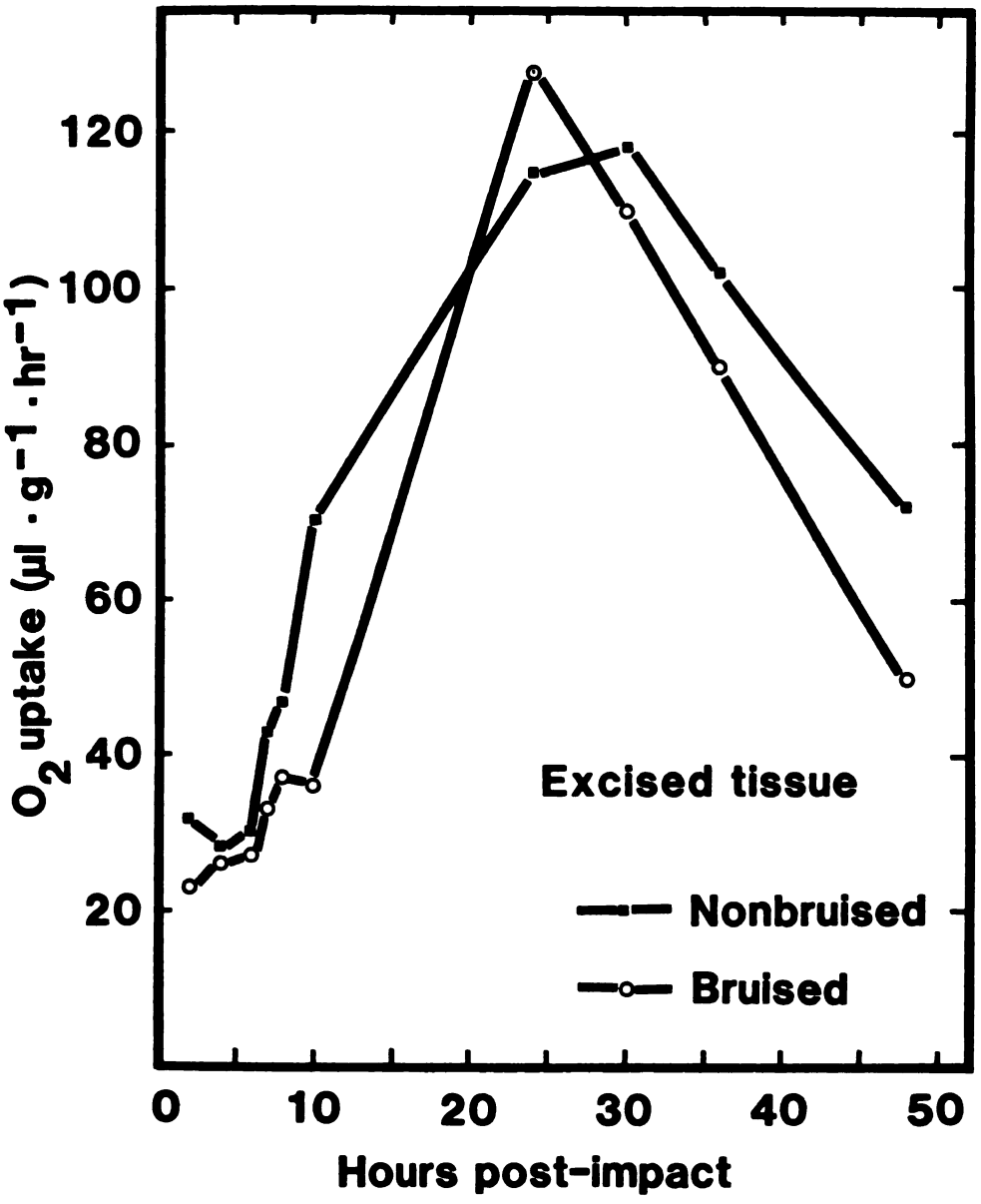


Figure 9. Oxygen uptake by excised bruised and nonbruised 'Empire' apple flesh tissue.

Table 4. Effect of atmospheric composition on volatile evolution by bruised and nonbruised 'Empire' apple tissues.[y,z]

	Ethylene	CO_2	Acetaldehyde	Ethanol
	($nl \cdot g^{-1} \cdot hr^{-1}$)	($\mu l \cdot g^{-1} \cdot hr^{-1}$)	($nl \cdot g^{-1} \cdot hr^{-1}$)	($nl \cdot g^{-1} \cdot hr^{-1}$)
Nitrogen				
Nonbruised	4.6a	37.3a	6.9a	25.4a
Bruised	5.9a	51.1b	10.3b	40.7b
Air				
Nonbruised	18.8c	48.5b	6.8a	< 5
Bruised	10.8b	70.ac	8.3ab	< 5

[y]Samples taken 3 hours post-impact.

[z]Mean separation by Duncan's multiple range test, 5% level.

Table 5. Effect of vacuum infiltration with 0.4 M mannitol and impact damage on volatile evolution by 'Empire' apple tissues.[y,z]

	Ethylene	CO_2	Acetaldehyde	Ethanol
	($nl \cdot g^{-1} \cdot hr^{-1}$)	($\mu l \cdot g^{-1} \cdot hr^{-1}$)	($nl \cdot g^{-1} \cdot hr^{-1}$)	($nl \cdot g^{-1} \cdot hr^{-1}$)
Nonbruised	33.4b	55.3a	7.6a	< 5
Bruised	16.2a	76.3b	19.4b	< 5
Infiltrated	14.2a	56.6a	15.8b	65.5

[y]Samples taken 3 hours post-impact or post-infiltration.

[z]Mean separation by Duncan's multiple range test, 5% level.

controls following impact damage (Table 6). The decline is clearly reflected in the ratio of bruised to control tissue malate concentration, which decreased to as low as 18 for apples tested 79 days after harvest. As was the case for ethylene, in some instances mean malate concentrations for bruised and nonbruised tissues do not correspond to the overall percentage decrease because of variability between fruits within sampling times. The percentage decrease among fruits, however, was consistent within sampling time. The decline in malate concentration was confirmed qualitatively by paper chromatograms. As illustrated in Figure 10, extracts from older bruised tissues (72 hours post-impact) showed smaller spots corresponding to malate than extracts taken from tissues 6 hours after bruising. A comparison of the malic acid and CO_2 concentrations in bruised tissue, expressed as percent of control (Fig. 11) shows that the initial decline in malic acid concentration due to bruising is inversely proportional to the rise in CO_2 evolution. It was not possible, however, to calculate a precise stoichiometric relationship for these two tissue constituents.

The evolution rates of both CO_2 and ethylene for bruised and nonbruised tissues increased with temperature increases from 0 to 30°C (Fig. 12, A & B). Regardless of temperature, the ratio of ethylene evolution from bruised tissues to that of control was always approximately 0.5 (Fig. 12, D). The CO_2 output varied with temperature, with the maximum differential between bruised and control tissues occurring at 20 and 30°C. These temperatures likewise

Table 6. Effect of fruit age on malic acid concentration in tissue extracts from impact-damaged 'Empire' apples[z]

Harvest	Days Cold Storage	Tissue	Malic Acid (mg/100 ml extract)						
			Hours post-impact						
			1	3	6	12	24	48	72
9/12	0	Nonbruised	454	--	492	482	504	380	532
			ns						
		Bruised	422	--	364	290	164	128	144
		% Nonbruised	93d	--	74c	60c	32a	34a	27a
10/12	0	Nonbruised	390	--	536	388	352	--	596
			ns						
		Bruised	346	--	390	242	204	--	268
		% Nonbruised	93c	--	76bc	69b	60b	--	28a
10/18	15	Nonbruised	352	336	342	352	278	346	414
			ns						
		Bruised	358	226	304	188	102	112	126
		% Nonbruised	102d	70bc	89cd	51ab	39a	33a	31a
10/18	50	Nonbruised	264	298	262	240	--	384	392
		Bruised	184	206	178	140	--	110	166
		% Nonbruised	71c	70c	68bc	61bc	--	22a	43ab
10/18	79	Nonbruised	186	324	346	306	260	400	--
			ns						
		Bruised	354	232	256	132	46	140	--
		% Nonbruised	92d	69c	74c	43b	18a	35b	--

[z]Mean separations within rows by Duncan's multiple range test, 5% level.

[y]Except where indicated ns, tissue differences within each sampling time and hour post-impact are significant at the 5% level.

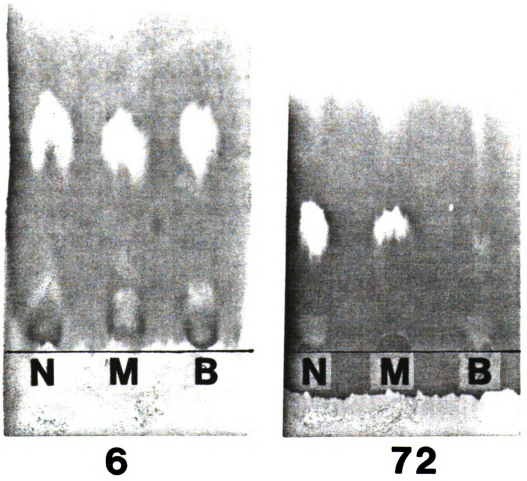
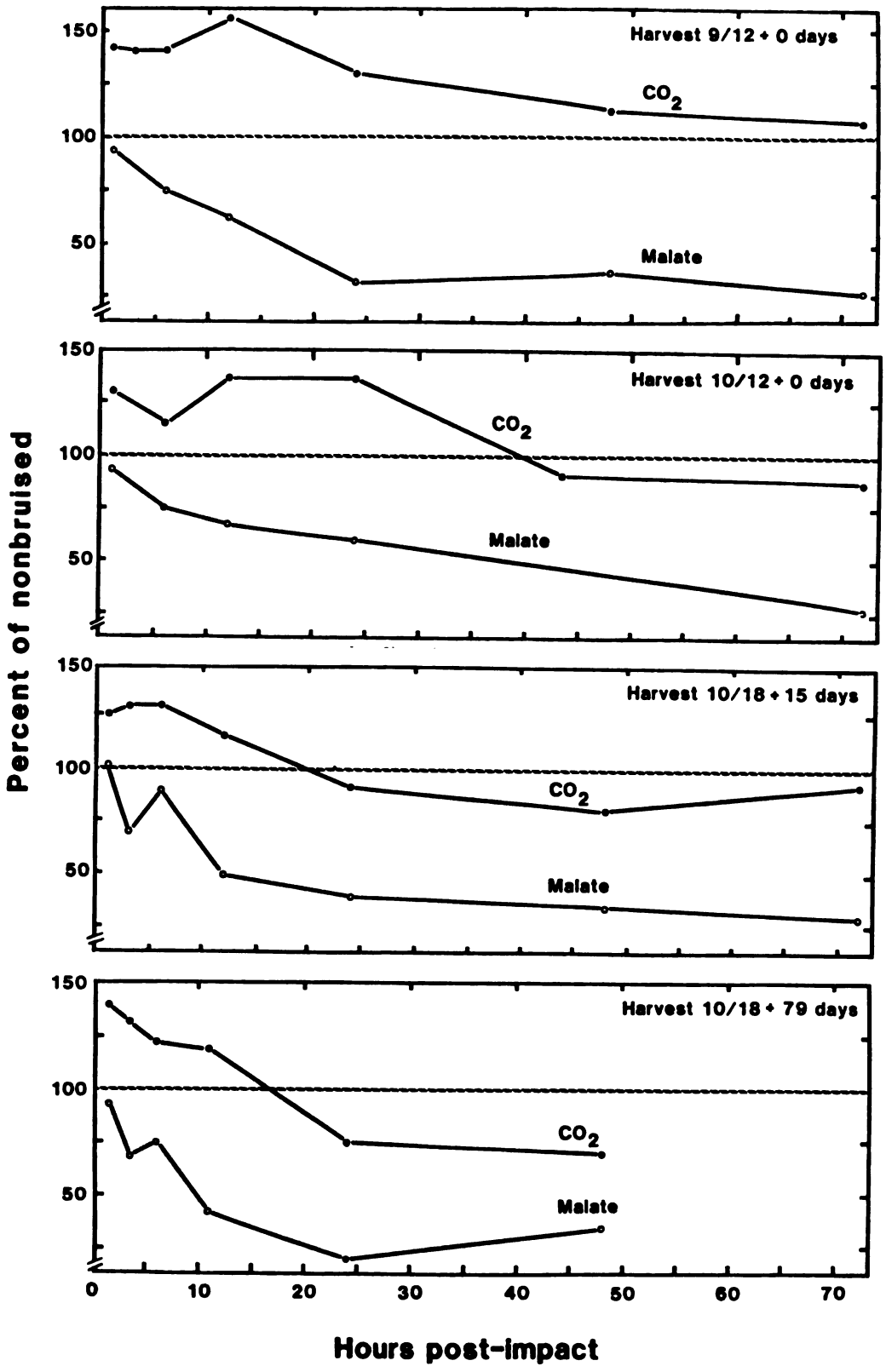


Figure 10. Chromatograms of extracts from bruised and nonbruised 'Empire' apple fruit tissues at 6 and 72 hours post-impact. N = nonbruised; B = bruised; M = malate standard (0.3%, w/v).

Figure 11. CO_2 production and malate concentration in excised bruised 'Empire' apple tissues from fruit harvested and stored at 0°C for various times.


Harvest 9/12 + 0 days
Harvest 10/12 + 0 days
Harvest 10/18 + 15 days
Harvest 10/18 + 79 days
CO2
Malate
150
100
50
0
10
20
30
40
50
60
70
Percent of nonbruised
Hours post-impact

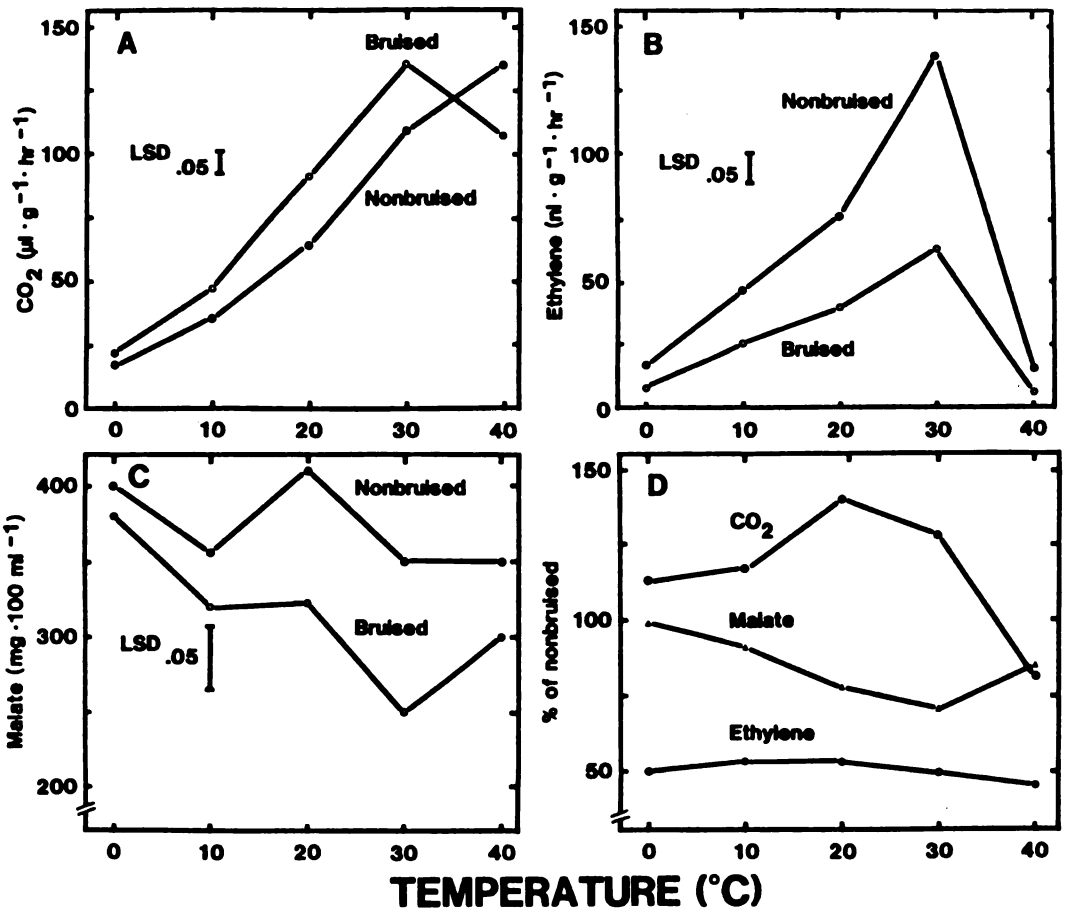


Figure 12. Effect of temperature on CO_2 production, (A), ethylene evolution, (B), and malic acid concentration; (C) of bruised and nonbruised excised 'Empire' apple fruit tissues; (D) CO_2, malate, and ethylene concentrations in bruised tissues as affected by temperature.

resulted in the greatest decrease in malate concentration in damaged tissue (Fig. 12,C&D). Ethylene evolution was greatly depressed at 40°C (Fig. 12,B).

Discussion

CO_2 evolution from impact-damaged whole apples increased during the initial 2 to 3 hours following damage, then declined to levels equivalent to those of nondamaged fruit 6 to 12 hours after impact. This effect due to damage was similar in excised bruised tissues, although the CO_2 response was more immediate, was sustained for a longer period of time, and was greater in magnitude when measured for bruised tissue alone than for whole fruit. Since nondamaged tissues comprise at least 90% of the weight of impact-damaged apples (M. Parker, unpublished observation), the lesser magnitude and shorter period of response of whole bruised fruit is probably due to the confounding presence of nondamaged tissues within the fruit, which dilute the overall increase in CO_2 emanating from the bruise.

Fruit with excised or occluded bruises generally evolved CO_2 in amounts similar to intact, nondamaged fruit, thereby demonstrating that the increased yield of CO_2 from impact-damaged whole fruit is localized at the site of the bruise. An exception to this was petrolatum/lanolin-treated fruit, which evolved CO_2 at an intermediate level between intact bruised and unbruised fruit 9 hours post-impact. This exception may have been due to a lag in diffusion of excess CO_2 from the bruise site through the relatively permeable cortex to an unoccluded site on the fruit before passing into the jar headspace (7). Within the bruise itself, the CO_2 increase was confined to the

cortical tissues closest to the site of impact, where damaged cells were probably greatest in number.

The increase in CO_2 evolution as a result of bruising does not appear to be a result of aerobic respiration, since there was no concomitant increase in O_2 uptake by damaged tissues. Oxygen uptake by both bruised and nonbruised tissues remained steady during the initial 6 hours of incubation in phosphate buffer, but subsequently increased dramatically before decreasing again. Burg and Thimann (8) found that soaking apple tissues in water had no effect on O_2 uptake, but only reported results for up to 90 minutes of soaking. Van Stevenink (34), however, cited several studies in which soaking tissues in various osmotica for greater lengths of time led to increased O_2 uptake due to induced activity of any of several enzyme systems. Incubation in buffer for more than 6 hours may therefore be responsible for the increased O_2 uptake by both bruised and nonbruised apple tissues. Nonetheless, the increases are not related to enhanced CO_2 production by the bruised tissue.

The possibility that rupture of the cells upon bruising caused localized flooding of intercellular spaces and a resultant enhanced production of CO_2 by anaerobic fermentaion was not substantiated when near-anaerobiosis was induced in a N_2 atmosphere or by mannitol-infiltration of tissue intercellular spaces. Neither procedure increased CO_2 evolution to levels comparable to those of bruised tissues. The increase in acetaldehyde concentrations in aerobic bruised apple tissues noted in these experiments was also reported by Thomas (31).

Impact damage to fruit probably ruptures the vacuolar membrane as well as the cell wall (14), resulting in a release of vacuolar malic acid into the cytoplasm, where it can be decarboxylated by cytoplasmic malic enzyme (10). This was reflected by a decrease in titratable acidity of bruise tissue extracts (expressed as malic acid) that was paralleled by an increase in CO_2 evolution from the bruise. Once the malate substrate is decarboxylated, CO_2 output by bruised tissues falls below that of intact tissue, since ruptured cells are unable to carry on normal respiratory activity.

Neal and Hulme (26) demonstrated a 1:2:1 stoichiometric relationship for the decarboxylation of malate to CO_2 and acetaldehyde in apple peel discs. They further showed that this enzymatic activity did not involve O_2 uptake. My findings support these conclusions, even though a stoichiometric relationship could not be demonstrated. The malic acid concentration in bruised tissues declined simultaneously with increased CO_2 and acetaldehyde evolution. The rate of decline in malate concentration was more rapid in older fruit, in keeping with the increased specific activity of malic enzyme as fruit ripen (10).

Temperature dependency of the increased rate of CO_2 production also indicates the enzymatic nature of the response. The decreased CO_2 evolution by bruised tissues compared to control at 40°C may have been due to temperature inhibition of enzymatic decarboxylase activity. The greater malic acid concentration in bruised tissues as a percent of control at 40°C compared to that at 20 or 30° is also indicative of less decarboxylation at higher temperatures.

Fruits dropped from greater heights are more severely damaged upon impact, and thus have more ruptured cells and vacuoles at the bruise site (13), with increased CO_2 evolution in proportion to the number of damaged cells. On this basis, CO_2 production could be used as a measure of the degree of injury sustained by the fruit and expressed as an index relative to the basal rate of CO_2 production by undamaged fruit. This technique could be particularly useful in determining the protection from damage afforded by different types of containers used in storage and transport of fruit.

Unlike the CO_2 response, the increase in ethylene production by impact-damaged preclimacteric apples is not due exclusively to increased production at the bruise site. The increased internal ethylene concentrations of bruised fruit over controls indicates that the entire fruit is affected rather than just the bruised tissue. Although the major barrier to gas diffusion in apples is the epidermis (7), gas diffusion from the cortex to the core is thought to be partially limited by vascular tissues (5). There is the possibility that enough impact energy was transmitted to the core tissues, which are metabolically more active than cortical tissues (28) to effect an increase in ethylene production. Energy transmission by vibration caused an increase in ethylene production by tomatoes (24,25).

Once fruit ripened enough to produce a quantity of endogenous ethylene, impact damage resulted in a 50% decrease in ethylene evolution from bruised tissues compared to controls. This percentage reduction remained consistent, although the Q_{10} for ethylene production by

both bruised and unbruised tissues (as calculated from data in Fig. 12 B) averaged 1.7 in the range of 10 to 30°C. I suggest that the similarity of Q_{10} in the two tissues is due to the presence of numerous intact cells within the bruise, whereas the overall reduction in ethylene evolution is due to actual physical damage to cells, rather than to a physiological response induced by the energy of impact. The reduction in ethylene evolution at 40°C noted here has been reported previously (22,36).

Increased ethylene production upon bruising of apples not yet producing endogenous ethylene could have been due to the stimulation of ACC synthase activity, followed by increased ACC and ethylene concentrations in the fruit, as demonstrated by Yu and Yang (38) in other injured plant tissues. However, as fruit gain the capacity to produce ethylene and proceed through ripening, the cell walls and membranes could be more susceptible to rupture upon impact (13,29). Disruption of the membranes, which are the site of ACC conversion to ethylene (4,22), would lead to an increase in ACC concentration at the bruise site, since ACC synthesis is not membrane bound (6), while ethylene production would be inhibited in the affected tissues. The increased ethylene evolution upon bruising noted by Massey[1] and Lougheed and Franklin (17) with preclimacteric apples, and the decrease in ethylene evolution from mature bruised apples out of storage found by Robitaille and Janick (28), lend support to the above hypothesis.

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[1]Ibid.

Conclusion

The increase in CO_2 evolution from apples after bruising is not due to enhanced aerobic or anaerobic respiratory activity, but rather is due to decarboxylation of malic acid in cortical tissues at the bruise site. Since the amount of CO_2 evolved due to bruising is proportional to the degree of damage to the fruit, it can perhaps be used as an index for injury that occurs during fruit handling and transport. In contrast to the enzymatic nature of the CO_2 response, the decrease in ethylene evolution from mature bruised apple tissues is due to physical disruption of the cells at the bruise site.

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SECTION II

LEVELS OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID AND ETHYLENE IN BRUISED APPLE TISSUE

Abstract

Concentrations of 1-aminocyclopropane-1-carboxylic acid (ACC) in bruised and nonbruised apple (Malus domestica Borkh., cv. 'Empire') fruit tissues were similar for 24 hours after impact-bruising, while there was a simultaneous 65% decrease in ethylene production by bruised tissues compared to nonbruised. ACC concentrations in bruised tissues declined to 60% of control levels by 48 hours post-impact. Bruised and nonbruised tissues were similar in relative ability to convert exogenously supplied ACC to ethylene, indicating a physical rather than a physiological cause for decreased ethylene production by bruised apple tissue.

Introduction

Wounding or stress leads to increased ethylene production by many plant tissues (1,10,14,15). Apple fruits that have begun producing endogenous ethylene, however, show a decrease in ethylene evolution as a result of bruising (11,14,17). The increase in ethylene production by wounded plant tissues is due to an induction of the synthesis of ACC synthase (6,20), which catalyzes the formation of ACC (5,6), which in turn is enzymatically converted to ethylene (2). Excessively high or low temperatures (5,18,19), osmotic shock (4), or anaerobiosis (18) lead to an accumulation of ACC in the tissue and an inhibition of ethylene production due to interference in the conversion of ACC to ethylene. Bruising causes the rupture of apple

cell walls and associated membranes (9), which are the presumed sites of ethylene synthesis from ACC (16). The purpose of this investigation was to determine if the reduction in ethylene production by bruised tissues of post-climacteric apples is caused by a change in ACC content or metabolism of these tissues.

Materials and methods

Plant material. Post-climacteric apples (average internal ethylene concentration of 180 $\mu l \cdot l^{-1}$), which do not form wound ethylene upon being cut (12), were taken from 0°C storage, equilibrated to 20°C in 15 hours, and dropped 1 m onto a hard, smooth surface to create an impact bruise approximately midway between the stem and the calyx. Fruits were selected randomly from the population of bruised apples at specific times post-impact. Discs of bruised and nonbruised tissue measuring 25 mm x 5 mm and weighing 3 g were then excised from a given apple with a cork borer.

Determination of ethylene and ACC. One-half of each tissue discs was incubated at 20°C in a 12 ml centrifuge tube sealed with a serum cap. At 1 hour, a 1-ml gas sample was taken with a syringe for ethylene analysis by gas chromatography. A 0.3 ml aliquot of juice expressed from the corresponding half of the tissue disc was used for determination of ACC by the method of Lizada and Yang (13). Unfiltered juice converted 60 to 80% of exogenously supplied ACC to ethylene.

Tissue capability to convert ACC to ethylene was determined with half-discs of bruised and nonbruised tissue excised at various

times after bruising and incubated for 1 hour at 20°C in 25 ml Erlenmyer flasks that were sealed with serum caps. Ethylene evolution was determined after 1 hour of incubation in 2.5 ml distilled deionized water with or without 0.4 mM ACC.

Results and Discussion

Ethylene production by bruised apple tissues decreased to 50% of the control by 1 hour post-impact (Fig. 1-A and Table 1) and eventually declined to 30% at the end of the experiment. Differences between ACC concentrations in bruised and nonbruised tissues were not significant in the first 24 hours after impact (Fig. 1-B and Table 1). Levels of ACC in bruised tissues decreased to 60% of nonbruised by 48 hours after bruising, although the corresponding ethylene production rates remained steady. The conversion of ACC to ethylene is a tightly coupled reaction with a rapid turnover rate (19). Thus, bruised apple tissues continued to produce ethylene at a rate of 1.8 $nmole \cdot g^{-1} \cdot hr^{-1}$, even while containing only 0.6 $nmole \cdot g^{-1}$ ACC.

The decline in ACC content of bruised tissues between 24 and 48 hours post-impact may have been due to decreased production of ACC from S-adenosylmethionine (SAM). An alternative possibility is that ACC was converted to its conjugate N-malonyl-ACC (MACC) (3,8) in cells no longer capable of converting ACC to ethylene. MACC, a poor source of ethylene compared to ACC (7), is not measured with 'free' ACC in the Lizada and Yang assay (13) unless previously hydrolyzed in hot HCl (8,7).

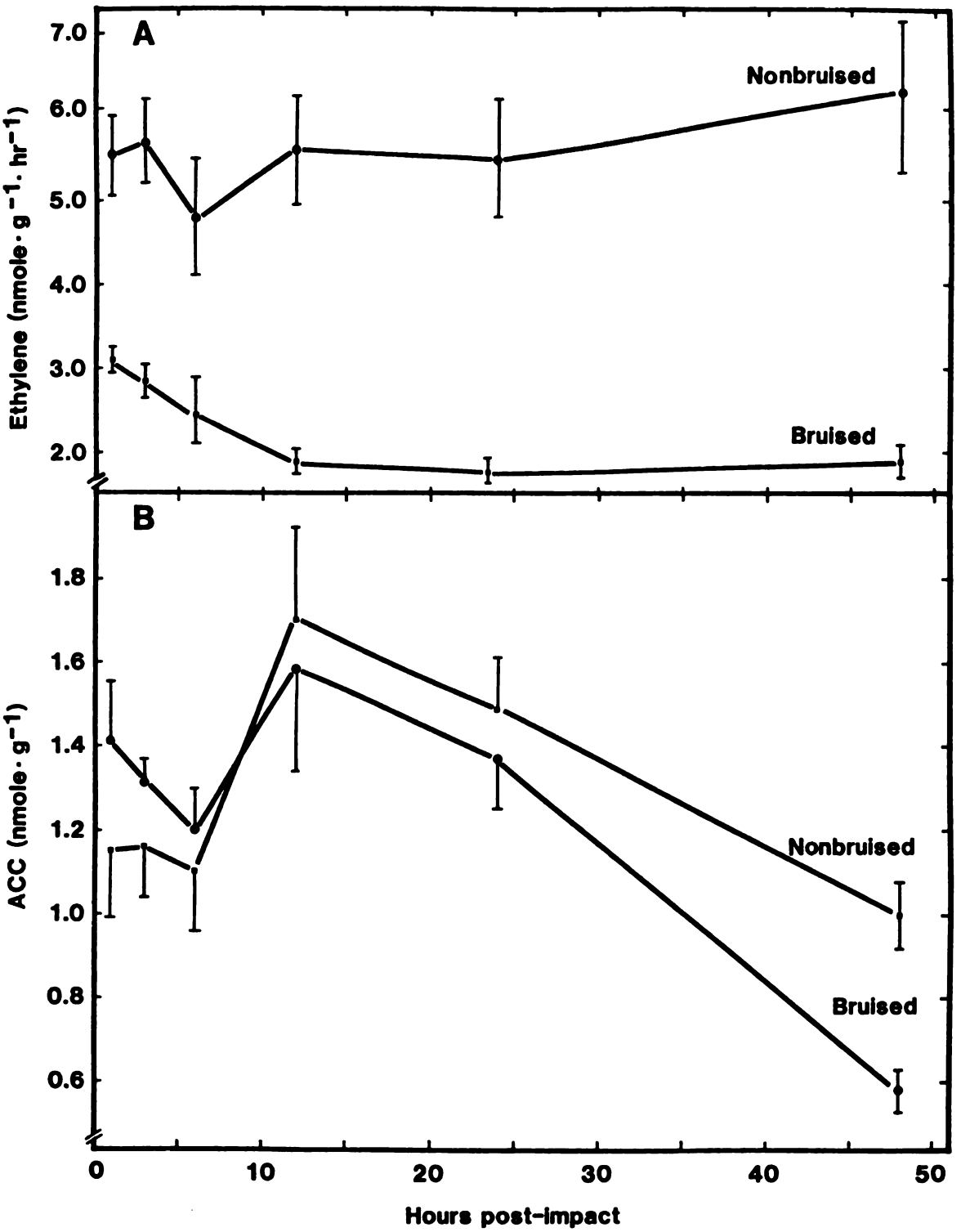

Figure 1. Ethylene (A) and ACC (B) concentrations in bruised and non-bruised 'Empire' apple fruit tissues excised at indicated times after bruising. Means are from 2 pooled experiments, ± S.E.

Table 1. Ethylene and ACC concentrations in bruised 'Empire' apple fruit tissues[z]

Hours post-impact	Ethylene ($nmole \cdot g^{-1} \cdot hr^{-1}$)	ACC ($nmole \cdot g^{-1}$)
	% of nonbruised	
1	53a	125a
3	49a	124a
6	47a	117ab
12	35b	94b
24	33b	97ab
48	30b	63c

[z]Data are from 2 pooled experiments; mean separations within columns by DMRT, 5% level.

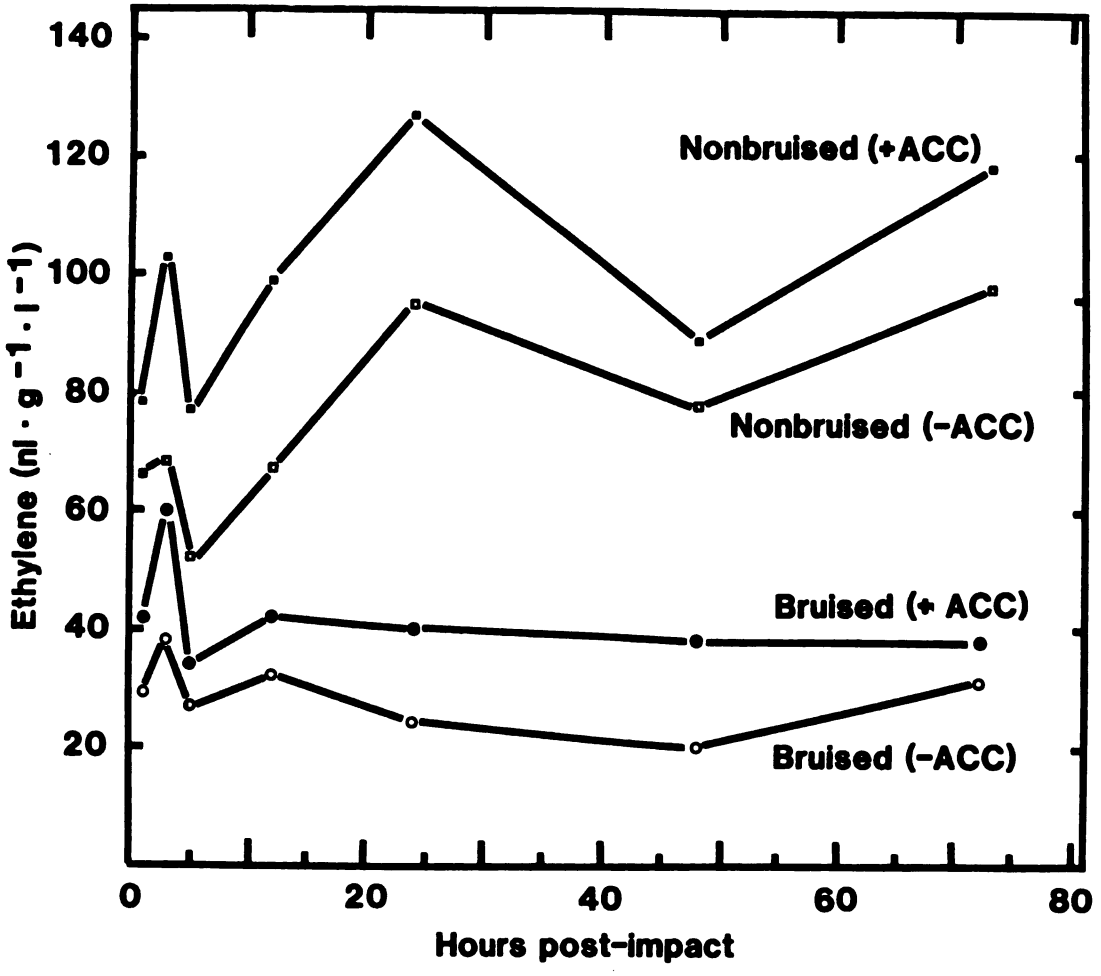

Figure 2. Ethylene evolution from bruised and nonbruised 'Empire' apple fruit tissues excised at indicated times after bruising and incubated for 1 hour with or without 0.4 mM ACC

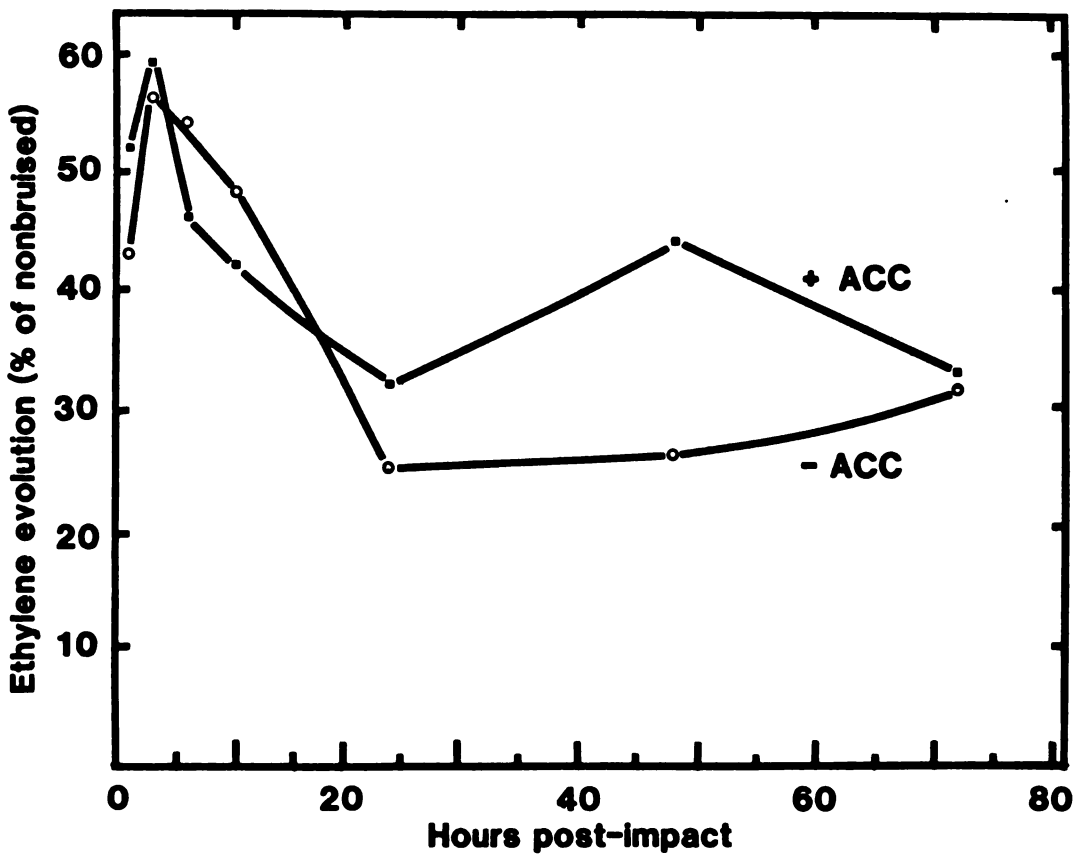

Figure 3. Ethylene evolution from bruised 'Empire' apple fruit tissues excised at indicated times after bruising and incubated for 1 hour with or without 0.4 mM ACC

Bruised and nonbruised tissues bathed in 0.4 mM ACC produced 20 to 90% more ethylene than tissues without exogenous ACC (Fig. 2). Both bruised and nonbruised tissues produced equal amounts of ethylene when cooked 4 minutes in a microwave oven prior to incubation without exogenously supplied ACC (11), thus eliminating the possibility of nonenzymatic conversion of ACC to ethylene.

Ethylene production ratios of bruised to nonbruised tissues were not affected by addition of ACC (Fig. 3), indicating that both tissues metabolized exogenous and endogenous ACC in a similar manner. Therefore, the 50% decrease in ethylene production by bruised tissues compared to controls must result from the physical destruction of cells within the bruised area rather than from differences in ethylene biosynthesis. Further evidence that the reduction is physical rather than physiological in nature is that the ratio of bruised to nonbruised ethylene production rates remains constant at 0.5 over a temperature range of 0° to 40°C (11). Although epidermal tissues from both nonbruised and bruised apples produce similar amounts of ethylene, there is a 50% reduction in ethylene production by the subjacent cortical tissues as a result of bruising (11,17). I conclude that the reduction in ethylene production by bruised cortical tissues of postclimacteric apple fruit is a consequence of cell destruction within the bruised area, since the surviving cells readily metabolize ACC to ethylene.

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CONCLUSIONS

1. Both the CO_2 and the ethylene response in bruised apples are due to disruption of the cells at the bruise site. The increase in CO_2 evolution from apples after bruising is not due to enhanced aerobic or anaerobic respiratory activity, but rather is due to decarboxylation of malic acid in the 0.5 cm or cortical tissue near the fruit surface at the point of impact.

2. The amount of CO_2 evolved due to bruising is proportional to the degree of damage and could be useful as an index for assessing physical injury that occurs during fruit handling and transport.

3. In contrast to the enzymatic nature of the CO_2 response, the decrease in ethylene evolution from mature bruised apple tissues is due to physical disruption of cells in the bruised areas. Intact cells in bruised tissues from mature apples produce ethylene from exogenously supplied ACC and over a range of temperatures in the same manner as nonbruised tissues.

APPENDIX

Appendix

Some experimental data, unnecessary for support of the proposed hypotheses, were omitted in the two journal manuscripts that comprise the majority of this thesis. They concern the ethylene production of preclimacteric fruit, the effect of exogenous malate on O_2 uptake and ethylene production by bruised and nonbruised tissues, and the lack of conversion of exogenous ACC to ethylene in dead tissues and are presented here with brief discussions to provide background material that supplements the manuscripts.

Fruit that were preclimacteric in ethylene production ($<0.1\ \mu l \cdot l^{-1}$ internal ethylene) did not have a consistent ethylene evolution response to bruising until approximately 24 hours after impact. The positive correlation of internal ethylene concentration with number of impact-drops shown in Table 1 occurred only 24 hours after bruising. Similarly, the linear regression of ethylene evolution on height to drop (Table 2) was not significant ($r = 0.14$), nor were there any significant differences between treatment means, when fruit were sealed 16 hours post-impact. When the sealed containers were opened, aerated thoroughly, and resealed with the same fruit 27 hours post-impact, the resulting regression of ethylene evolution on drop height was highly significant ($r = 0.75$, significant at the 0.1% level), as shown in Fig. 6 in the first manuscript. The

physiological basis for the development of the ethylene response over time in bruised preclimacteric apples bears further investigation.

Oxygen uptake rates of bruised and nonbruised tissues were similar when followed over 48 hours post-impact. The addition of 0.1 M malic acid to the incubation medium of nonbruised tissues 6 hours after initial measurements had no effect on O_2 uptake rate, compared to tissues without exogenous malate (Table 3). The statistically significant difference in O_2 uptake rate of bruised tissues with exogenous malate compared to other tissue treatments also existed before malate was added. Exogenous malate was not likely a factor in the lower rate of O_2 uptake in this case, as noted in the last 14 hours of this experiment, when there were no statistical differences between treatments. As noted in the first manuscript, these data support the concept that the enzymatic decarboxylation of malate by mature apple tissues does not require O_2 uptake.

Neither the decrease in ethylene evolution by bruised apple tissues compared to controls nor the overall ethylene output of both tissues were affected by exogenous malate (Table 4).

Both bruised and nonbruised tissue samples heated 4 minutes at 'high' setting in a microwave oven had only residual ethylene production capacity, and no ability to convert exogenous ACC to ethylene (Table 5). Since there is evidently no system capable of converting exogenous ACC to ethylene in cooked ("dead") tissue pieces, the ability of noncooked bruised tissues to effect the conversion indicates that at least some cells in those tissues were "alive". I have no

explanation for the apparently greater production of ethylene by cooked bruised tissues compared to cooked nonbruised.

Table 1. Effect of 0, 1, and 3 drop-impacts from a height of 1 m on internal ethylene concentration in preclimacteric 'Empire' apples[y,z]

Number of impacts	Internal ethylene concentration ($nl \cdot l^{-1}$)				
	Hours post-impact				
	1	3	6	12	24
0	19a	--	23 ns	15a	20a
1	18a	21	28	31b	30b
3	29b	20	26	24ab	74c

[y]Fruit harvested Aug. 31, bruised Sept. 2. Different lots of bruised fruit sampled at a given time.

[z]Mean separations within column by Duncan's multiple range test, 5% level.

Table 2. Ethylene evolution from preclimacteric 'Empire' apples after impact drops from various heights[y,z]

	Drop Height (cm)				
	0	12.5	25	50	100
Ethylene ($nl \cdot kg^{-1} \cdot hr^{-1}$)	11.0	10.0	13.6	14.6	14.1 ns

[y]Fruit harvested Aug. 31, bruised Sept. 1

[z]No significant difference between means by Duncan's multiple range test, 5% level

Table 3. Oxygen uptake by bruised and nonbruised 'Empire' apple tissues as affected by exogenous malic acid (0.1 M)[y,z]

Tissue	Oxygen uptake ($\mu l \cdot g^{-1} \cdot hr^{-1}$)								
	Hours post-impact								
	2	4	6	8	10	24	30	36	48
Bruised	23ns	26a	27a	37ab	36b	128a	111ns	90 ns	50ns
Nonbruised	32	28a	30a	47a	70a	115a	117	103	77
Bruised + malate	29	19b	16b	17b	13b	49b	99	106	70
Nonbruised + malate	28	36a	42a	55a	65a	117a	122	104	84

[y]Malate added to incubation medium 6.25 hours post-impact

[z]Mean separations within columns by Duncan's multiple range test, 5% level

Table 4. Ethylene evolution from bruised and nonbruised 'Empire' apple tissues as affected by exogenous malic acid (0.1M)[y,z]

Tissue	Ethylene evolution ($nl \cdot g^{-1} \cdot hr^{-1}$)								
	Hours post-impact								
	2	4	6	8	10	24	30	36	48
Bruised	45ns	45a	45a	49a	47a	52a	47a	43a	36a
Nonbruised	68	86b	92b	97b	139b	228b	153b	213b	186b
Bruised + malate	40	50ab	51a	50ab	56a	59a	53a	49a	45a
Nonbruised + malate	55	72a	80b	80ab	125b	186b	156b	161b	130b

[y]Malate added to incubation medium 6.25 hours post-impact

[z]Mean separations within columns by Duncan's multiple range test, 5% level

Table 5. Ethylene evolution from bruised and nonbruised 'Empire' apple tissues as affected by incubation with or without 0.4 mM ACC after heating in a microwave oven for 4 minutes

Treatment	Tissue	
	Nonbruised	Bruised
	Ethylene ($nl \cdot g^{-1} \cdot hr^{-1}$)	
-ACC	0.44	1.50
+ACC	0.47	1.30