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THE EFFECT OF FRUIT REMOVAL ON LEAF PHOTOSYNTHESIS, WATER RELATIONS, AND CARBOHYDRATE PARTITIONING IN SOUR CHERRY AND PLUM.

presented by

Riccardo Gucci

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James A. Flore
Major professor

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THE EFFECT OF FRUIT REMOVAL ON LEAF PHOTOSYNTHESIS, WATER RELATIONS, AND CARBOHYDRATE PARTITIONING IN SOUR CHERRY AND PLUM

By

Riccardo Gucci

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ABSTRACT

THE EFFECT OF FRUIT REMOVAL ON LEAF PHOTOSYNTHESIS, WATER RELATIONS, AND CARBOHYDRATE PARTITIONING IN SOUR CHERRY AND PLUM

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The effect of fruit removal on photosynthesis and gas exchange parameters of leaves from field-grown, mature sour cherry (Prunus cerasus L. 'Montmorency') and plum (Prunus domestica L. 'Stanley') trees was studied with respect to environmental conditions, and carbon and water status of the leaf.

Removal of fruits at the end of stage III caused an immediate (within 48 h) decrease of net photosynthesis (Pn) equal on average to 41% in 1985, 26% in 1986, and 27% in 1987 for sour cherry, 25% in 1986 and 0% in 1987 for plum. Removal of plum fruits during stage II reduced Pn 32% in 1986 and 16% in 1987. Pn inhibition after fruit removal persisted 2-4 wks in sour cherry, but only 7-10 days in plum when fruits were removed during stage II. In both species Pn inhibition was greatest in the afternoon, lower or negligible in the morning.

Pn decrease of defruited trees was paralleled by similar, simultaneous changes in stomatal conductance (g_s), whereas calculated levels of intercellular CO_2 (Ci) did not change significantly after fruit removal. Defruiting did not significantly affect chlorophyll content or leaf water

potential, but defruited trees had lower osmotic potentials. Stomatal closure and lower osmotic potentials of defruited trees did not appear to be related to loss of leaf turgor.

Non-stomatal contribution to the post-harvest decrease of Pn calculated from field and laboratory measurements of assimilation-internal CO_2-response curves ranged from 55% to 76%. Photorespiration rates were similar for fruiting and defruited plum trees, while in sour cherry Pn inhibition due to fruit removal decreased from 27% of the control at 21% O_2 to 14% at 2.5% O_2.

Levels of soluble sugars (fructose, glucose, inositol, sorbitol, and sucrose) in the leaf were not significantly different before and after fruit removal. Leaf starch content increased 2-3-fold within 24 h from fruit removal. Analysis of transmission electron micrographs of leaf mesophyll cells of sour cherry did not reveal disruption of thylakoid membranes due to enlargement of starch grains in the chloroplast.

The results are interpreted as evidence for sink-induced inhibition of leaf Pn after fruit removal and support the hypothesis of feedback regulation of Pn by levels of non-structural carbohydrates in the leaf.

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Guidance Committee:

The journal paper format was chosen for this dissertation in accordance with departmental and university regulations. The dissertation is divided into four sections and an appendix. Section one and two are intended for publication in The Journal of the American Society for Horticultural Science, sections three and four are intended for publication in Physiologia Plantarum.

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LIST OF ABBREVIATIONS

A/Ci	CO_2 assimilation response curve to internal CO_2
Ca	ambient CO_2
CE	carboxylation efficiency
Ci	intercellular CO_2
CSA	cross sectional area
Gc	stomatal contribution to a change in Pn
g_s	stomatal conductance
IC	cloudiness index
L/F	leaf to fruit ratio
Lg	stomatal limitation to Pn
J	molar concentration of cell sap
OP	leaf osmotic potential
PAR	photosynthetic active radiation
Pi	inorganic phosphate
Pn	net photosynthesis
R	gas constant
RH	relative humidity
RuBP	ribulose bisphosphate
Rubisco	ribulose bisphosphate carboxylase/oxygenase
T	air temperature
TEM	transmission electron microscopy
TP	turgor pressure
Tr	transpiration
VPD	vapor pressure deficit
WP	leaf water potential
WUE	water use efficiency

INTRODUCTION

In order to maximize yields and resistance to various stresses of fruit trees it is necessary a better understanding of the relationship between sources and sinks for assimilates.

Compensatory changes of Pn in response to source-sink manipulations and environmental conditions have been shown to occur in several species.

Evidence in favor or against changes of leaf net photosynthesis after removal of the reproductive sink has been reported for many Prunus species. However, data reported in the literature are seldom comparable because of differences in plant material (species, age, cultural practices), experimental methods, and/or environmental conditions. In addition, in only a few cases a clear distinction has been made between the effect on photosynthesis due to the elimination of the reproductive sink (blossoms, flowers, or fruitlets at an early stage), and changes of photosynthesis caused by removal of fruits when they are strong sinks for assimilates. These two situations may have a different impact on gas exchange parameters of the tree. In fact, if compensatory changes of Pn occur in response to altered sink strength, then the

stage at which fruits are removed is critical to detect any effect of the fruit on gas exchange variables.

Determining the physiological response of fruit trees to removal of fruits in the field is important not only to optimize cultural practices like pruning, fruit thinning, and irrigation, but also to understand phenomena like June drop and alternate bearing. For this reason, gas exchange parameters, water relations, and carbohydrate partitioning were measured when developing or mature fruits were removed from sour cherry or plum trees in the field. Sour cherry and plum were chosen because of their different rate of fruit growth and period of development that allowed to study the "fruit effect on Pn" under different climatic conditions during the growing season.

The objective of this study was to characterize the physiological and environmental conditions under which the "fruit effect" can be detected in sour cherry and plum and to determine the mechanism of regulation of photosynthesis by the fruit.

Section I

THE EFFECT OF FRUIT REMOVAL ON PHOTOSYNTHESIS AND WATER RELATIONS IN SOUR CHERRY.

ABSTRACT

Removal of mature fruits from field-grown sour cherry trees (Prunus cerasus L. cv. 'Montmorency') caused an immediate (within 24-48 h) decrease in leaf net photosynthesis (Pn) over 3 growing seasons. Pn inhibition averaged to 41% in 1985, 0% and 51% in 1986, and 27% in 1987 (afternoon measurements). The decrease of Pn appeared as early as 24 h after harvest and was particularly evident in the afternoon, or when air temperatures were above 28 C and light intensity high (PAR 1500-2000 μmols m^{-2} s^{-1}). Pn rates of harvested trees remained significantly lower than fruiting ones for 2-3 weeks after removal of fruits. The decline of Pn after fruit removal was similar in spur and terminal leaves and was not associated with changes in leaf chlorophyll content. Pn rates were higher in both treatments when the source was limited with respect to sink strength (leaf/fruit ratio < 2). Despite symptoms of apparent wilting of the foliage after fruit harvest, there was no significant decrease in turgor due to treatment.

Changes in Pn rate were paralleled by similar, simultaneous changes in stomatal conductance (g_s). Stomatal conductance of trees with fruits removed also diminished sharply in the afternoon. Calculated levels of intercellular CO_2 (Ci) remained approximately constant (190-220 μl l^{-1}) for both treatments during the course of the experiment. In addition, Ci of defruited trees was usually equal to or higher than that of fruiting trees. Therefore, both

stomatal and non stomatal factors seem to be responsible for the observed inhibition of leaf photosynthesis following fruit removal.

INTRODUCTION

The leaves of sour cherry trees appear cupped after fruits are removed, as if the tree is under water stress (Flore and Gucci 1986). Fruit removal has been reported to cause a decrease of photosynthesis in many annual (Hall and Milthorpe 1975; Kriedemann et al. 1976; Lenz 1979; Plaut and Mayoral 1984) and perennial species (Downton et al. 1987; Fujii and Kennedy 1985; Lenz 1979; Schaffer et al. 1987). On the other hand, removal of flowers or fruitlets (stage I) only slightly reduced leaf photosynthesis of peach (DeJong 1986), sweet cherry (Roper et al. 1988), and sour cherry (Sams and Flore 1983). Other physiological responses to fruit removal include decreased stomatal conductance and increased leaf water potential (DeJong 1986; Downton et al. 1987; Kriedemann et al. 1976; Schaffer et al. 1987), altered levels of phytohormones (Hoad et al. 1977; Kriedemann et al. 1976; Setter et al. 1980), and altered carbohydrate partitioning within the leaf and among different plant organs (Hall and Milthorpe 1978; Roper et al. 1988; Schaffer et al. 1987). The decrease in stomatal conductance and the change in Pn appear to be simultaneous, but it is still not clear whether the decrease in Pn is caused by stomatal closure or also by nonstomatal factors. Further, is stomatal closure driven directly by substances translocated to or from the fruit or by changes in carbon metabolism? Since ABA accumulation in detached grapevine leaves may be triggered by loss of turgor (Loveys

and During 1984), extended periods of water stress may account for ABA-induced stomatal closure and the cupped appearance of sour cherry leaves after harvest. On the other hand, Pn regulation by carbon metabolism would be achieved either because high levels of intercellular CO_2 cause stomatal closure, or because of direct feedback inhibition in the photosynthetic reduction cycle to regenerate ribulose-1,5-bisphosphate.

Field studies on the effect of fruit removal on Pn of woody species are often inconclusive because experimental conditions are not well defined. Yet, environmental and experimental conditions may limit Pn rates of woody species more than internal mechanisms (Nelson 1984). Pn rates of sour cherry, under controlled or natural conditions, may be limited by both environmental and internal factors (Flore and Sams 1986; Sams and Flore 1983; Roper et al.1988).

In this study I report on the effect of fruit removal on gas exchange of sour cherry, with particular emphasis on the interaction between changes in photosynthetic response and environmental parameters, and differences in source to sink (leaf/fruit) relationships. Further, I have attempted to determine if the wilted appearance of sour cherry foliage after fruit harvest was due to water stress. Fruit removal was performed at maturity, when the fruit still presented a relatively strong sink. To assess environmental variability, fruit removal was repeated over 3 growing seasons.

MATERIALS AND METHODS

Plant material. Three pairs of adjacent sour cherry trees (*Prunus cerasus* L. cv. Montmorency on Mahaleb rootstock), planted in 1979, were selected similar in size, vigor and crop load in an orchard at the Horticultural Research Center (HRC) of Michigan State University, East Lansing (latitude 43°N, altitude 288 m) in spring 1985. Rows were oriented north-south, trees were spaced at 8.2 x 3.0 m, and soil type was a Miami loam of pH = 5.5-6.0. Six trees were used in 1985, four in 1986 and 1987. In 1986 additional measurements were taken on four trees, spaced 2.0 x 6.0 m, planted in 1981 on an Emmet sandy loam (pH=6.2) at the Northwestern Horticultural Experiment Station (NWHES), Traverse City (latitude 45°N, altitude 245 m). At both locations, trees were randomized in blocks, each containing 2 trees. Pesticides were applied according to commercial recommendations (Mich. Ext. Bul. E154, Fruit Pesticide Handbook). Pruning, fertilization, and other cultural practices were performed according to standard practices.

Leaf/fruit ratio, fruit growth and removal. Leaf/fruit ratios for each tree were estimated yearly by counting leaves and fruits on 4-5 limbs on the east and west sides of the canopy of each tree after fruit set (mid stage II of fruit development). Leaves from these same shoots were later measured for gas exchange, water relations and chlorophyll

content. Shoots for gas exchange measurements were chosen based on position, girth, length, and crop load.

Leaf lamella angles were measured with a protractor 7 days after fruit removal, considering the leaf midvein as the origin of the angle, and the 2 half blades as the sides.

Fruits (n=20-25) were collected weekly from about 35 days after full bloom until fruit maturity for growth measurements. Estimated dates of full bloom at the HRC location were: April 24, 1985; April 26, 1986; April 26, 1987. Fruits were transported, sealed in a plastic bag under ice, to the laboratory where fresh weights were measured with a Mettler AE 163 analytical balance (Mettler Instruments AG, Greifensee, Switzerland). Longitudinal, cheek and suture diameters were measured with a precision caliper. Dry weights were determined after drying the fruits to constant weight at 105° C. During this study 2 trees were completely harvested at fruit maturity each year. Dates of fruit harvest were: July 3, 1985; June 30, 1986; July 7, 1987. One additional tree was defruited on July 14, 1985. Trees were harvested by hand from 1700 to 2100 hr. Two trees were also harvested at NWHES on July 16, 1986. Yields per tree amounted to 25 and 36 kg in 1985, 13 and 7 kg in 1986, and 44 and 30 kg in 1987 (HRC, East Lansing).

Environmental parameters. Values of minimum/maximum temperatures, rainfall and degree of cloudiness were taken from daily weather records from each station.

Gas exchange parameters. Leaf net photosynthesis (Pn), stomatal conductance (g_s), PAR, leaf temperature, and air relative humidity (RH) were measured with a portable open gas exchange system equipped with a Parkinson broad leaf chamber (model ADC LCA-2, Analytical Development Co., Hoddesdon, England) operated at the following conditions: saturating light intensity (PAR > 1000 μmols m^{-2} s^{-1}), ambient CO_2 of 330-340 μl l^{-1}, flow rate 0.4 l/min, inlet RH 6-11%, unless otherwise stated. The cuvette was clamped onto the leaves and readings were taken after differential CO_2 had equilibrated (within 30-50 seconds). Relative humidity of the outgoing air and cuvette temperature were also monitored for calculation of g_s. One spur leaf and one leaf apical to the fruit on 1-year-old wood were measured on 3-5 shoots per tree for a total of 12-20 leaves per treatment at each determination. Leaves were tagged at the beginning of the experiment and subsequent measurements were made on the same leaves. Adjacent leaves were chosen in those cases in which physical damage had occurred. Pn measurements started between 1200 and 1600 hr in 1985. In 1986 and 1987, weather permitting, Pn was measured on opposite sides of the canopy twice a day (morning and afternoon). Leaf photosynthesis, conductances and internal CO_2 were calculated as previously described (Moon and Flore, 1986). The experimental component of changes in gas-exchange parameters was calculated by subtracting the decrease (or summing the increase) of the

control value for that specific variable from the change observed for the defruited treatment.

The experimental design was a randomized complete block. Treatment comparisons were made at each date by ANOVA, where appropriate, and means were separated by LSD or SE.

Leaf chlorophyll. Chlorophyll content was determined according to the method of Moran (1982). Five to ten leaves per tree were collected at each time of Pn measurement, and immediately frozen in dry ice. Three 1-cm diameter discs per sample were punched from the lamella, weighed, and suspended in N,N-dimethyl-formamide (0.5-0.6% w/v). The remaining tissue was stored at -17°C until the osmotic potential was determined. Analyses were repeated 3 times per tree. Leaf discs were recovered after extraction and their weights recorded after drying to constant weight at 105° C. Results were calculated on the basis of both weight and area.

Leaf potentials. Leaf water potential of 3 to 4 leaves per tree was determined with a portable pressure bomb (PMS Instrument Company, Corvallis, Oregon) at each time of Pn measurement. Leaves were similar in size, position on the spur or shoot, distance from fruits, apparent turgor, and exposure to light to those used to measure photosynthesis. Two procedures for water potential measurement were employed. That of Turner and Long (1980) was used in 1986

and 1987, whereby the leaf was enclosed in a zip-seal plastic bag prior to collection. In 1985 the leaf was harvested without using the zip-seal plastic bag. The time between detachment of the leaf and placing it into the pressure chamber never exceeded 30 seconds. Comparisons between water potential of harvested and fruiting trees were not affected by the change in method.

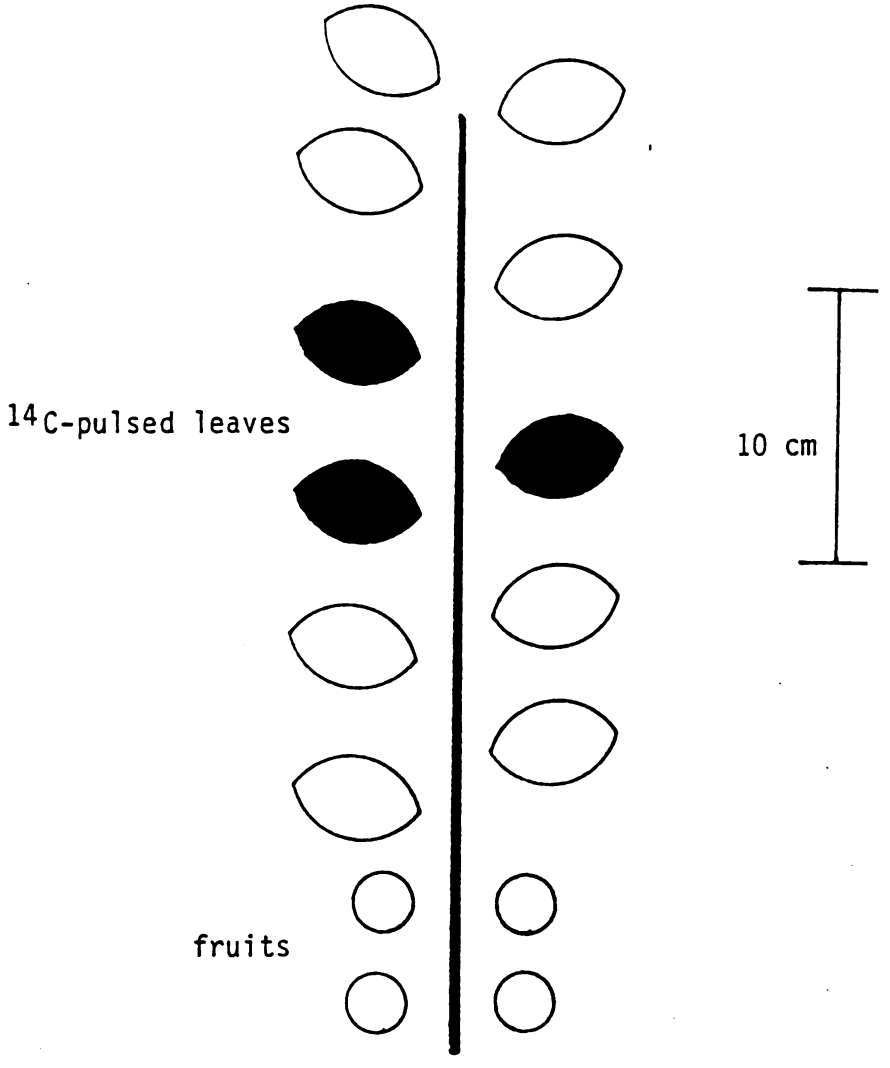
Leaf osmotic potential was measured on frozen tissue leftover from chlorophyll analysis. Major veins were excised, the thawed tissue was placed in a 5 cc syringe, and 10 ul of the cell sap was forced out with the plunger and used for solute determination with a vapor pressure osmometer (5500 M2448 Wescor Inc, Logan, Utah) at 37° C. Solute concentration (mmol/kg) was converted into osmotic potential using the Van't Hoff equation (OP= R x T x J), where R= gas constant, T= temperature at which the measurement was made, and J= molar concentration of cell sap. Osmotic potential determinations were made in triplicate for each tree at each date. Turgor pressure was calculated as the difference between water and osmotic potentials.

^{14}C studies. Shoots from defruited and control trees were excised at the base between 800 and 900 hr and immediately transported with bases in water to the laboratory (< 10 min.), where they were recut under water to a maximum length of 45 cm and allowed to equilibrate in a stainless steel

ventilated hood for 4 hours (light intensity = 600-700 µmols m^{-2} s^{-1}; T = 26°C; ambient CO_2= 350-370 µl l^{-1}). Light was provided by four 400 W high pressure sodium vapor lamps (General Electric, LU 400/40). Three leaves from the median portion of the shoot were chosen so to have 3-4 leaves apical and 4-5 basal to them. Fruiting shoots bore 5 to 10 fruits in the lower part of the shoot (Figure 1). $^{14}CO_2$ pulsing was performed as described by Kappes (1985) with the following modifications: a) three adjacent leaves were enclosed in bags of approximately 1.5 liter capacity containing ambient air. Bags were prepared from sheets of Mylar 30 (polyethylene terephthalate polyvinylidene chloride coated) (DuPont, Wilmington, Delaware). This material was chosen for its low permeability to CO_2; b) $^{14}CO_2$ was released by reacting 0.7 ml of ^{14}C-sodium bicarbonate diluted to 10 µCi/ml (56 mCi/mmol, in sterile aqueous solution obtained from ICN, Irvine, California) and 1 ml lactic acid (20% v/v in deionized water). Leaves were exposed to $^{14}CO_2$ for 30 minutes, then bags were removed. CO_2 uptake estimated from radioactivity incorporated into the pulsed leaves ranged from 85% to 95%. Translocation patterns were determined by autoradiography. Shoots were sampled 3 hrs after pulsing, mounted on paper and dried for 48 hours at 75 C in a forced draft oven. The dry samples were exposed to X-Omat AR film (Eastman Kodak Co., Rochester, NY) for 10 days and then developed according to manufacturer guidelines. Levels of detection by autoradiography and

Figure 1. Schematic representation of leaves and fruits on excised shoots from fruiting and defruited sour cherry trees used for ^{14}C pulsing.

Figure 1



time-course study (0.5-24 hours) of transport after pulsing were determined using a Biological Oxidizer - OX400 (R. J. Harvey Instrument Corporation, Hillsdale, New Jersey) operated at O_2 and N_2 flows of 0.3 l/min. Leaf discs (30-60 mg dry tissue), and shoot and fruit sections (70-130 mg dry tissue) were combusted at 2 and 4 minute cycles respectively, the products trapped in 15 ml of Carbon-14-Cocktail (R.J. Harvey Instrument Company, Hillsdale, New Jersey), and the radioactivity counted in a liquid scintillation counter (1211 Rackbeta, LKB-Wallac, Turku, Finland). Combined efficiency of combustion and counting estimated by carbon-14 standards (CFR.101, Amersham International plc, UK) ranged from 65 to 80%.

RESULTS

Fruits were removed at the end of stage III in all 3 years of the study. At this stage cherry fruits were still relatively strong sinks for assimilates because of their size and relative activity. Changes in both diameter and dry weight in 1987 are illustrated in Figure 2.

Fruit effect in 1985

During the first 2 weeks of July, average maximum and minimum air temperatures (T) were 26.5° and 12.1° C respectively for the 10 days preceding harvest. Maximum and minimum T for the month were 27.5°C and 13.9°C (figure 3a), while average leaf temperature at time of measurement was

Figure 2. Growth curve of sour cherry fruits in 1987. Date of full bloom: April 26, 1987; Date of fruit harvest: July 7, 1987 (indicated by arrow). Vertical bars indicate ± SE (n=25).

Figure 2

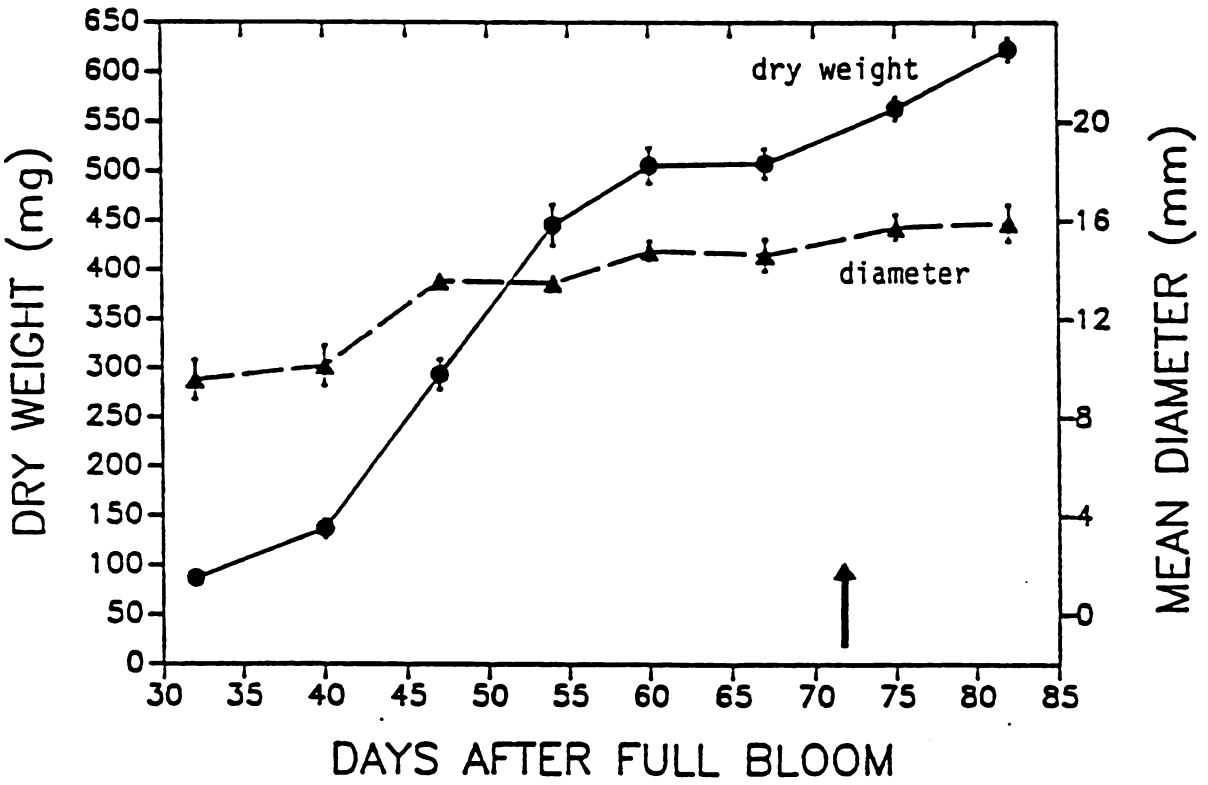
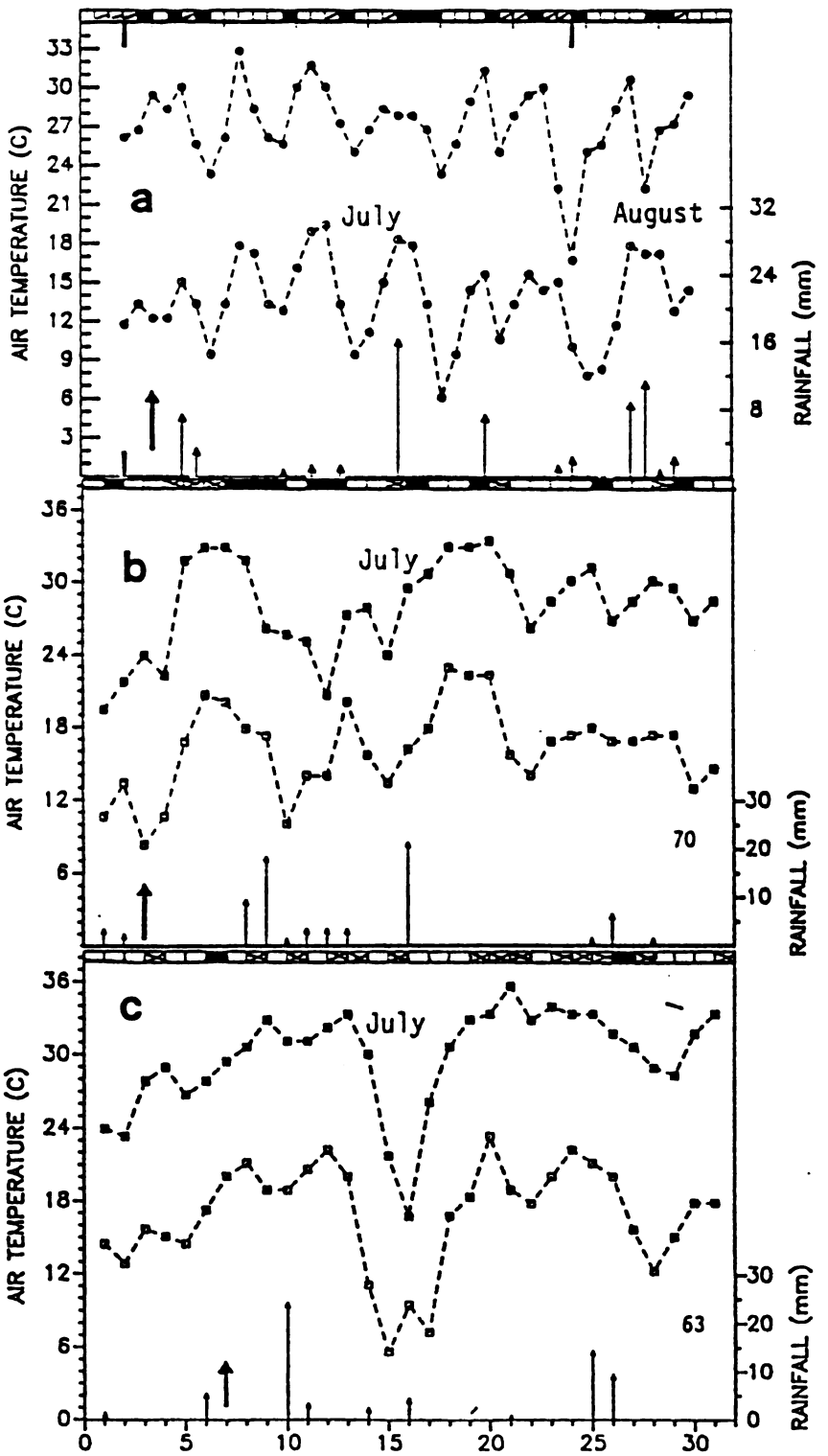

DRY WEIGHT (mg)
650
600
550
500
450
400
350
300
250
200
150
100
50
0
dry weight
diameter
20
16
12
8
4
0
MEAN DIAMETER (mm)
30
35
40
45
50
55
60
65
70
75
80
85
DAYS AFTER FULL BLOOM

Figure 3. Minimum and maximum temperatures during the course of field gas exchange measurements in 1985 (a), 1986 (b), 1987 (c). Arrows indicate the date of harvest. Needle bars indicate daily rainfall, squares at the top of the graph indicate cloud cover (■ cloudy; ⊠ partially cloudy; □ clear). The number shown at the right of each graph denotes monthly precipitation in mm.

Figure 3


a
July
August
AIR TEMPERATURE (C)
RAINFALL (mm)
b
July
70
c
July
63
0
5
10
15
20
25
30

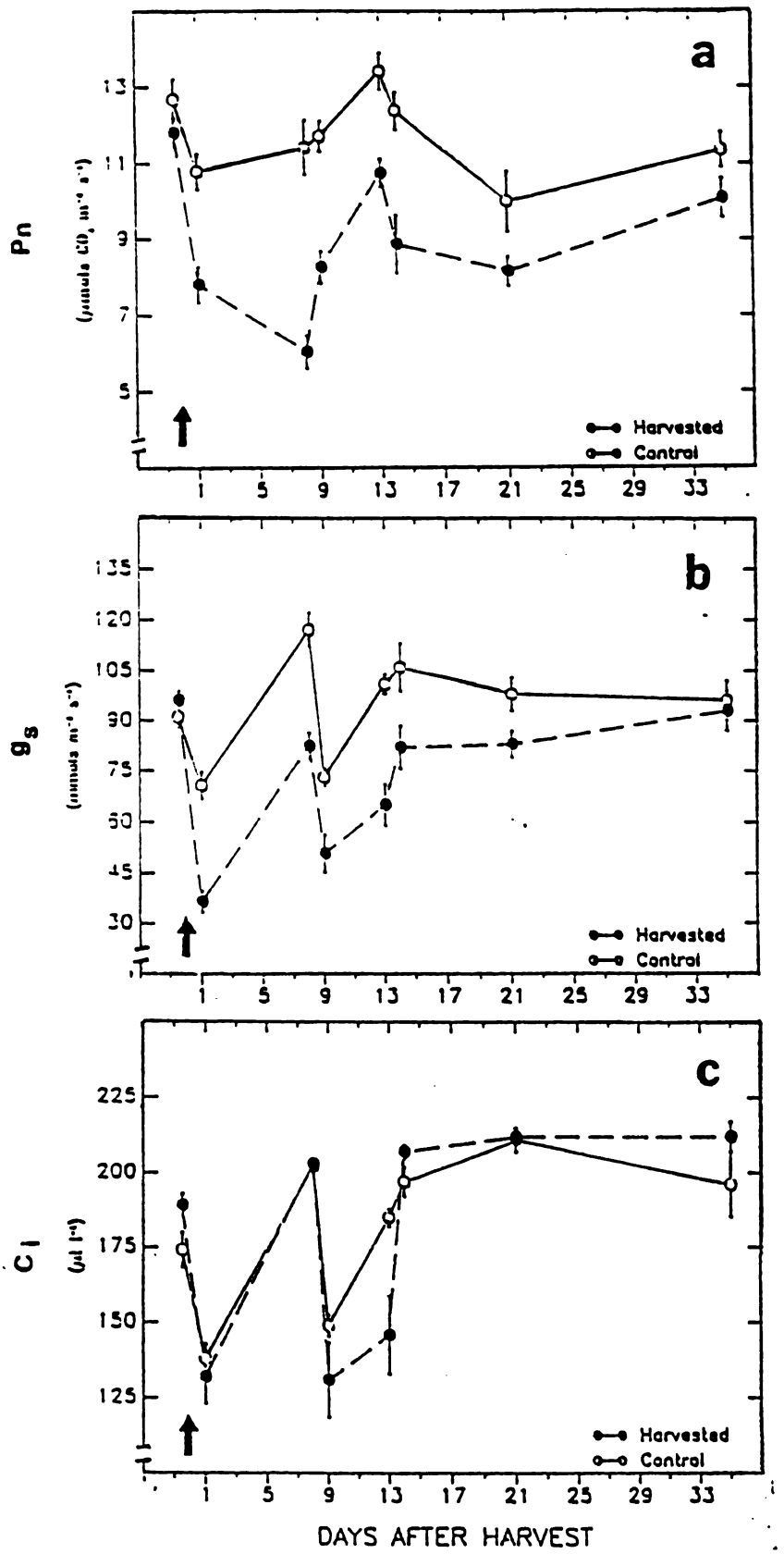
31.3°C, within the optimal range for Pn in sour cherry (Sams and Flore 1983). Most days were cloudless and PAR was usually > 1500 μmols m^{-2} s^{-1} at the time of Pn measurements, well above the light saturation level for Pn (22-30° C at 1200 μmols m^{-2} s^{-1} and VPD=0-6.6 kPa; Sams and Flore 1982). Precipitation totalled 20 mm in the first 15 days of July, and 32 mm in the second 15 days. Rains were uniformly distributed in July (Figure 3a) and usually occurred at night.

Fruit removal caused a 34% decrease in Pn rates (from 11.8 to 7.8 μmols m^{-2} s^{-1}) within 24 h from treatment, and a 49% decrease after 8 days (Figure 4a). Such differences persisted for the next 15 days, then diminished. By the 4th week after harvest differences were no longer significant (P=0.05) and Pn rates of harvested trees approached pre-harvest values (Figure 4a). Leaf Pn did not decrease when fruits were removed on July 17, 1985 (data not shown).

Average Pn rates of fruiting trees were 11.7 μmols m^{-2} s^{-1}, comparable with those reported in the literature for cherry (Sams and Flore 1983; Roper and Kennedy 1986). Stomatal conductance (g_s) was also reduced by defruiting, and decreased from 96 to 36 mmols m^{-2} s^{-1} 24 h after harvest. The experimental component of g_s decrease was estimated to be about 20 mmols m^{-2} s^{-1} and was particularly evident 1 and 9 days after harvest. Values of g_s for fruiting trees ranged from 70 to 120 mmols m^{-2} s^{-1} over the duration of the study (Figure 4b). Calculated values of

Figure 4. Changes in net photosynthesis (a), stomatal conductance (b) and intercellular CO_2 (c) of fruiting and defruited sour cherry trees in 1985. Date of harvest: July 3, 1985. Vertical bars are ± SE (n=12-20). Measurements started between 1230 and 1430 hr.

Figure 4



intercellular CO_2 (Ci) fluctuated from about 190 $\mu l\ l^{-1}$ to 135 $\mu l\ l^{-1}$, mainly due to the large decrease of g_s on day 1, 9, and 13 after harvest (Figure 4b-4c). Leaf water potentials (WP) were about -2.0 MPa for both treatments at the beginning of the experiment, remained approximately constant from day 5 to day 9, then decreased as low as -4.0 MPa (day 21-26) (data not shown). Nevertheless, no WP differences between harvested and fruiting trees were found. Osmotic potentials were not recorded in 1985.

The apparent cupping of leaves on harvested trees was confirmed by measurements of leaf angles 7 days after harvest. Mean angles were significantly narrower in defruited than in fruiting trees (P=0.05). Differences were larger for spur than for terminal leaves (Table 1).

No differences in Pn inhibition due to leaf type, spur or terminal were found. Pn of terminal leaves was 66, 75, 72, and 74% of that of control 1, 9, 14, and 21 days after harvest respectively. Percentages for spur leaves were 80, 66, 70, and 89% at those same dates (Table 2). Pn rates were consistently higher in trees which had a low leaf/fruit (L/F) ratio (Figure 5). Inhibition of Pn after harvest was similar at the different L/F ratios , except at 14 and 21 days after harvest, when Pn decrease was greater for trees with a low source/sink ratio.

Table 1. Angles of mature leaves from fruiting and harvested sour cherry trees measured 7 days after removal of fruits at the HRC, East Lansing, 1985.

Leaf position	Defruited	Control	LSD[z]
Spur	96 a	113 b	9.3
Terminal	82 a	86 a	10.1
Mean	89 a	98 b	7.7

[z] Each number is the average of 30 leaves per treatment. Letters indicate differences within rows at the 0.05 level using LSD mean separation test. Measurement taken with a protractor on July 7, 1985.

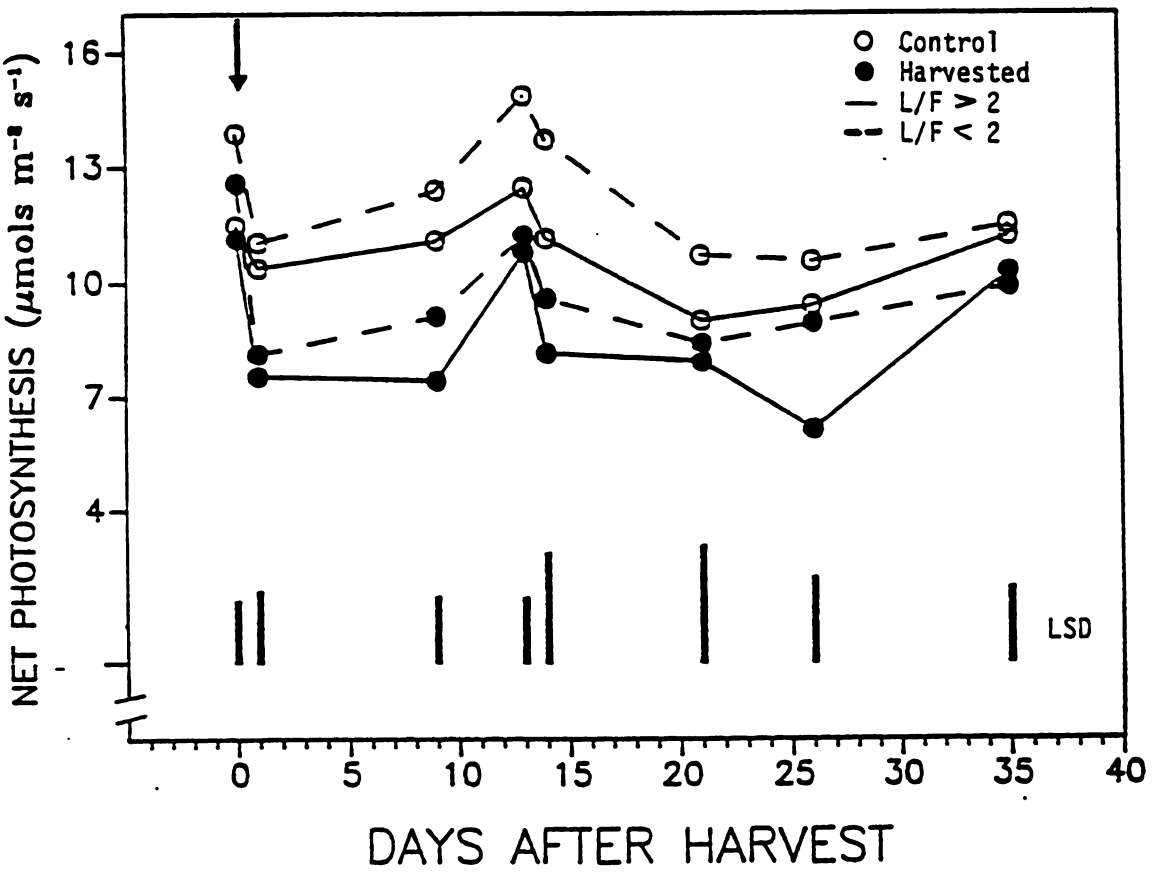
Table 2. Inhibition of photosynthesis in leaves from spurs and terminal shoots of sour cherry following harvest of mature fruits, HRC, East Lansing in 1985.

Days after harvest	Net Photosynthesis[z] (μmols m^{-2} s^{-1})			
	Terminal shoot		Spur	
	Defruited	Control	Defruited	Control
- 0.5	12.3	13.2	11.4	12.1
1	7.5a	11.4b	8.1a	10.1b
9	8.7a	11.6b	7.8a	11.8b
14	8.9a	12.3b	8.8a	12.5b
21	7.8a	10.5b	8.6a	9.7ab

[z] Each number represents the average of 6-10 leaves/ treatment. Letters indicate statistical differences within rows at the 0.05 level using Duncan's Multiple Range Test. Absence of letters within a row indicates lack of significant differences.

Figure 5. The effect of different leaf/fruit ratio on net photosynthesis of fruiting and defruited sour cherry trees in 1985. Values are means of 6-10 leaves. Vertical bars indicate Least Significant Differences at the 5% level.

Figure 5


Control
Harvested
L/F > 2
L/F < 2
LSD
NET PHOTOSYNTHESIS (μmols m⁻² s⁻¹)
16
13
10
7
4
0
5
10
15
20
25
30
35
40
DAYS AFTER HARVEST

Fruit effect in 1986

In 1986 fruit load was only 10 kg/tree and L/F ratio between 5 and 10 for all four trees. Environmental conditions were often sub-optimal for Pn during the experimental period (Figure 3b). Average maximum and minimum T during the 10 days before harvest were respectively 24.4° and 13° C, during the 10 days after harvest 27.2° C and 13.9° C. Cloudy conditions occurred on 10 out of 13 days (Figure 3b) and under such conditions PAR was usually about 100-300 μmols m^{-2} s^{-1}. Total rainfall in July was 70 mm. No significant differences in Pn were found between control and defruited trees following treatment (Table 3). A similar experiment was conducted at NWHES two weeks later when environmental conditions were similar to those recorded in East Lansing in 1985, cherry fruits were at the same stage as those harvested at the beginning of July in 1985, and L/F ratios were less than 5. The experiment was conducted for only four days. Mean leaf temperatures were 28.6°, 27.6°, 33.5°, 36.6°, 35° C respectively at the times of measurement. VPD inside the cuvette varied from 2.3 kPa on day 0 to 4.2 kPa on day 3 (afternoon).

Pn was reduced 14 to 51% during the 3 days following fruit removal (Figure 6a). Pn rates of fruiting trees, comparable with those recorded in 1985, increased from 11.3 to 13.2 μmols m^{-2} s^{-1} after the first 24 h, but declined to 8.9 μmols m^{-2} s^{-1} on day 2. The experimental component of the Pn change was therefore approximately 22%. Values of

Table 3. Changes in net photosynthesis (Pn), stomatal conductance (g_s) and intercellular CO_2 (Ci) of fruiting and defruited sour cherry trees located at the Horticultural Research Center, East Lansing, in 1986. Fruits removed on July 2, 1986. Measurements made in the afternoon between 1300 and 1700 hr.

Gas exchange parameter[z]	Fruits	Days after fruit removal				
		-12	-9	-1	+1	+7
Pn (μmols m^{-2} s^{-1})	+	16.1a	14.2	17.3	16.8	11.3
	-	14.6b	15.0	18.1	16.3	11.0
Gs (mmols m^{-2} s^{-1})	+	130	116	102	98	117a
	-	154	128	114	127	141b
Ci (μl l^{-1})	+	216a	216	175	179a	231
	-	246b	221	186	221b	251

[z] Different letters indicate significant differences (P=0.05) between treatments within parameters and dates. No letter indicates means not statistically different. Each value is the mean of 10 replications.

stomatal conductance were low for both treatments (50-100 mmols m^{-2} s^{-1}), but g_s was significantly higher in fruiting trees (Figure 6b). Ci did not differ significantly between the 2 treatments Figure 6c).

Fruit effect in 1987

In 1987 Pn did not decrease significantly until 3 days after harvest. The decrease amounted to 27% in the afternoon (from 9.8 to 8.2 μmols m^{-2} s^{-1}, plus an experimental component equal to 10%) but was not apparent in the morning. The 2-day delay for the appearance of the fruit effect can be explained by the limited light and sub-optimal temperatures prevalent on the date of harvest and immediately thereafter (for this reason, fruit removal was postponed to July 7th; Figure 3c). The inhibition was considerably lower than that observed in 1985 (20% vs 40%) (note that the same trees were used in both years). The effect of fruit removal was lower in the morning than in the afternoon (Figure 7a, 7b). No differences were found in the morning (measurements starting between 900 and 1100 hr), but Pn rates dropped 27% in the afternoon (measurements started from 1300 to 1600 hr) (Figure 7b). The effect remained significant in the afternoon for 18 days after harvest, when the measurements were terminated. Pn was not measured in both morning and afternoon on every day because variable environmental conditions prevented replication of measurements at a different time of the day. Average Pn

Figure 6. Changes in net photosynthesis (a), stomatal conductance (b), and intercellular CO_2 (c) of fruiting and defruited sour cherry trees located at the Northwestern Horticultural Experimental Station, Traverse City, in 1986. Date of fruit harvest: July 15, 1986 (indicated by arrow). Vertical bars indicate $\pm$ SE (n=18-20). Pre-harvest measurements started at 1400 hr; post-harvest measurements started for each date at 1300, 900, 1500, and 900 hr respectively.

Figure 6

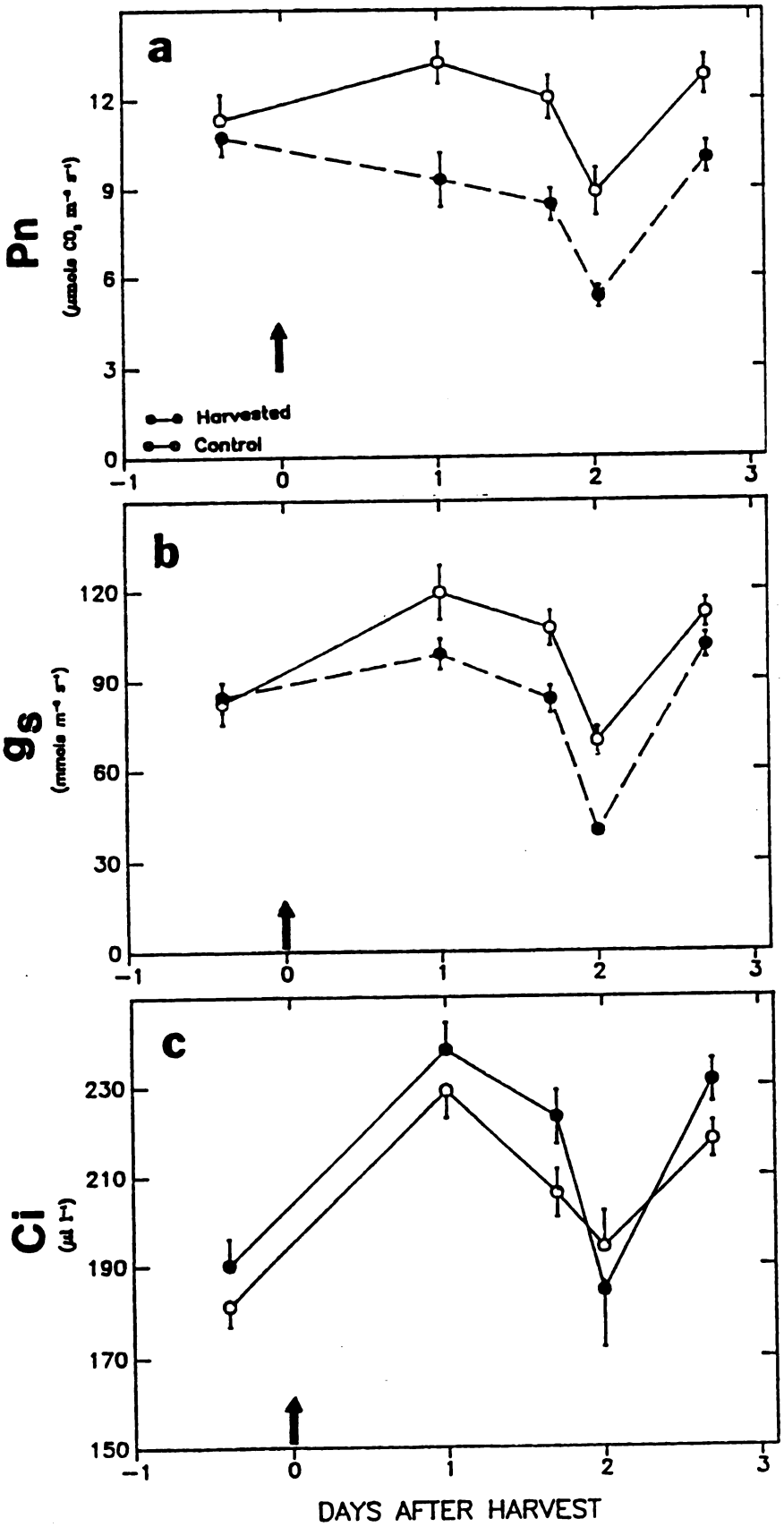
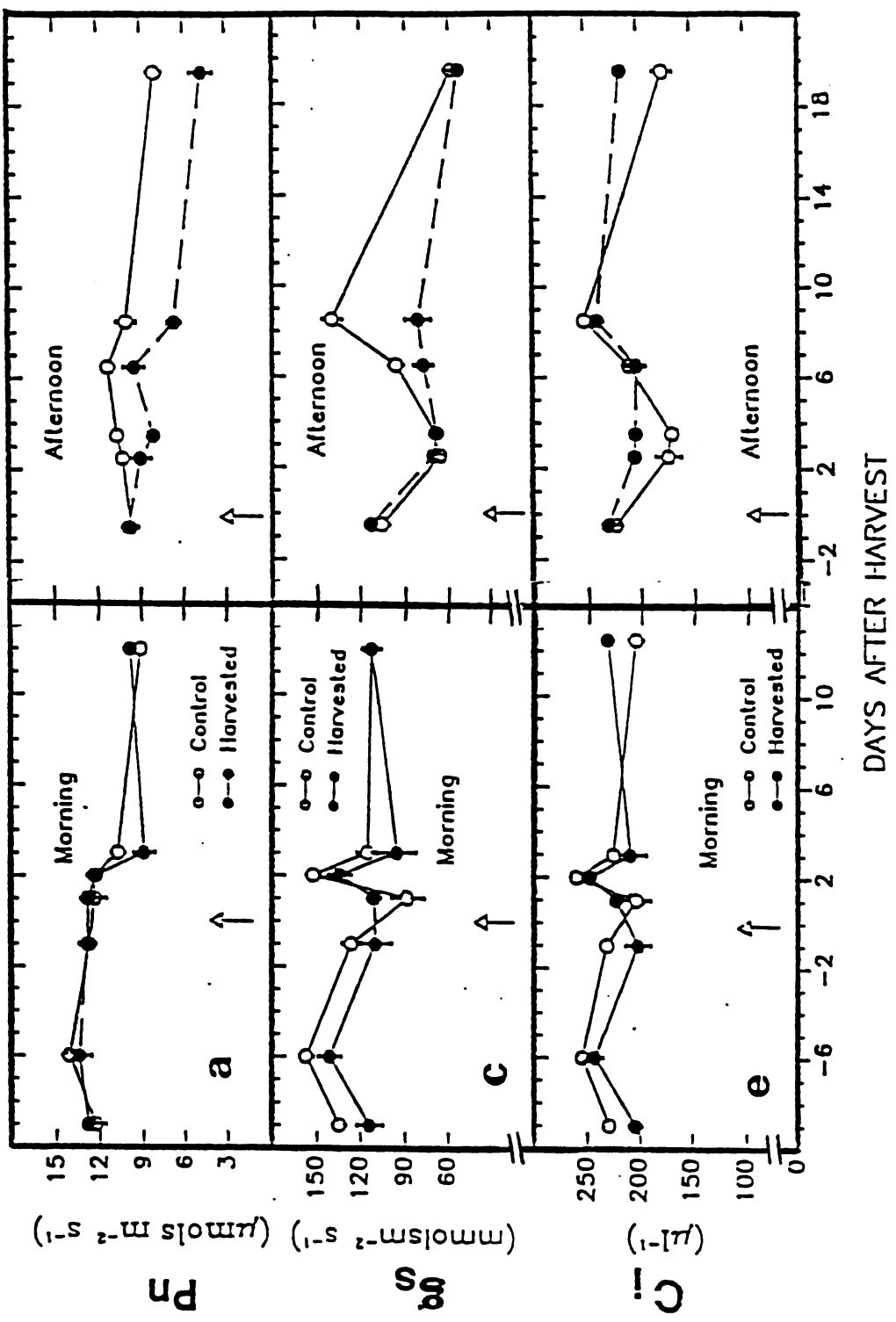

a
b
c
Pn
(μmols CO2 m-2 s-1)
gs
(mmols m-2 s-1)
Ci
(μl l-1)
Harvested
Control
12
9
6
3
0
120
90
60
30
230
210
190
170
150
-1
0
1
2
3
DAYS AFTER HARVEST

Figure 7. Morning (a,c,e) and afternoon (b,d,f) changes in net photosynthesis (a,b), stomatal conductance (c, d), and intercellular CO_2 (e, f) of leaves from fruiting and defruited sour cherry trees in 1987. Date of harvest: July 7, 1987. (indicated by arrow). Morning measurements started between 930 and 1130 hr; afternoon measurements started between 1300 and 1500 hr. Vertical bars are $\pm$ SE (n=16).

Figure 7


Pn
(μmols m⁻² s⁻¹)
gs
(mmolsm⁻² s⁻¹)
Ci
(μl⁻¹)
Morning
Afternoon
Control
Harvested
a
c
e
DAYS AFTER HARVEST

rates were 14% higher in the morning (about 11.9 μmols m^{-2} s^{-1}) than in the afternoon (10.2 μmols m^{-2} s^{-1}).

Changes of g_s followed a pattern similar to those of Pn (Figure 7c). The difference between treatments was maximum (58 mmols m-2 s-1) 9 days after fruit removal and was significant at 6 and 9 days. Ci was about 223 and 229 μl l^{-1} in the morning for defruited and fruiting trees respectively, 215 (Defruited) and 201 μl l^{-1} (Control) in the afternoon (Figure 7e; 7f). Fruit removal did not affect Ci of defruited trees in the morning but increased Ci about 40 μl l^{-1} in the afternoon (days 2 , 3, and 19). No difference was apparent on day 7 and 9.

Inhibition of Pn was not the result of early chlorophyll loss. Chlorophyll content did not vary appreciably over time and was almost identical for the 2 treatments, when expressed on a leaf area basis (Table 4). Lower values, although not significant at 0.05 level, for harvested trees were observed when chlorophyll content was calculated an a dry weight basis (Table 4). Since water content was not affected the difference may have reflected thicker leaf mesophyll in harvested trees.

Water potential in the morning decreased from -1.43 MPa to -1.87 MPa during the period of study, but differences between the 2 treatments were not consistent (Table 5). Similar changes occurred for leaf osmotic potential (OP), whose values fluctuated little over time. Moderate accumulation of solutes may have occurred as a result of

Table 4. Chlorophyll content in fruiting and defruited sour cherry trees, HRC , East Lansing, 1987.

Date	Defruited				Control			
	g/mg dr.wt.	mg/ dm2	chl. a/b	dry/ fr.wt.	g/mg dr.wt.	mg/ dm2	chl. a/b	dry wt./ fr.wt.
July 2	10.4	6.0	3.0	0.29	10.6	5.9	3.0	0.30
July 8	9.3	5.6	2.8	0.28	10.2	5.9	2.9	0.31
July 13	10.5	6.2	2.9	0.30	11.4	6.1	2.8	0.30
Aug. 7	9.6	5.7	3.0	0.31	10.3	6.1	2.9	0.31

Date of fruit removal: July 7, 1987 at 1700 hr. No comparisons between defruited and control were statistically significant (P=0.05).

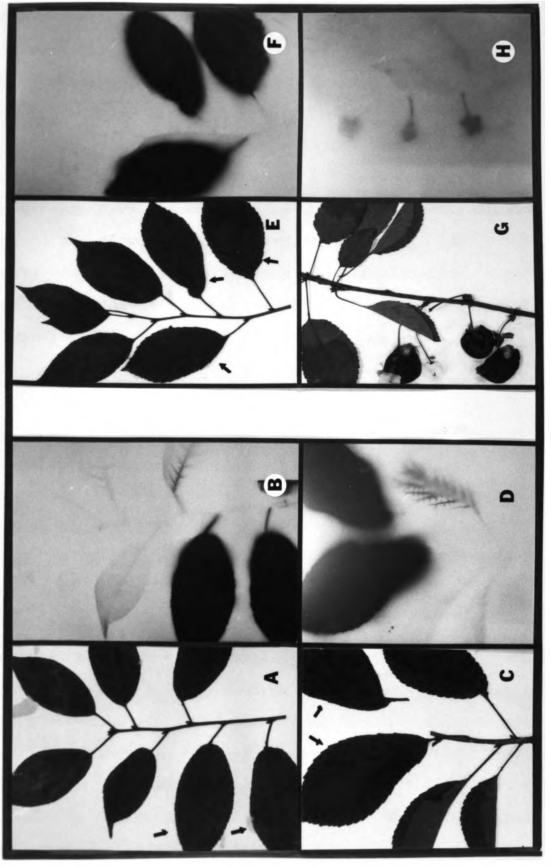
Table 5. Leaf water potential, osmotic potential and turgor pressure of fruiting and defruited trees, East Lansing Date of fruit removal: July 7, 1987.

Date	Water Potential (MPa)			Osmotic Potential (MPa)			Turgor Pressure (MPa)	
	Defruit.	Control	LSD	Defruit.	Control	LSD	Defruit.	Control
July 2	- 1.43	- 1.42	NS	- 2.46	- 2.56	NS	1.03	1.14
July 9	- 1.26a	- 1.13b	0.08	- 2.51a	- 2.28b	0.20	1.25	1.15
July 12	- 1.74a	- 1.97b	0.20	- 2.49	- 2.72	NS	0.75	0.85
July 18	- 1.82	- 1.87	NS	- 2.62	- 2.65	NS	0.80	0.78

Each number is the average of 6-8 leaves/treatment (WP) or 4-6 leaves/treatment (OP). Comparisons of statistical significance are separate for water and osmotic potential. Turgor pressure calculated as the difference between WP and OP. Letters indicate statistical differences at the 0.05 level. No letter indicates differences not statistically significant.

Figure 8. Patterns of carbon translocation in shoots excised from defruited (A, B, C, D) and fruiting (E, F, G, H), mature sour cherry trees in 1987. C^{14} pulsing performed 3, 4, 10, and 11 days after harvest. (A, B, E, G) represent patterns in the upper half of the shoot, (C, D, G, H) in the lower half. Pulsed leaves indicated by arrows (see also Figure 1). Details of experimental procedure are reported in Materials and Methods and Figure 1.

Figure 8



water stress or water loss. Turgor was always maintained (minimum of 0.7 MPA on July 12 and 18) and there were no apparent differences between the treatments.

^{14}C studies showed that ripe cherry fruits continued to import assimilates from fully expanded leaves (Figure 8). Translocation from the leaves on the median portion of the shoot was directed acropetally in shoots from defruited trees, and/or basipetally (towards the fruit) in fruiting shoots Figure 8). This pattern of translocation occurred even when shoots were pulsed 2 weeks after the date of harvest).

DISCUSSION

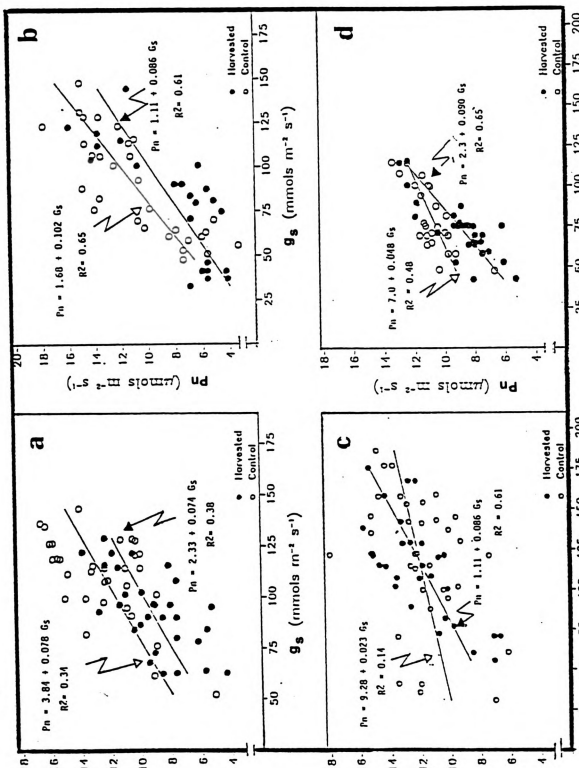
Leaf Pn of sour cherry trees decreased up to 51% of pre-harvest values 48 h after fruit removal under field conditions. The decline of Pn was not always evident in the 3 years of study. For example, defruiting did not reduce Pn rates of defruited trees at the East Lansing location in 1986, but had a marked effect on the same trees in 1985 and 1987. Environmental variables were different between 1985 and 1986. Climatic conditions intermediate between 1985 and 1986 occurred in 1987, when Pn inhibition became evident only three days after harvest (after two cloudless days).

Pn inhibition of defruited trees was particularly evident in the afternoon, and was not associated with changes in chlorophyll content. Fruiting and defruited trees showed similar changes of Pn per unit variation of g_s

(slopes of the regression lines) both in the morning and the afternoon of 1986 (Figure 9a, 9b); Pn increase per unit Gs of defruited trees was greater than fruiting ones in 1987 (Figure 9c, 9d). Since positive correlation between Pn and g_s was lower in the morning than in the afternoon and average g_s values were lower in the afternoon than in the morning for both treatments in both years, Pn was probably limited by stomatal aperture in the afternoon. Decrease of g_s associated with fruit removal (Downton et al. 1987; Kriedemann et al. 1976; Schaffer et al. 1987) or midday depression of photosynthesis (Raschke and Resemann 1986; Tenhunen et al. 1981) have also been reported for several woody species. Midday stomatal closure of Arbutus and Quercus has been related to increasing VPD in the afternoon in natural environments (Tenhunen et al. 1980). Under simulated natural conditions g_s of hazelnut leaves was as low as 25 mmols m^{-2} s^{-1} at a VPD of 3.0 kPa and a WP of -1.9 MPa (Schulze and Kuppers 1979). In hazelnut g_s progressively decreased with long-term water stress, but not with short-term water stress. Stomatal closure during short-term water stress seemed to be entirely dependent on air humidity and temperature (Schulze and Kuppers 1979). Because of the type of experiment conducted in this study I do not have conclusive evidence as to whether stomatal closure in sour cherry in the afternoon was regulated by environmental parameters alone. Further studies will have to be conducted in a controlled environment. Although transient periods of

Figure 9. Relationship between leaf net photosynthesis (Pn) and stomatal conductance (g_s) for fruiting and defruited sour cherry trees in the morning (a, c) and afternoon (b, d) of 1986 (a, b) and 1987 (c, d). Each symbol is the mean of 6-10 observations. Measurements were made at the Northwestern Horticultural Station, Traverse City, Michigan, in 1986, and at the Horticultural Research Center, East Lansing, Michigan, in 1987.

Figure 9


a
Pn = 3.84 + 0.078 Gs
R2= 0.34
Pn = 2.33 + 0.074 Gs
R2= 0.38
b
Pn = 1.68 + 0.102 Gs
R2= 0.65
Pn = 1.11 + 0.086 Gs
R2= 0.61
c
Pn = 9.28 + 0.023 Gs
R2 = 0.14
Pn = 1.11 + 0.086 Gs
R2 = 0.61
d
Pn = 7.0 + 0.048 Gs
R2 = 0.48
Pn = 2.3 + 0.090 Gs
R2= 0.65
Harvested
Control
Pn (μmols m-2 s-1)
gs (mmols m-2 s-1)

mild water stress may have occurred especially during the hottest part of day, there was no evidence of symptoms of long-term water stress. Therefore, lack of turgor is probably not responsible for the decrease of g_s after fruit removal in sour cherry.

Diurnal stomatal movements have also been reported to be regulated by levels of ABA. Stomatal closure following accumulation of ABA in the leaf occurs in soybean (Setter et al. 1980), grapevine (Loveys 1984), and apricot (Loveys et al. 1987). In grapevines grown in a semiarid environment (South Australia), ABA seemed to account for midday stomatal closure, whereas air temperature and humidity appeared responsible for stomatal behavior later in the day (Loveys and During 1984). In both grapevine and apricot grown in a similar environment ABA accumulation was not the result of loss of turgor (Loveys 1984; Loveys et al. 1987). Since afternoon stomatal closure was particularly evident only for defruited sour cherry trees grown in a much more humid environment (Michigan), further studies on the effect of fruit removal will have to consider changes of ABA levels in the leaf in response to fruit harvest.

It has also been hypothesized that growth regulators other than ABA may regulate leaf photosynthesis and the flow of assimilates to the fruit by stimulating fruit growth and sink strength (Chalmers et al. 1976; Herold 1980; Hoad et al. 1977; Neales and Incoll 1968). From the data presented thus far it is not possible to determine whether the change

of Pn rate after removal of cherry fruits was caused by direct action of phytohormones or was the consequence of feedback inhibition due to carbohydrate accumulation (for a review of both hypotheses and their implications see Neales and Incoll 1968, or Herold 1980).

Calculated Ci levels of defruited sour cherry trees equalled or exceeded Ci levels of fruiting trees, which suggests that rates of Pn and g_s may regulate Ci/Ca ratio and maintain approximately constant. Evidence for Pn regulation by endogenous factors after fruit removal also derives from different Pn rates when L/F ratios are above a certain threshold (Figure 5 and Table 3). In 1986 all trees had a high L/F ratio (range 5-10) and bore a small crop (about 10 kg/tree), which could have resulted in compensatory changes of Pn in response to sink strength modifications similar to those described in bean, pepper, cucumber,and apple (Borchers-Zampini et al. 1980 ;Kriedemann et al. 1976; Hansen 1969; Plaut and Mayoral 1984). Since vegetative growth was vigorous, assimilate supply and demand might have been influenced more by the growth of shoots, stems, and leaves in those relatively young trees. It is also interesting that, in sour cherry, the flow of assimilates from leaves located in the central portion of the shoot was reoriented towards the vegetative apex after fruit removal even when fruits were beyond the stage of full maturity (Figure 8). The presence of ^{14}C-activity in overripe fruits indicated that even at this late stage of

development the fruit was still a relatively active sink for assimilation products. Analogous redistribution of assimilates has been previously reported for apple and grapevine at times of rapid fruit growth and import of assimilates into the fruit (Calo' and Iannini 1975; Hansen 1970). The effect of L/F ratio, high levels of Ci for defruited trees, and changes in carbon allocation due to the presence of mature fruit seem to indicate that nonstomatal components and carbon partitioning are affected by fruit removal and may be the primary determinants of post-harvest Pn inhibition.

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KEYWORDS: Prunus cerasus, inhibition of photosynthesis, stomatal conductance, intercellular CO_2, environment, fruit removal.

ABBREVIATIONS: Pn, net photosynthesis; g_s, stomatal conductance; Ci, internal CO_2; VPD, vapor pressure deficit; L/F, leaf/fruit ratio; T, air temperature; PAR, photosynthetic active radiation; WP, water potential; OP, osmotic potential; TP, turgor pressure; R, gas constant; J, molar concentration of cell sap.

Section II

NON-STOMATAL LIMITATIONS, PHOTORESPIRATION, AND CARBOHYDRATE PARTITIONING DURING INHIBITION OF PHOTOSYNTHESIS FOLLOWING FRUIT HARVEST IN SOUR CHERRY

ABSTRACT

Nonstomatal limitations and leaf carbohydrate partitioning during inhibition of net photosynthesis (Pn) occurring after fruit harvest were studied in sour cherry (Prunus cerasus L.) over two growing seasons. Stomatal contribution to the decrease of Pn ranged from 32 to 40% in a controlled environment and from 25 to 45% under field conditions. Under laboratory conditions, reducing the O_2 concentration from 21% (air) to 2.5% increased Pn 73% in defruited trees, but only 47% in controls. Similar results were obtained under field conditions at O_2 concentrations of 21% vs 0.5%. Both harvested and control treatments showed evidence of O_2-sensitivity of Pn. The levels of soluble sugars (fructose, glucose, inositol, sorbitol, and sucrose) in leaves were not significantly affected by defruiting. In contrast, starch levels in the leaf increased about 3-fold within 48 h after fruit harvest in both years (from 0.8 to 2.9% dry wt. in 1986 and from 0.6 to 1.9% dry wt. in 1987), but dropped to pre-harvest levels 3 days after harvest in 1987. The large non-stomatal contribution to Pn decrease after harvest and the changes in starch levels support the hypothesis that defruiting results in end-product inhibition of photosynthesis.

INTRODUCTION

I have shown in the previous section that stomatal behavior does not appear to account completely for the inhibition of Pn caused by fruit harvest in sour cherry. Nonstomatal limitations are reportedly the most relevant component in the afternooon depression of Pn in grapevine (Downton et al 1987) and Quercus (Tenhunen et al. 1984). However, stomatal limitations have been claimed to be totally responsible for changes in Pn in other systems or for other types of manipulations (Downton et al. 1988; Kriedemann et al. 1976; Setter et al. 1980). Obstructed translocation by chilling or girdling, fruit removal, or continuous illumination have all been shown to decrease Pn rate in different species (Azcon-Bieto 1983; Claussen et al. 1985; Mayoral et al. 1985; Setter et al. 1980).

Photorespiration has been implicated in Pn decrease after fruit removal in citrus (Lenz 1979), but not in other systems (Peet and Kramer 1980). Accumulation of non-structural carbohydrates due to a reduction in sink strength or excessive assimilate supply has been hypothesized to regulate Pn rates via end-product inhibition at the source level (Azcon-Bieto 1983; Neales and Incoll 1968). There is evidence for changes both at the level of sucrose synthesizing enzymes affecting the rate of carbohydrate export out of the leaf (Claussen et al. 1985; Ho 1979; Pharr et al. 1985; Rufty and Huber 1983) and Pn inhibition caused physically by accumulation of starch

(Milford and Pearman 1975; Nafziger and Koller 1976; Schaffer et al. 1986).

The objectives of this study were to quantify the non-stomatal limitations to Pn after fruit harvest in field-grown 'Montmorency' sour cherry trees, to determine if photorespiration contributed to changes in Pn, and to measure changes in non-structural carbohydrates in the leaf mesophyll.

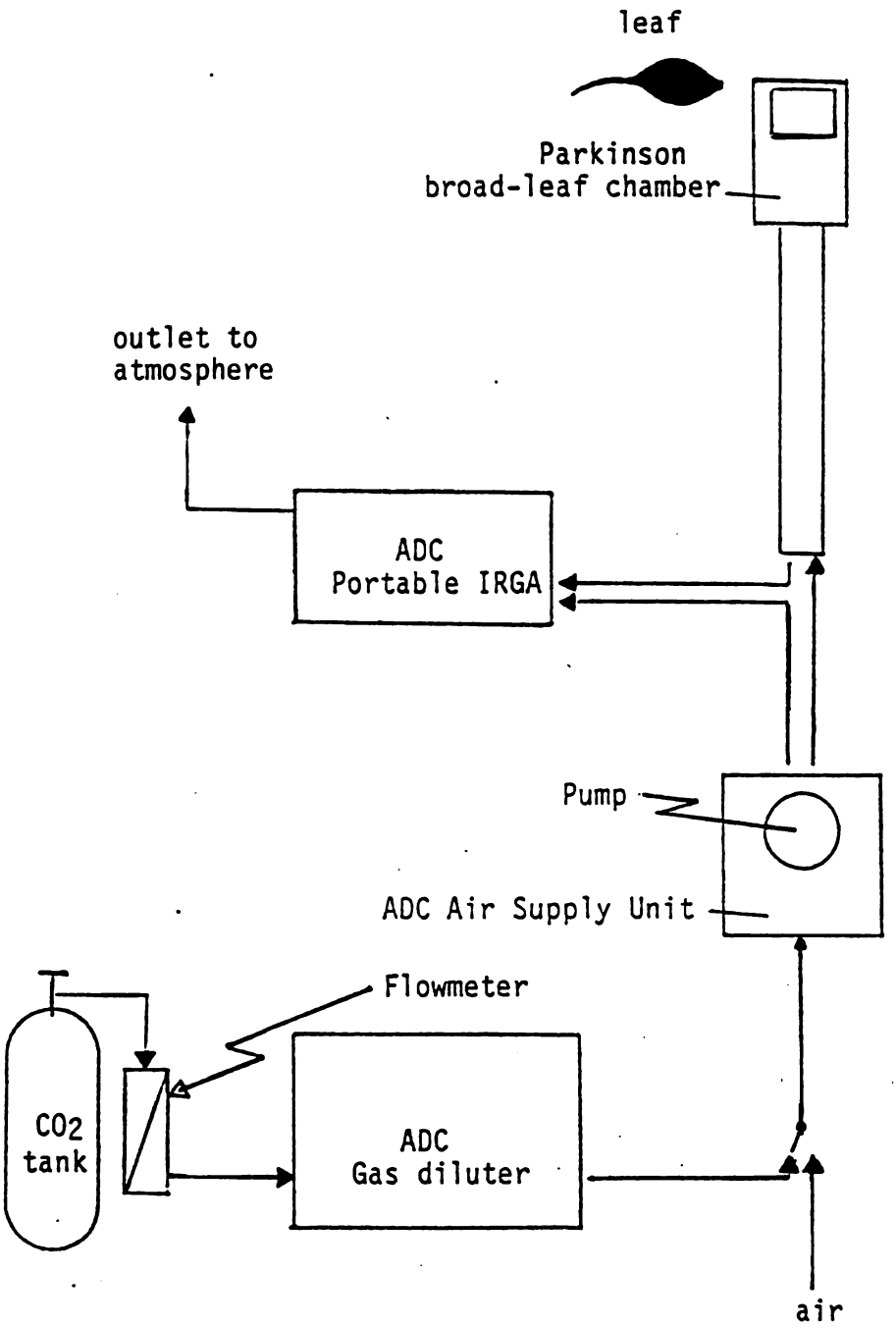
MATERIALS AND METHODS

Plant material. Four 8-year-old sour cherry trees (Prunus cerasus L. cv. Montmorency) were chosen in an orchard at the Horticultural Research Center (HRC) of Michigan State University, East Lansing, Michigan, in 1987, and four 5-year old sour cherry trees at the Northwestern Horticultural Experiment Station (NWHES), Traverse City, Michigan, in 1986. Trees were spaced 8.2 x 3.0 m at the HRC and 6.0 x 2.0 m at the NWHES. Fruits were removed on July 16, 1986 at NWHES, and July 7, 1987 at HRC. Pesticides were applied according to commercial recommendations (Mich. Ext. Bul. E154, Fruit Pesticide Handbook). Pruning, fertilization and other cultural practices were performed according to standard practices. Other details about plant material, location, and experimental conditions are as reported in Section I.

Gas exchange measurements. All measurements in the field were made in an open system using an ADC LCA2 portable infrared gas analyzer equipped with a Parkinson broad leaf chamber (Analytical Development Company, Hoddesdon, UK) at saturating light intensity (PAR > 1000 μmols m^{-2} s^{-1}) and air temperatures between 24 and 32 C. Field measurements of photorespiratory rates and CO_2-response curves were taken using a gas tank containing approximately 2000 μl l^{-1} CO_2 in nitrogen and an ADC GD600 gas diluter (Analytical Development Company, Hoddesdon, UK) connected in series to an ADC air supply unit, chamber and analyzer (Figure 1). Photorespiration was estimated by comparing Pn rates at ambient CO_2 (325-340 μl l^{-1}) and O_2 (21%) with rates at low O_2 (0.5%). CO_2-response curves were obtained by decreasing the CO_2 concentration of ingoing air from 330 μl l^{-1} to about 50 μl l^{-1} with the gas diluter. Levels of CO_2 in the chamber were monitored with an ADC LCA2 portable infrared analyzer. Flow rate was 0.6 l/min, and pressure in the gas diluter was maintained above 0.1 MPa. Measurements were taken on four leaves of those regularly measured for gas exchange (see also Section I) from one tree/treatment at PAR > 1500 μmols m^{-2} s^{-1}, cuvette temperature 31-35° C, air relative humidity 60-70%, air temperature 28-32° C, and relative humidity of incoming air 15-25%. Measurements started at 1200 hr and were run on 2 consecutive days (9-10 days after defruiting). Photorespiration and CO_2 response curves were measured in the laboratory using the open system

Figure 1. Schematic diagram of gas-exchange equipment used to measure CO_2 curves and estimate photorespiration under field conditions. Abbreviations: ADC, Analytical Development Company, Hoddesdon, England; IRGA, infra-red gas analyzer.

Figure 1


leaf
Parkinson
broad-leaf chamber
outlet to
atmosphere
ADC
Portable IRGA
Pump
ADC Air Supply Unit
Flowmeter
CO2
tank
ADC
Gas diluter
air

described by Sams and Flore (1982) and modified as follows: a) An ADC 225 Mk3 infrared Gas Analyzer (Analytical Development Company, Hoddesdon, UK) was used to measure differential CO_2 concentrations at the inlet and outlet of leaf chambers; b) air flow entering the chambers was regulated with Matheson 8100 series mass flowmeters and Matheson 8200 series mass flow controllers connected to a Matheson multichannel Dyna-Blender 8219 (Matheson, Instruments, Horsham, Pennsylvania). Four shoots were excised, using the precautions recommended by Lakso (1980), from fruiting and defruited trees at 900 hr and taken to the laboratory where they were left to acclimate for 3 hours at air temperature of 23° C and 1000 μmols m^{-2} s^{-1} PAR. Light was provided by GE 400 W multivapor lamps (metal halide, General Electric). Once acclimated, one leaf (4th or 5th from apex) per shoot was inserted into a leaf chamber. Four chambers were used at a time. CO_2 response curves were determined by decreasing the CO_2 concentration to the compensation point. CO_2 concentrations at the chambers exits were monitored with an ADC LCA2 portable infrared analyzer, O_2 concentrations with a O-260 Beckman oxygen analyzer (Beckman Instruments Inc., Irvine, California). Measured leaves were maintained at 1000 μmols m^{-2} s^{-1} PAR, 27.5-28.5° C leaf temperature, and dew point of 1-1.5° C equivalent to a VPD of about 2.85 kPa. Flow rates to the chambers were 2.3 ± 0.1 l/min depending on leaf size and photosynthesis rate. Measured leaves were harvested and

their area determined with a LICOR LI 3000 leaf area meter (LI-COR Inc, Lincoln, Nebraska).

Calculations, statistical analysis, and curve fitting Gas exchange parameters were calculated according to the equations by vonCaemmerer and Farquhar (1981) as previously described (Moon and Flore, 1985). Stomatal contributions to the change of Pn after fruit harvest were calculated according to the methods proposed by Farquhar and Sharkey (1982) and Jones (1985). The following equations were used:

a) $Lg = Pn^0 - Pn \;/\; Pn^0$ (Farquhar and Sharkey 1982)

where Lg is the stomatal limitation to Pn, Pn is the assimilation rate that actually occurs, and Pn^0 the assimilation rate that would occur if resistance to CO_2 diffusion was zero;

b) $$Gc = \frac{1}{2}\left\{\frac{Pn_1}{rg_1 + rm_1} + \frac{Pn_2}{rg_2 + rm_2}\right\}\frac{rg_1 - rg_2}{Pn_2 - Pn_1}$$ (Jones 1985)

where Gc is the stomatal contribution to the change in occurring from condition 1 to 2, r_g the stomatal resistance, and r_m the residual resistance to CO_2 diffusion. Photosynthesis, and stomatal and mesophyll resistances used in the equations refer to means of values at 330 $\mu l\ l^{-1}$ ambient CO_2. Curve fitting was done by computer using the Marquardt method of successive approximations. The best fit for demand curves (A/Ci), evaluated by R^2 and analysis of residuals, was given by a nonlinear model of the type:

$$Pn = B(1) \times \exp\{B(2)\ Ci\} + B(3)\}$$

Table 1 . Coefficients of estimated demand (a) and supply (b) gas exchange curves and relative coefficients of determination for fruiting and defruited treatment, HRC East Lansing, 1987.

Coefficient[z]	Treatment			
	Defruited	R^2	Fruiting	R^2
B(1)	589.79	0.81	- 28.14	0.77
B(2)	1.13×10^{-4}		1.81×10^{-2}	
B(3)	- 591.61		14.13	
C(1)	22.14	0.99	27.69	0.98
C(2)	- 0.065		- 0.082	

[z] Coefficients refer to the following equations:
(a) Pn = B(1) x exp {B(2) x Ci} + B(3)
(b) Pn = C(1) + C(2) x Ci
where Pn is net photosynthesis, Ci is intercellular CO_2, and R^2 is the coefficient of determination of the regression curves. Measurements made in the laboratory on excised shoots 9 days after fruit removal.

The estimated coefficients of demand curves for fruiting and harvested trees are reported in Table 1.

Carboxylation efficiencies at each concentration of Ci were calculated from derivatives of the demand curves at the point of the chosen Ci concentration, as follows:

$$d(Pn)/d(Ci) = B(1) \times \exp\{B(2) \times Ci\} \times B(2)$$

Gas supply curves for each treatment were calculated by the equation $Pn=(Ca-Ci) \times g_s$ (Farquhar and Sharkey 1982) using g_s values at Ca= 330 $\mu l\ l^{-1}$.

Gas exchange measurements in the field were made according to a randomized complete block design (2 blocks, each consisting of 2 trees; 6-10 replications per tree). The design for gas exchange in the lab was completely randomized with leaves as replications. Means were separated by LSD ($P < 0.05$). Comparisons were made only between treatments at each single date.

Carbohydrate analysis . Analyses of soluble sugars (glucose, fructose, sucrose, sorbitol, and inositol) were performed by gas chromatography, analysis of starch by enzymatic degradation and color reaction. Details about the methods used are reported in Section IV.

Transmission electron microscopy. Samples for TEM were collected from 3 leaves per treatment at 1400 hr immediately before and 24 h after fruits were removed. Samples were fixed in 4% glutaraldehyde on ice and postfixed in 2% OsO_4 at room temperature. Samples were dehydrated with ethanol

and embedded in Mollenhauer mixture resin (Mollenhauer 1964). Sections were stained with uranyl acetate and Reynolds lead (Reynolds 1963).

RESULTS

Calculated stomatal contribution to the post-harvest decrease of photosynthesis ranged from 32% to 40% in a controlled environment and from 25% to 45% in the field. Carboxylation efficiency (CE) of fruiting sour cherry trees was about 20% higher than that of defruited ones at 100 $\mu l\ l^{-1}$ Ci, corresponding to an ambient CO_2 (Ca) of 180-220 $\mu l\ l^{-1}$ (Figure 2).

Photorespiration estimated from Pn rates at 21% O_2 and 2.5% O_2 in the laboratory and in the field (0.5%) are reported in Table 2. The ratio of Pn values from harvested and control trees measured in the laboratory was slightly higher at 2.5% O_2 (86%) than at 21% O_2 (73%). The difference of Pn at 2.5% O_2 and 21% O_2 expressed as a percentage of values at 21% O_2 was 73% for harvested and 47% for control treatment when measured in the laboratory, 71% and 42% when measured in the field. Leaf content of soluble sugars was not significantly affected by defruiting in 1986 and 1987 (Figure 3). Sorbitol was the most abundant among the soluble sugars in the leaf (about 56%), followed by sucrose (27%), glucose (9%), and fructose (7%). Inositol was only found in traces (< 0.5%). There was no clear trend

Figure 2. CO_2 response curves of fruiting and defruited sour cherry trees measured with an open gas exchange system (Sams and Flore 1982). Measurements were made in the laboratory 9 days after fruits were harvested on July 7, 1987.
Environmental conditions: leaf T 28-29°C; dew point of air going into the cuvette 0.6°C; ambient CO_2 from 340 µl l^{-1} to 50 µl l^{-1}; PAR 1000 µmols m^{-2} s^{-1}.
Equation at top of the graph refers to the model used to fit demand curves for gas exchange. Gas supply curves for each treatment were fitted by linear regression using values of stomatal conductance of fruiting or defruited treatment at 340 µl l^{-1}.

Figure 2

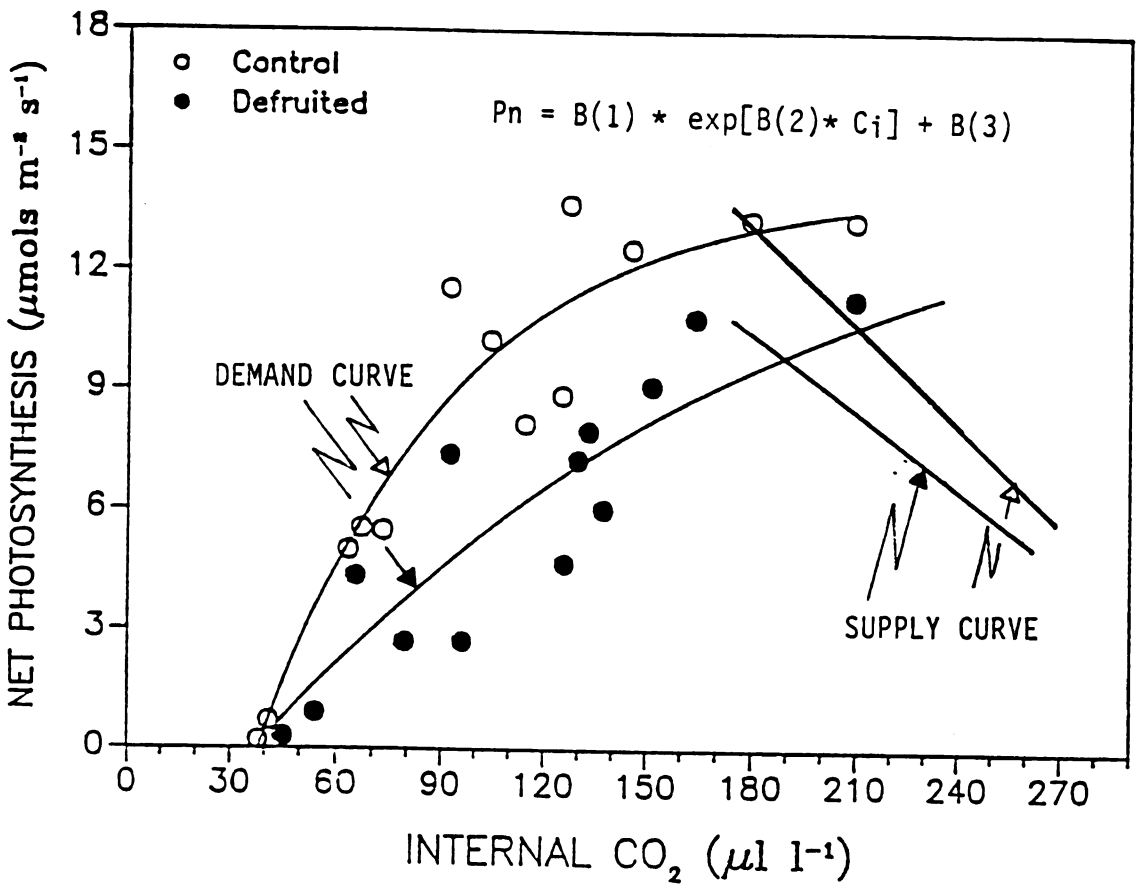

Control
Defruited
Pn = B(1) * exp[B(2)* Ci] + B(3)
DEMAND CURVE
SUPPLY CURVE
NET PHOTOSYNTHESIS (μmols m⁻² s⁻¹)
INTERNAL CO₂ (μl l⁻¹)
0
3
6
9
12
15
18
0
30
60
90
120
150
180
210
240
270

Table 2 . Photosynthesis and estimated photorespiration of fruiting and defruited sour cherry shoots in the laboratory and in the field at HRC, East Lansing, in 1987.

Laboratory				Field			
O_2 (%)	Net Photosynthesis (μmols m^{-2} s^{-1})		D/C (%)	O_2 (%)	Net Photosynthesis (μmols m^{-2} s^{-1})		D/C (%)
	Defruited (D)	Control (C)			Defruited (D)	Control (C)	
21 (a)	9.65	13.18	73	21	3.13	7.19	43
2.5 (b)	16.71	19.36	86	0.5	5.34	10.19	52
(b-a)	7.06	6.18		(b-a)	2.21	3.00	
$\frac{(b-a)}{a}$ (%)	73	47		$\frac{(b-a)}{a}$ (%)	71	42	

Data are means of values from 2 experiments. Data collected on on July 17 and 18, 1987; fruits harvested on July 8, 1987. Measurements made from 1130 to 1330 in the laboratory, from 1130 to 1530 hr in the field.
Environmental conditions in the laboratory: PAR = 1000 μmols m^{-2} s^{-1}; T = 28.2- 28.6°C; CO_2 = 320-355 μl l-1; VPD = 2.95 kPa.
Environmental conditions in the field: PAR > 1500 μmols m^{-2} s^{-1}; T = 30-34°C; CO_2=320-325 μl l-1; VPD = 3.6 kPa. VPD refers to the deficit between air at the inlet and outlet of the leaf chamber.

in levels of soluble sugars over time due to fruit harvest (Figure 3).

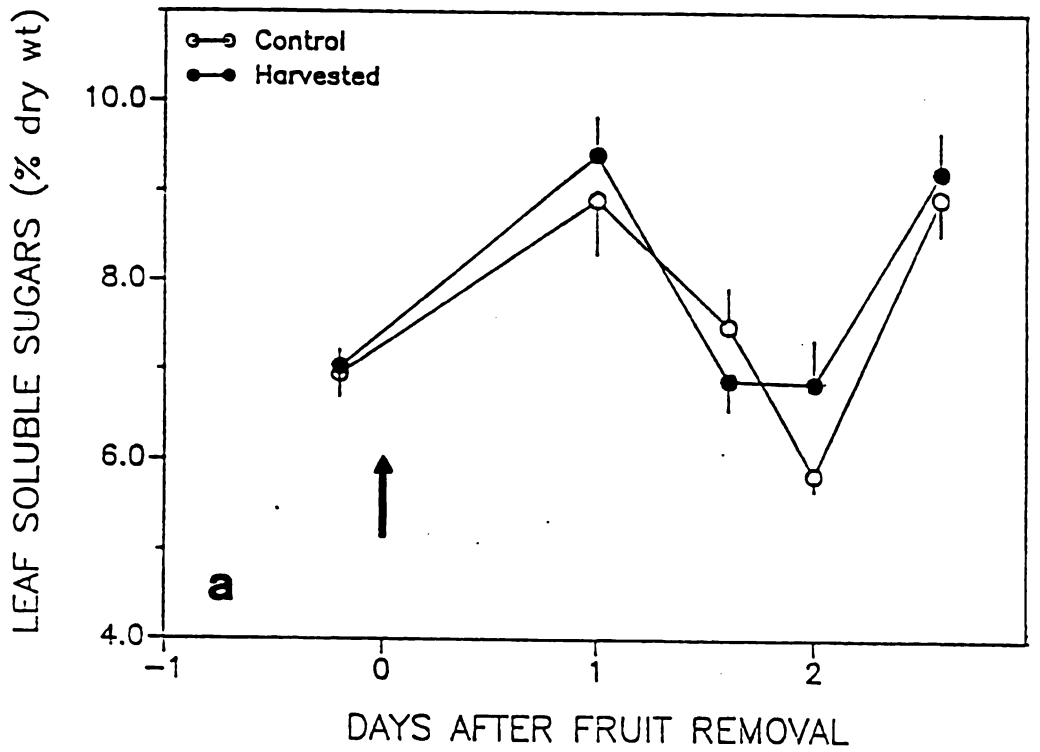
Starch levels increased on average 350% times within 48 h after harvest in 1986 (Figure 4). In 1987 leaf starch levels of harvested trees increased from 0.6% to 1.95% 24 h after harvest, but decreased to pre-harvest value 3 days after harvest (0.66% dry wt.). Starch levels in control leaves also increased 24 h after harvest (from 0.60% to 1.46% dry wt.), but dropped to 0.33 and 0.37% 3 and 5 days after harvest respectively (58% of pre-harvest values). The differences between control and defruited trees were significant at all times of sampling after fruit harvest in 1986 (Figure 4a), and 4 out of 5 times in 1987 (Figure 4b). Increase in leaf starch was confirmed by enlargement of starch grains in chloroplasts of mesophyll cells (Figure 5). Analysis of about 45 cells (spongy and mesophyll) from TEM micrographs revealed a 3-fold increase in the area of the chloroplast occupied by starch grains after fruit removal. However, no breakage of chloroplasts or disruption of membranes could be observed due to the enlargement of such grains (Figure 6).

DISCUSSION

The decrease in leaf Pn following removal of fruits in sour cherry appeared to be regulated by several factors.

Figure 3 . Changes in leaf content of soluble sugars after fruit harvest in sour cherry: a) 1986, NWHES, Traverse City, Michigan; b), HRC, East Lansing. Soluble sugars were fructose, glucose, inositol, sorbitol, and sucrose. Arrows indicate date of harvest. Vertical bars ± SE.

Figure 3


Control
Harvested
LEAF SOLUBLE SUGARS (% dry wt)
10.0
8.0
6.0
4.0
−1
0
1
2
a
DAYS AFTER FRUIT REMOVAL

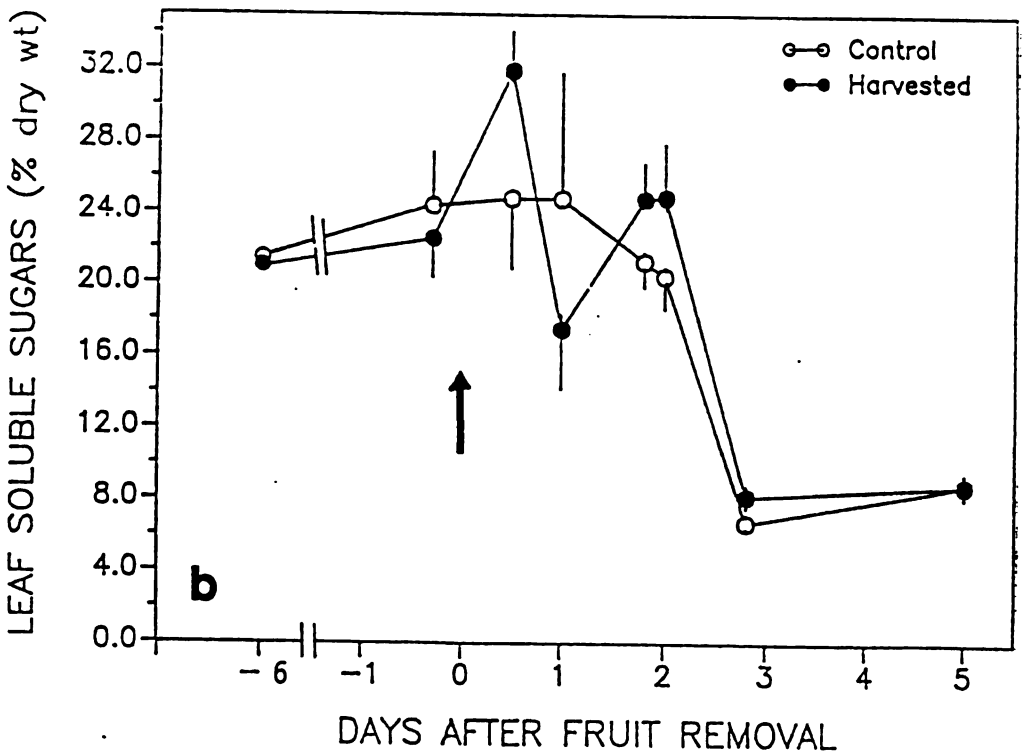

Control
Harvested
LEAF SOLUBLE SUGARS (% dry wt)
32.0
28.0
24.0
20.0
16.0
12.0
8.0
4.0
0.0
− 6
−1
0
1
2
3
4
5
b
DAYS AFTER FRUIT REMOVAL

Figure 4 . Changes in leaf starch content after fruit harvest in sour cherry: a) 1986, NWHES, Traverse City, Michigan; b) 1987, HRC, East Lansing. Arrows indicate date of harvest. Vertical bars ± SE.

Figure 4

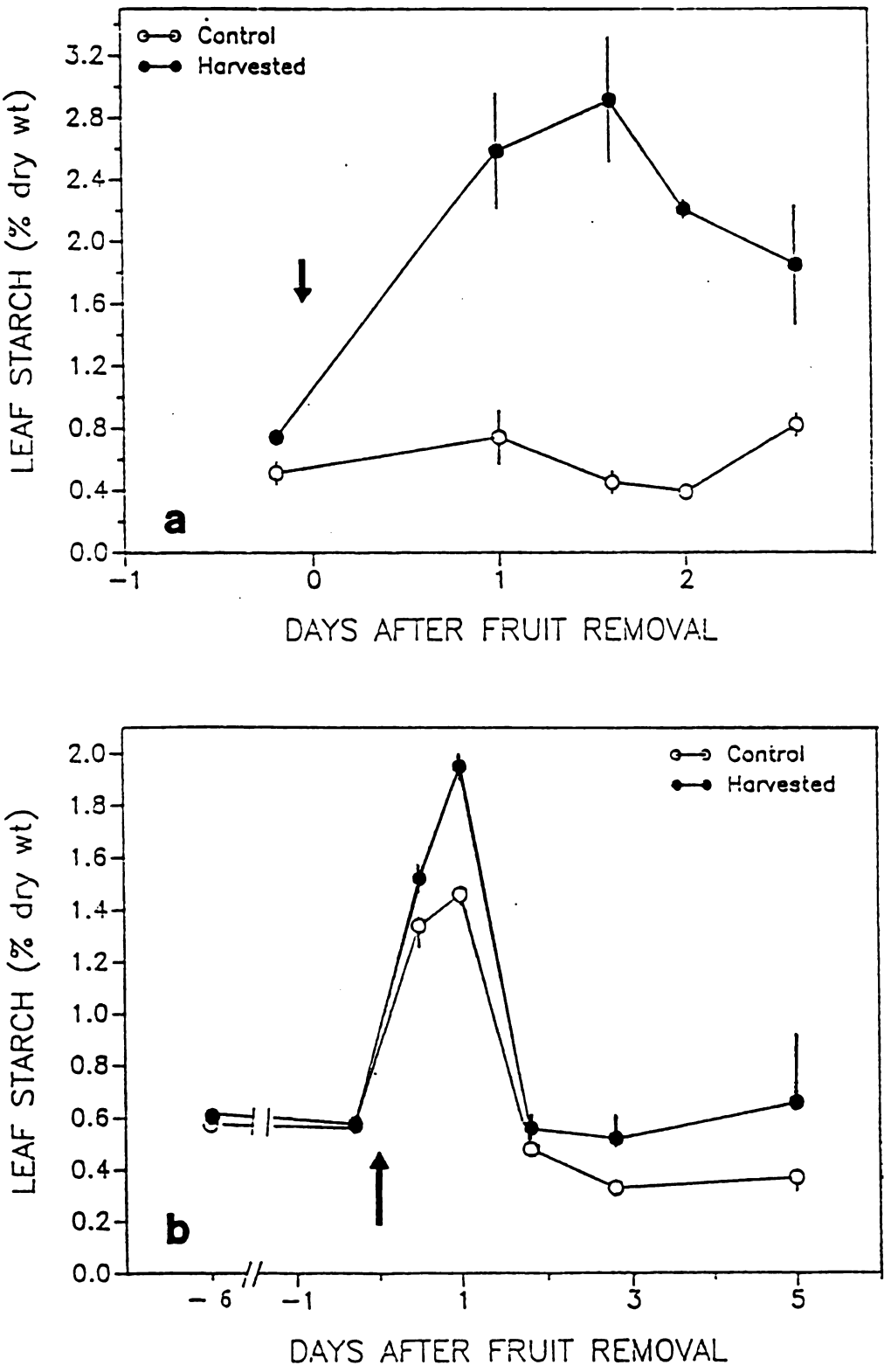

Control
Harvested
LEAF STARCH (% dry wt)
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
a
−1
0
1
2
DAYS AFTER FRUIT REMOVAL
Control
Harvested
LEAF STARCH (% dry wt)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b
−6
−1
1
3
5
DAYS AFTER FRUIT REMOVAL

Figure 5 . Transmission electron micrographs of palisade (A, B) and spongy (C, D) mesophyll cells of leaves collected from sour cherry trees 1 h before (A, C) and 24 h after (B, D) fruit removal at HRC in 1987. Magnification: A. 5800x; B. 4100x; C. 11800x; D. 6300x. Legend: c) chloroplast; s) starch grain; w) cell wall.

Figure 5

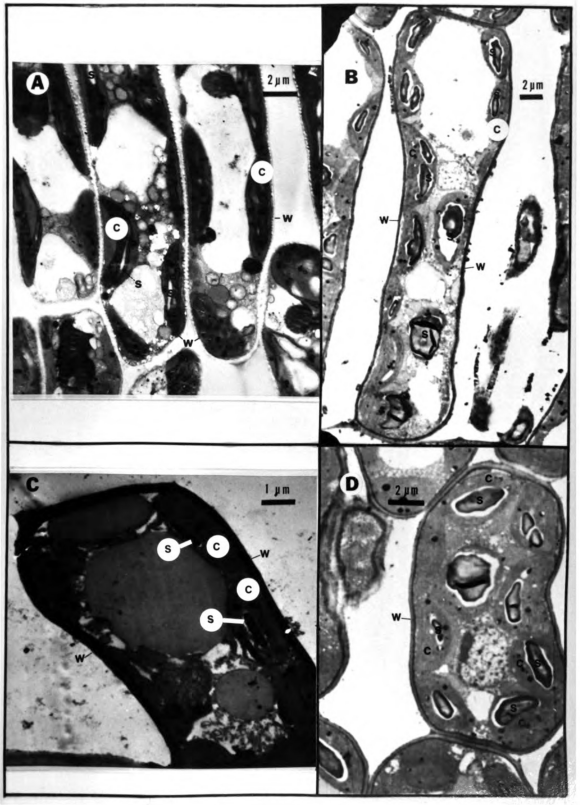
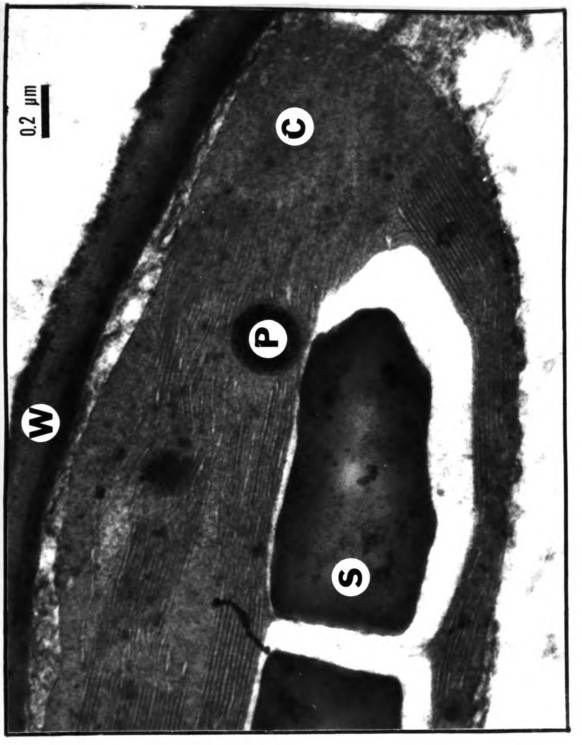


Figure 6 . Transmission electron micrograph of an enlarged starch grain in the chloroplast of a spongy mesophyll cell from leaves of sour cherry 24 h after fruit removal. Note the apparently undamaged arrangement of the thylakoid membranes in the chloroplast. Sampling done at 1400 hr. Legend: c) chloroplast; p) plastoglobule; s) starch grain; w) cell wall. Magnification: 57700x.

Figure 6



Nonstomatal components contributed more than stomatal ones. Analysis of A/Ci curves for harvested and control treatment seemed to indicate that major limitations to Pn were in the region of RuBP regeneration rate (Farquhar and Sharkey 1982; Jones 1973), whereas lower CE of leaves from harvested trees only occurred at Ci < 150 $\mu l\ l^{-1}$ (Figure 2). O_2-sensitivity of Pn was not lost after fruit removal (Table 2). Since percentages of Pn ratios of harvested and control treatment decreased from 27% at 21% O_2 to 14% at 2.6% O_2, post-harvest Pn inhibition was slightly relieved at low O_2. This also seemed to be confirmed by percentages of photosynthetic enhancement at low O_2 (72% of Pn at high O_2 for harvested trees, 45% for control trees) (Table 2) and the slight increase of the CO_2 compensation point after harvest (Figure 2). Limitations of RuBP regeneration capacity, RuBP carboxylation activity, and increase in CO_2 compensation point have also been reported during midday depression of Pn in Quercus (Tenhunen et al. 1984). This may indicate that the post-harvest decline of Pn is not a phenomenon specifically induced by fruit removal, but it may represent a general type of response to environmental conditions or lack of sink strength. The involvement of photorespiration in the Pn response to fruit removal has also been reported in citrus (Lenz 1979) and it is probably effective through a release of competitive inhibition of O_2 for Rubisco (Ogren and Bowes 1971). Involvement of photorespiration is also in

agreement with the hypothesis of RuBP limitations, since the RuBP oxygenase also uses RuBP and, if RuBP is in limited supply , the presence of O_2 can rapidly reduce the rate of carboxylation.

Increases in levels of non-structural carbohydrates after fruit removal have been reported for citrus (Schaffer et al. 1986), plum (Section III), pepper (Hall and Milthorpe 1978), eggplant (Claussen et al. 1985), and cucumber (Barrett and Hamling 1978), but not for sweet cherry (Roper et al. 1988). In sour cherry, only starch increased after harvest, whereas soluble sugars showed no clear trend (Figures 3 and 4). Changes in leaf starch content not accompanied by changes of soluble sugars have also been reported for many species in response to various source-sink manipulations (Maidsen 1968; Milford and Pearman 1975; Nafziger and Koller 1976; Ho 1979). Such a trend could be due to the rapid metabolism of soluble sugars and their conversion into starch. Such changes have also been related to changes in the activity of sucrose or starch synthesizing enzymes (Claussen et al. 1985; Pharr et al. 1983; Rufty and Huber 1985) and/or reduced rates of translocation and export of assimilates from the leaf (Ho 1979; Fondy and Geiger 1980).

Stitt (1986) has recently shown that leaves may have an excess capacity for electron transport so that Pn rate can be enhanced if the rates of RuBP regeneration and carboxylation are increased. He also suggested that the

rate of sucrose synthesis may be the ultimate limitation for photosynthesis since it may prevent the recycling of Pi (Stitt 1986). The discussion of the hypothesis of Pi limitations on Pn is beyond the scope of this paper (see the following references for details: Sharkey 1985; Sharkey et al. 1986; Stitt 1986; Sivak and Walker 1986). However, limitations in the utilization of triose phosphate have also been confirmed during Pn inhibition by ABA feeding or exposure to dry air in Arbutus (Loske and Raschke 1988). Since in this study we did not attempt to determine the activity of enzymes involved in carbon photosynthetic metabolism or the role of metabolites other than the major non-structural carbohydrates, it is impossible to say whether Pn was inhibited by synthesis of translocatable sugars or by utilization of triose phosphates.

On the other hand, we did investigate whether the post-harvest increase in starch might have resulted in physical damage to the chloroplasts of mesophyll cells. Analysis of TEM micrographs showed enlargement of starch grains consistent with the increase of starch content determined by enzymatic analysis, but no disruption of chloroplast or thylakoid membranes was apparent (Figures 5 and 6). Physical damage to the chloroplast by starch grains has been suggested as a possible mechanism of Pn inhibition and cause for leaf chlorosis in response to various source-sink manipulations (Cave et al. 1981; Nafziger and Koller 1976;

Schaffer et al. 1986), but there is still contradictory evidence about the regulatory role of starch on leaf Pn (Little and Loach 1973; Potter and Breen 1980). Because of the low starch content of sour cherry leaves and the absence of chloroplast breakage, starch probably plays no major role in the post-harvest photosynthetic decline, but may be an additional factor playing on the overall depression of photosynthesis.

In conclusion, although the data presented here are not conclusive, A/Ci curves, photorespiration, CO_2 compensation point, and increase in leaf starch content all seem to point towards limitations in the rate of RuBP regeneration rate as a likely mechanism of inhibition. Nevertheless, further experiments on the effect of elevated concentrations of CO_2 (> 350 $\mu l\ l^{-1}$) on the stomatal and non-stomatal response of sour cherry leaves and the activity of enzymes of carboxylation and regeneration of RuBP are needed to elucidate the mechanism of Pn inhibition after fruit harvest.

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KEYWORDS: Stomatal limitations, Prunus cerasus , soluble sugars, starch.

ABBREVIATIONS: Pn, net photosynthesis; g_s, stomatal conductance; Ci, intercellular CO_2; Ca, ambient CO_2; PAR, photosynthetic active radiation; T, leaf temperature; A/Ci, response curve of CO_2 assimilation to internal CO_2; Lg, stomatal limitations; Gc, stomatal contribution to a change in Pn; TEM, transmission electron microscopy; CE, carboxylation efficiency; RuBP, ribulose bisphosphate; Rubisco, ribulose bisphosphate carboxylase/oxygenase; Pi, inorganic phosphate.

Section III

DIURNAL AND SEASONAL CHANGES IN LEAF NET PHOTOSYNTHESIS FOLLOWING FRUIT REMOVAL IN PLUM. I. THE INFLUENCE OF STAGE OF FRUIT DEVELOPMENT AND ENVIRONMENTAL CONDITIONS

ABSTRACT

The effect of fruit removal on net photosynthesis (Pn), stomatal conductance (g_s), internal CO_2 (Ci), and chlorophyll content of leaves from mature plum trees (Prunus domestica L. cv. Stanley) was determined in the field at two stages of fruit development over 2 consecutive growing seasons. Gas exchange parameters were measured with a portable infrared open system. When fruits were removed during stage II (mid-June), Pn decreased from 12.6 to 8.5 μmols m^{-2} s^{-1} 24 h after harvest in 1986, and from 12.1 to 10.2 μmols m^{-2} s^{-1} in 1987. Pn inhibition was greatest in the early afternoon (30-45% of pre-harvest values), less (15-25%) or absent in the morning. Lower Pn rates in defruited trees persisted for 6-8 days in both years. Recovery of Pn in harvested trees coincided with vegetative growth 6 times more vigorous than that of fruiting trees in the first 6 weeks after fruit removal. Leaf chlorophyll content was similar (2.2 μg mg^{-1} fresh wt.) in defruited and fruiting trees. Fruit harvest at the end of stage III reduced Pn 25% after 24 h in 1986, but had no effect in 1987. The difference between years was probably related to both sink activity and environmental factors The decline of Pn following fruit harvest appeared to be confined to the defruited sector of the canopy. Midday depression of photosynthesis was observed only in harvested trees. Calculated levels of intercellular CO_2 (Ci) were not significantly affected by defruiting. Water relations,

feedback inhibition by assimilation products, and direct action of phytohormones are suggested as mechanisms responsible for reduced Pn after fruit removal in plum.

INTRODUCTION

The presence of the fruit reportedly enhances leaf net photosynthesis in eggplant (Lenz 1979), pepper (Hall & Brady 1977), soybean (Mondal et al. 1978), strawberry (Choma et al. 1982), citrus (Lenz 1979), and grapevine (Downton et al. 1987). On the contrary, no consistent effect has been shown in sour cherry (Sams & Flore 1983) or sweet cherry (Roper et al. 1988). In most crops the "fruit effect" on photosynthesis seems to be largely dependent on the particular stage of fruit development at which gas exchange measurements are taken. Higher photosynthetic rates have been correlated with the presence of fruits during periods of maximum fruit growth rate and consequent demand for assimilates in tomato (Starck et al. 1979) and peach (Chalmers et al. 1975; DeJong 1986). Fruiting reduces above- and/or below-ground vegetative growth in tomato (Starck et al. 1979), pepper (Hall and Milthorpe 1978), strawberry (Forney and Breen 1985; Schaffer et al. 1985), peach (Crews et al. 1975; DeJong 1986), and apple (Hansen 1967; Maggs 1963).

Certain conditions may be necessary before a positive sink effect on photosynthesis is observed. Data reported in the literature are seldom comparable because of plant material, environmental differences during growth, and methods used to measure gas exchange (Roper et al. 1988). Furthermore, the physiological mechanism of sink-dependent regulation of Pn is still unclear (Guinn and Mauney 1981).

Plum is a suitable and convenient system for studying the effect of fruit removal on photosynthesis of fruit trees, because of its long period of fruit development and potential for heavy cropping. Photosynthetic characteristics of plum trees have been described to be very similar to those of peach (DeJong 1983), which have been extensively characterized (Crews et al. 1975; DeJong 1983), but few studies have been reported for plum. In peach the presence of the fruit reportedly stimulates photosynthesis even to the point of overcoming light limitations for leaves in the inner part of the canopy (Chalmers et al. 1975). The same authors found a significant decline in photosynthesis in those leaves following harvest of mature fruits. On the contrary, DeJong (1986) noticed lower rates of photosynthesis in nonfruiting peach trees only during periods of high fruit growth. However, differences between fruiting and non-fruiting trees were small.

There are no known studies on the effect of fruit removal on photosynthesis and gas exchange of plum. An understanding of the dynamics of vegetative and reproductive development is particularly important in view of the alternate bearing habit of some plum varieties. It is also important to determine the interaction between fruit removal and with environmental and physiological parameters. For the above reasons, I measured gas-exchange parameters following removal of fruits at pit hardening or at maturity over two years.

The purpose of the present study was to characterize the short- and long-term photosynthetic responses to fruit removal in plum, with particular emphasis on the environmental and physiological conditions under which such responses occur in the field.

MATERIALS AND METHODS

Plant material. Three pairs of plum trees (Prunus domestica L. cv. Stanley) planted in 1976 on a Miami loam at the Horticultural Research Center of Michigan State University, East Lansing (latitude 43° N, altitude 288 m) were selected based on similarity in vigor, crop load, leaf/fruit ratio and pre-harvest Pn rates in spring 1986. Trees were spaced 3.3 x 8.2 m, aligned in 4 rows oriented north-south, and trained to an open center. The same trees were utilized in 1987. The six trees were randomly assigned to treatments according to a randomized block design. Fertilizers and pesticides were applied according to commercial recommendations.

Fruit growth and harvest. A sample of 20-25 fruits were collected weekly from 2 weeks after full bloom to maturity for growth measurements. Fruits were immediately weighed, their length, suture and cheek diameters measured with a precision caliper, and then oven dried at 105°C to constant weight and dry weights recorded. Full bloom dates were estimated to be April 27, 1986 and April 29, 1987. In both

years fruits were removed by hand in stage II and at the end of stage III. Dates of harvest were: June 15, 1986; June 16, 1987; September 2, 1986 and 1987. Fruit densities were established by counting all the fruits on each scaffold and removing the selected percentage. Experiments on partial harvest were performed only at fruit maturity in both years. Percentages of harvest were 50, 75, and 100% in 1986, and 60, 75, 90, and 100% in 1987. Average yield per tree was 17 kg in June 1986, 21 kg in June 1987, and 24 kg in September 1987 (yield data not available for September 1986). Fruiting shoots of 2 additional plum trees on the same site were girdled with a knife by removing 0.5 cm of bark at approximately 50 cm below apex. Girdling was performed on June 10, 1986. Fruits were not removed from girdled shoots.

Leaf/fruit ratio and vegetative growth. A leaf/fruit ratio equal to 4.1 for each tree was estimated by counting leaves and fruits on 4-5 limbs on the east and west sides of the canopy. Vegetative growth of fruiting and harvested trees was estimated from cross-sectional areas and elongation of at least 3 differently oriented scaffold-limbs per tree. Cross-sectional area of the limb was calculated from 2 diameters measured 50 cm distal to the insertion of each scaffold on the trunk. Vegetative elongation was calculated by summing the terminal growth of all 1-year-old shoots longer than 10 cm present on the same limb.

Environmental parameters. Daily records of minimum and maximum temperatures, rainfall, and degree of cloudiness were taken from the records of the local weather station. The cloudiness index was calculated as the sum of the number of cloudy days divided by the number of days in the period of study. The number of cloudy days was calculated using the following scale: clear= 0; partially cloudy = 0.5; cloudy = 1.

Gas exchange parameters. Net photosynthesis, stomatal conductance, PAR, leaf T, and air relative humidity were measured with an ADC LCA-2 portable open gas exchange system equipped with a Parkinson broad leaf chamber (Analytical Development Company, Hoddesdon, UK) on 6-8 leaves distributed on 3-4 limbs (shoots) per tree at each time of measurement. Two fully expanded leaves were used on each shoot, one on a fruit-bearing spur, the other apical to the spur. Measurements were always taken on the same leaves, unless leaves were inadvertently damaged during one of the measurements (damaged leaves amounted to less than 5% for each time course study). Leaves measured in September were not the same as those measured in June of the same year. In the partial harvest experiment, 2 trees were used as controls and 2 were totally harvested, but only one tree was used for intermediate treatments. Diurnal variation of gas exchange parameters was measured twice in June 1986 (1 and 4 days after harvest, from 600 to 1900 hr), and once in June

1987 (1 day after harvest from 800 to 1800 hr). In 1987 gas exchange was always measured twice a day (morning and afternoon).

In order to investigate how localized the effect of fruit removal on Pn was, opposite sides of the canopy were partially harvested in 1986 and Pn measured on the west side to allow afternoon measurements. In 1987 Pn was measured only on the defruited side. Time of measurement for experiment 1 (trees defruited on the east side) was between 1215 and 1430 hr, for experiment 2 (trees defruited on the west side) between 1430 and 1630 hr. Unless otherwise noted the Pn unit was operated as follows: flow rate= 0.4 l/min; ambient CO_2 = 325-345 µl l^{-1}; PAR > 1000 µmols m^{-2} s^{-1}. Gas exchange parameters were calculated as previously described (Moon & Flore 1986). Data were analyzed statistically by ANOVA within dates according to a randomized complete block design. Means were separated by LSD (P=0.05) unless otherwise indicated.

Chlorophyll. Chlorophyll content was determined in 1987 using the method of Moran (1982) as described in Section I. Data were blocked by date .

RESULTS

The effect of fruit removal on gas exchange of plum was studied at two different times of the year. Fruits were removed on June 15-16, or September 2, these dates

corresponding respectively to stage II and the end of stage III on the double sigmoid curve typical of stonefruits (Lilleland 1932) (Figure 1). The warmest part of the year begins in Michigan in mid-June (Figure 2a). Average minimum and maximum temperatures were respectively 12.1° and 25.6 °C in June 1986, 16.1° and 28°C in July 1986, 14° and 28°C in June 1987, 17° and 29.8°C in July 1987 (figure 2a). Rainfall was relatively abundant in these months averaging 112 mm in June and 65 mm in July, sufficient for growth and production of fruit trees without irrigation.

Fruit removal during stage II reduced Pn rates 35% within 24 h in 1986 (from 12.6 to 8.5 μmols m^{-2} s^{-1}) (Figure 2b), 20% within 48 h in 1987 (Figure 2d). Significant differences (P=0.05) between Pn rates of defruited and fruiting trees remained apparent for approximately 7-9 days. The decline of Pn was due to neither early senescence of leaves from defruited trees nor changes in chlorophyll content. Leaf chlorophyll content was about 2.27 μg mg^{-1} fresh wt (5.61 mg dm^{-2}), and the chlorophyll [a/b] ratio approximately 3.5 at each date of sampling for each treatment. Chlorophyll content did not vary diurnally (data not shown). In both years, Pn rates of defruited trees returned to pre-harvest values 9-10 days after harvest (Figure 2b). Recovery was associated with vigorous growth of the canopy of defruited trees (Table 1). Total shoot length per cm^2 CSA of defruited trees was 6 times greater than that of fruiting trees when measured on Aug. 1,

Figure 1. Fresh and dry weights of plum fruits in 1986 and 1987. Full bloom dates: April 27, 1986; April 29, 1987. Arrows indicate times of fruit removal. Vertical bars indicate ± SE (n= 20-25).

Figure 1

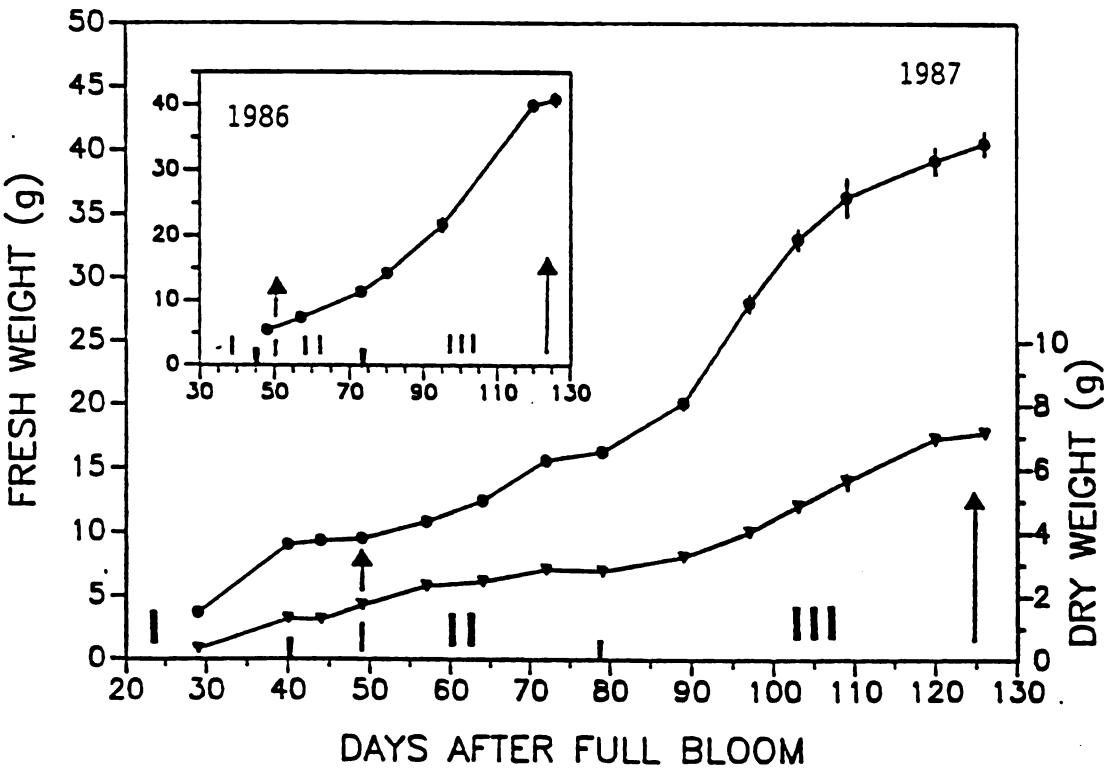

1986
1987
I
II
III
FRESH WEIGHT (g)
DRY WEIGHT (g)
DAYS AFTER FULL BLOOM

Figure 2. (a, c) Minimum and maximum temperatures, precipitation, and cloud cover during the first period (June) of fruit removal in 1986 and 1987. Arrows indicate the date of harvest. Needle bars indicate daily rainfall. Squares on top of the graph cloud cover (■ cloudy; ◪ partially cloudy; □ clear). Thick bars separate months; the number shown at the end of the month denotes monthly precipitation. (b, d) Inhibition of photosynthesis after fruit removal during stage II of development. Vertical bars indicate ± SE.

Figure 2

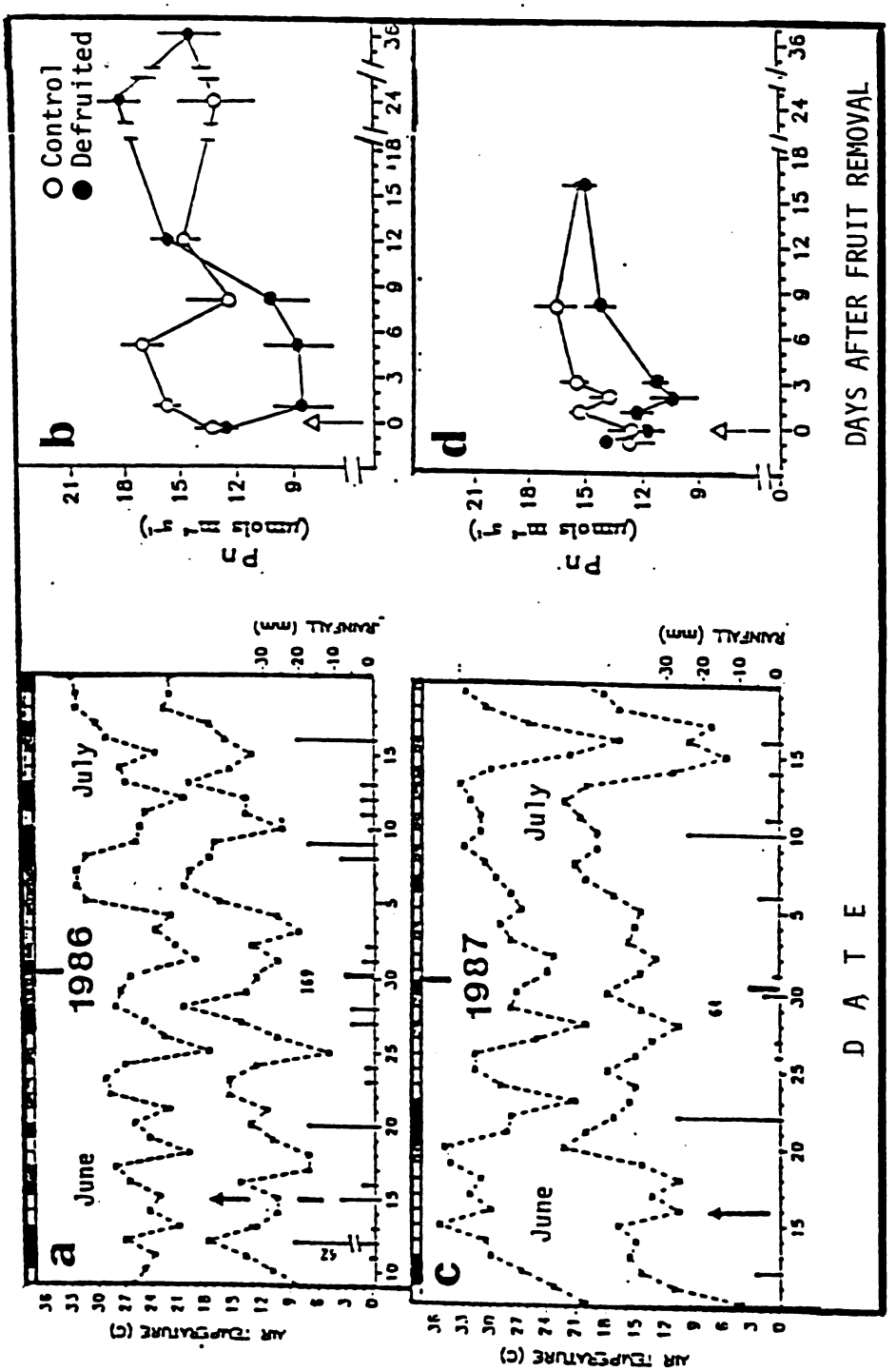

a
June
1986
July
b
Control
Defruited
c
June
1987
July
d
DAYS AFTER FRUIT REMOVAL
DATE
AIR TEMPERATURE (C)
RAINFALL (mm)
Pn

Table 1. Increase in shoot elongation and cross sectional diameters of limbs from fruiting and defruited plum trees.

Period[z]	Treatment	Mean shoot length (cm)	Cross sect. area (CSA) (cm^2)	Total length /CSA (cm^{-1})
(a) Mid summer	Control	20.5 ± 2.1	5.25 ± 0.8	54.7
	Defruited	46.1 ± 4.7	7.29 ± 1.2	297.0
(b) End of growing season	Control	37.8 ± 3.3	5.52 ± 0.9	171.4
	Defruited	68.4 ± 4.3	7.87 ± 3.6	400.0
End-Mid (b-a)	Control	17.3	0.17	116.7
	Defruited	22.3	0.58	103.0

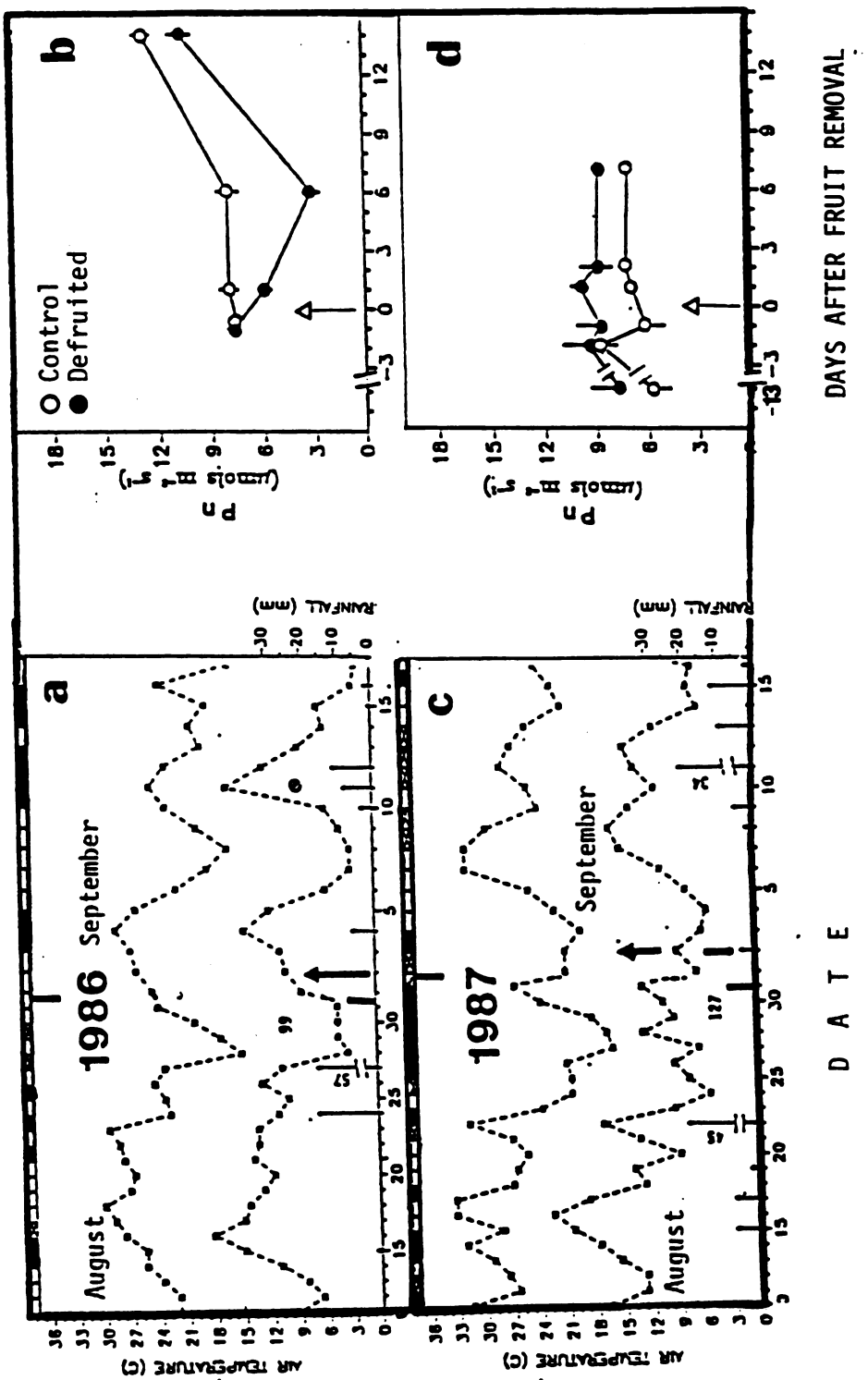
[z] Date of harvest: June 15, 1986. Control trees were harvested on September 30, 1986. Vegetative growth assessed at mid-summer and during winter rest. Values are means ± SE.

1986. Vegetative growth was the same for both treatments in the second part of the growing season (from Aug. 1, 1986 until leaf abscission) (Table 1). Mean shoot length of harvested trees was more than twice that of fruiting trees in midsummer (46.1 vs 20.5 cm), but only 29% greater in the second part of the growing season (23 vs 17 cm).

Diurnal differences in gas exchange parameters were evident because of fruit removal in 1987 (Figure 4). Pn decreased 25% in the afternoon, but no significant differences were apparent in the morning (Figures 4 and 5). Afternoon differences were significant (P=0.05) for 8 days. In 1987 Pn inhibition in the afternoon was about 45% of the pre-harvest value. Pn rates of control trees increased about 20% (from 12.6 to 15.1 μmols m^{-2} s^{-1}) during the period of rapid fruit growth (between June 15 and July 2) (60 mg dry wt/ day; 158 mg fresh wt/ day). Stomatal conductance (g_s) in the morning was not significantly affected by defruiting, but was higher for control trees in the afternoon. Stomatal conductance was higher in the morning than in the afternoon for both treatments until 4 days after harvest, when max T declined from 30-36 to 22-28 C. G_s of control increased about 70% (from 90 to 150 mmols m^{-2} s^{-1}), showing positive correlation with Pn. When expressed as a percentage of pre-harvest values g_s of defruited trees increased about 40%. Values of intercellular CO_2 (Ci) were similar for both treatments in the morning and afternoon, indicating that both stomatal and mesophyll

Figure 3. (a, c) Environmental variables during the second period of fruit removal (September) in 1986 and 1987; (b, d) changes of photosynthesis after removal of mature fruits in 1986 and 1987. Symbols are the same as in Figure 2. Vertical bars indicate ± SE.

Figure 3


a
1986
August
September
AIR TEMPERATURE (C)
RAINFALL (mm)
b
Control
Defruited
c
1987
d
DAYS AFTER FRUIT REMOVAL
D A T E

factors were involved in the observed decrease of Pn after harvest (Figures 4e; 4f; 5e; 5f).

Midday depression of Pn occurred in both defruited and girdled shoots (Figure 6). The effect of defruiting was significant only at 1300 hr and thereafter (differences at 1400 hr turned out to be significant in two other other days, data not shown). In the first hours of the morning (600 and 800 hr) Pn was limited mainly by light (PAR= 50 and 350 μmols m^{-2} s^{-1} respectively), then increased until 1200 hr and remained constant until late afternoon when light again became limiting. G_s was significantly different only at 1800 hr , Ci of defruited trees was significantly higher than that of control in the afternoon.

Branches were girdled to determine whether the decrease in Pn observed after harvest depended solely on a chemical inhibitor translocated from or to the fruit. The presence of fruit on girdled shoots did not prevent Pn inhibition. Girdling reduced Pn rates significantly (P=0.05) and was more effective than defruiting in the afternoon. Ci levels were also higher for the girdling treatment. In addition, leaves from girdled shoots showed signs of premature senescence and, for this reason, gas exchange measurements were abandoned 10 days after treatment.

Fruit removal late in the season (September 2) not only occurred at a period of slower fruit growth rate, but also at a time when environmental conditions were very different from those in June. Sub-optimal Pn rates could be explained

Figure 4. Changes of photosynthetic rate (Pn), stomatal conductance (g_s), and intercellular CO_2 (Ci) following defruiting during stage II in the morning and afternoon in 1987. Vertical bars $\pm$ SE (n= 16-20).

Figure 4

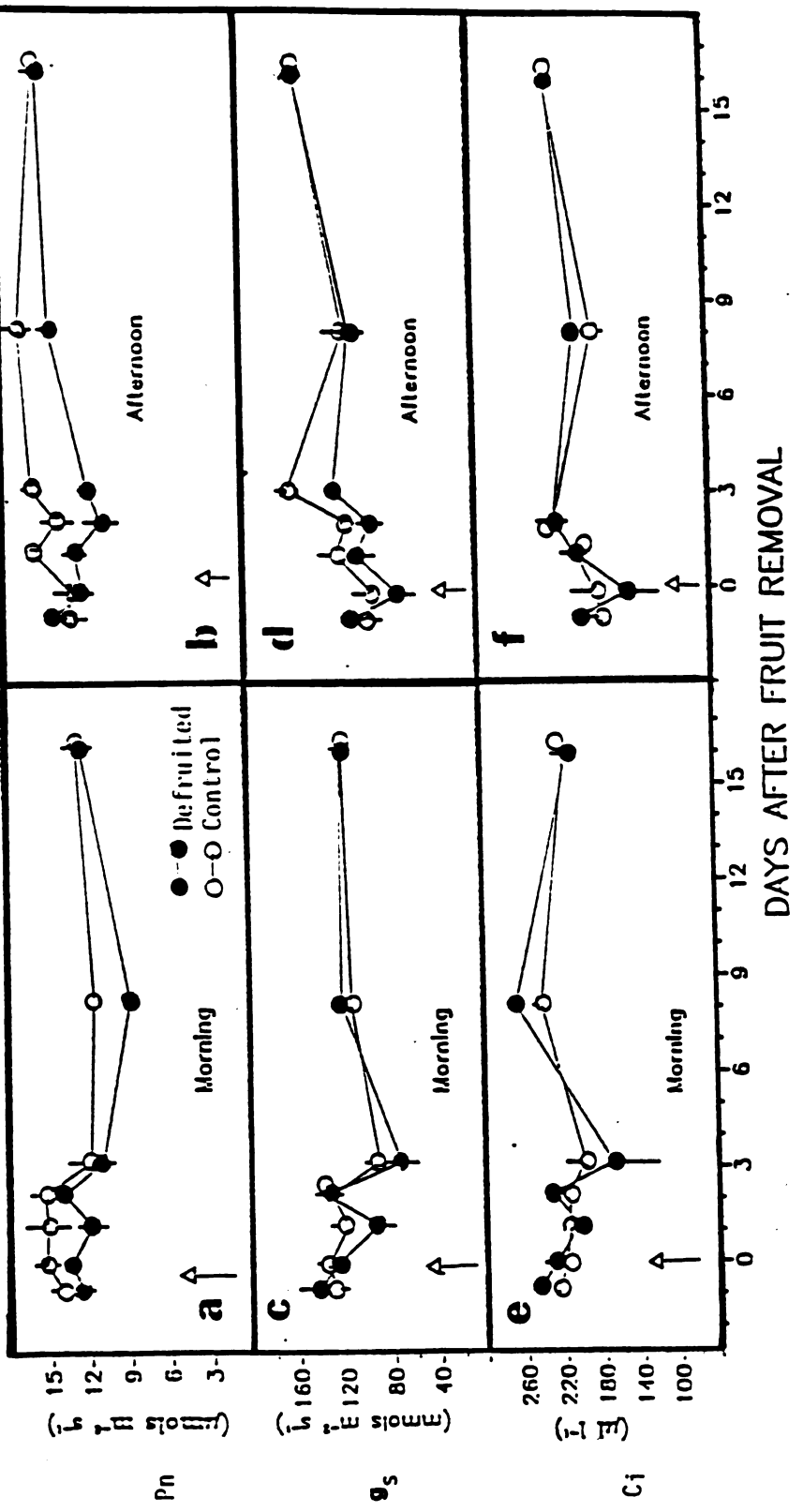

Pn
gs
Ci
Morning
Afternoon
Defruited
Control
DAYS AFTER FRUIT REMOVAL

Figure 5. Changes of photosynthetic rate (Pn), stomatal conductance (g_s), and intercellular CO_2 following defruiting during stage II in the morning and afternoon in 1987. Gas exchange parameters expressed as percentage of the mean of pre-harvest values.

Figure 5

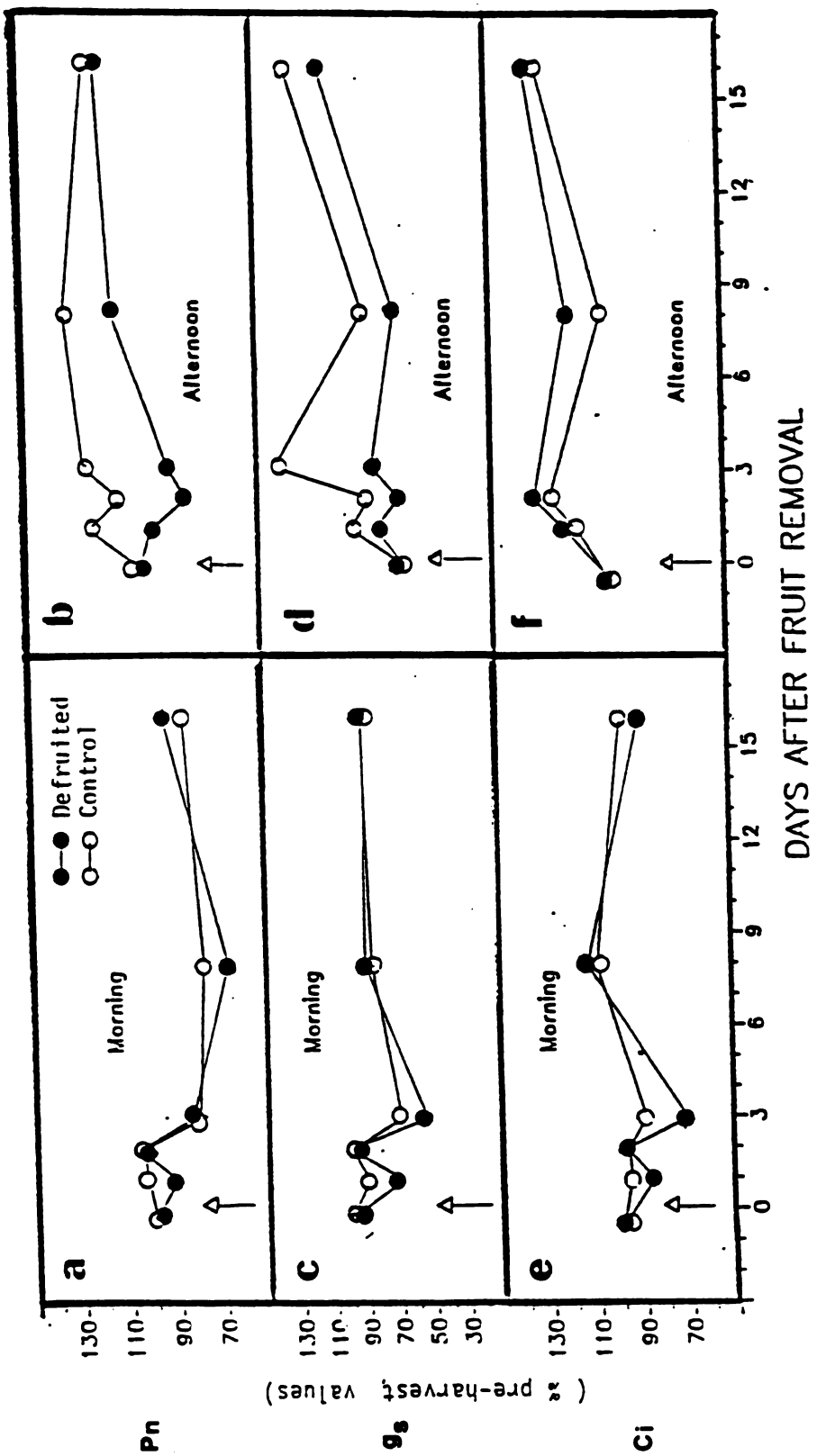
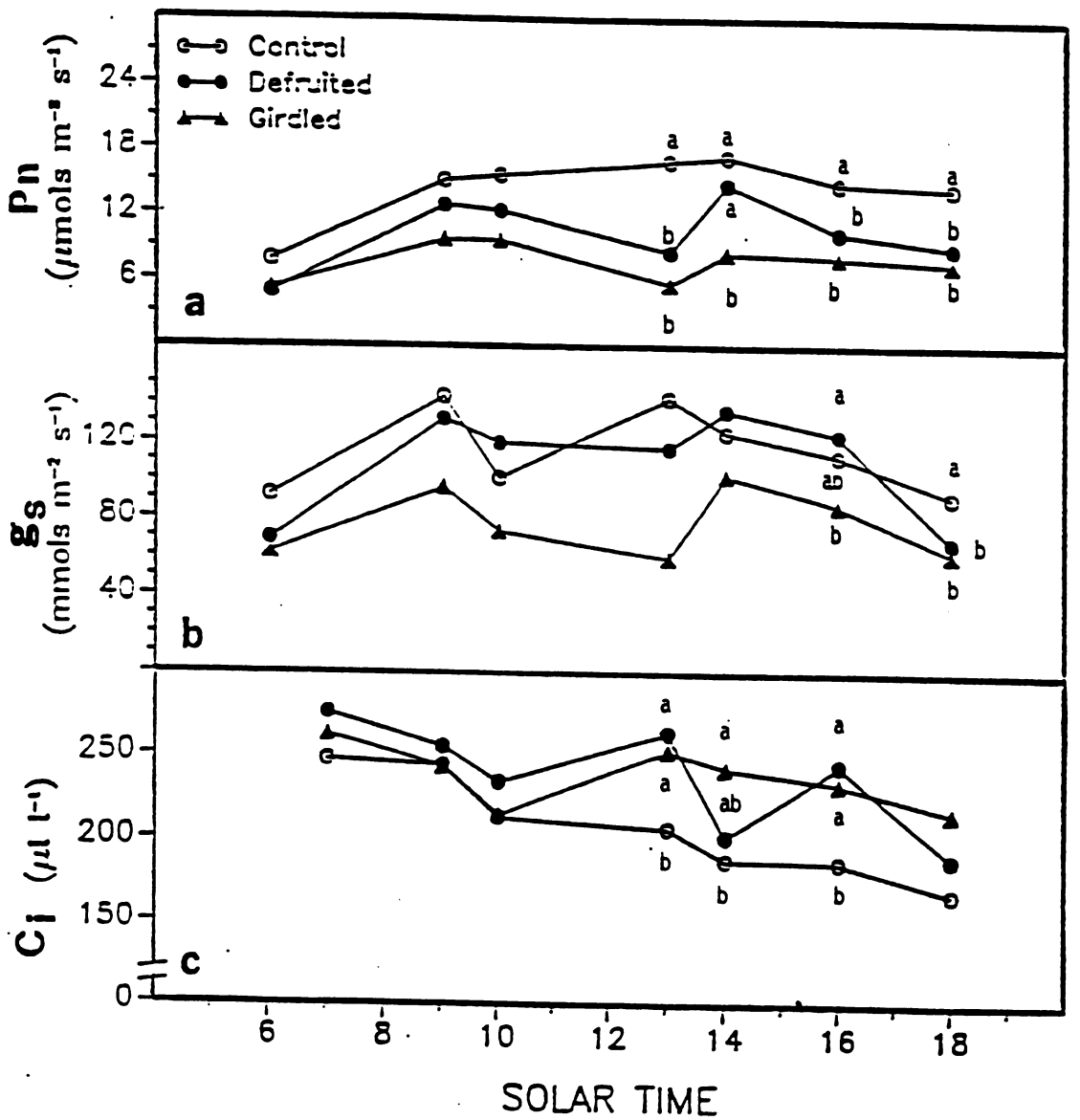

Defruited
Control
Morning
Afternoon
a
b
c
d
e
f
Pn
gs
Ci
(% pre-harvest values)
DAYS AFTER FRUIT REMOVAL

Figure 6. Diurnal changes of Pn, g_s, and Ci of leaves from fruiting, defruited, or girdled shoots of field-grown plum trees. Branches defruited on June 15, 1986. Different letters indicate significant differences between treatments at each single date.

Figure 6


Control
Defruited
Girdled
Pn (μmols m⁻² s⁻¹)
gs (mmols m⁻² s⁻¹)
Ci (μl l⁻¹)
a
b
c
SOLAR TIME
6
8
10
12
14
16
18

by decreasing T and PAR, or by leaf age. In fact, averages of min/ max T were respectively 10.6°and 22.8°C in September 1986, and 8.6°and 23.4°C in 1987. Rainfall was 142 mm from Aug. 24 1986 to Sept. 9, 1986, 144 mm from Aug. 19, 1987 to Sept 11, 1987. The cloudiness index (IC) was about 0.5 in both years, higher than the mean for June (0.45) or July (0.37) (Figures 3a and 3c). The variability of response to fruit removal in the 2 years of study is shown in Figures 3b and 3d. Pn rates were constant over the period August-September, but lower than in June-July (about 9 µmols vs 14-15 µmols m^{-2} s^{-1}). No symptoms of leaf senescence were observed until later in the season (October). In 1986 fruit removal reduced Pn 25% after 24 h and 63% 6 days after harvest. The fruit effect was visible only at high T and clear sky. In 1987 fruit removal had no apparent effect, despite the similarity of T and rainfall. The only difference was in the cloudiness index (only 2 sunny days from Aug. 19, 1987 to Sept 3, 1987) and so light (250-350 µmols m^{-2} s^{-1}) may have been limiting.

In order to evaluate whether a quantitative relationship existed between fruit removal and Pn inhibition, trees were sectorially harvested (Table 2). When fruits were removed from the east side, Pn on the west side appeared to decrease linearly with increasing amount of fruits removed (Table 2, column c). Similarly Pn varied inversely with percentage of fruit removal. Although initial Pn rates were not equal in all trees, comparisons between

100% and 0%, and 50% and 75% still remained consistently quantitative. The effect was largely localized (see experiment 2), but also dependent on total sink strength, since the largest drop in Pn was observed when all fruits were removed (no fruits were near). In experiment 2 the greatest decline from pre-harvest values occurred when only 50% of the crop was removed from the side of the tree used for measuring Pn. However, variability of initial Pn rates, inadequate replication due to the kind of experiment and type of data analysis can explain part of the inconsistencies for which 100% treatment was less inhibited than 75% or 50%.

We have already pointed out the poor relationship between g_s and Pn changes after fruit removal. Correlation analysis for some of the experiments carried out in this study are reported in Table 3 (other experiments gave negative correlations!). Correlation is high only for September 1986 experiment 2, experiment 1 and diurnal (control, June 16, 1986).

DISCUSSION

There are similarities and differences between the effect of fruit removal at pit hardening and at fruit maturity in plum. The intensity of the effect is intermediate between that reported for peach by DeJong (1985) vs Chalmers et al. (1975). Peach and plum have similar photosynthetic characteristics and pattern of fruit

Table 2. Total sink strength of plum fruits and amount of Pn inhibition following partial localized harvests at fruit maturity.

EXPERIMENT 1 - Photosynthesis measured between 1115 and 1330 hr on side opposite to the harvested one.

EXP 1	Net Photosynthesis					
Fruits[y] removed	DAYS AFTER HARVEST (μmols m^{-2} s^{-1})	(% pre-harvest)				SDPH[z]
(%)	0	0	1	6	14	
0	8.63	100	90	95a	145	+ 30
50	10.26	100	73	88a	137	- 2
75	10.19	100	67	80a	124	- 29
100	8.59	100	64	49b	102	- 85

EXPERIMENT 2 - Photosynthesis measured between 1230 and 1500 hr on defruited side.

EXP 2	Net Photosynthesis					
Fruits[y] removed	DAYS AFTER HARVEST (μmols m^{-2} s^{-1})	(% pre-harvest)				SDPH[z]
(%)	0	0	1	6	14	
0	6.52	100	125a	119a	199	+ 145
50	8.55	100	86ab	23b	147	- 44
75	10.70	100	47b	36b	120	- 132
100	6.82	100	92a	30b	190	+ 12

[y] Date of harvest: September 2, 1986. Photosynthesis expressed as % of pre-harvest rates for each treatment. Different letters indicate statistical differences (P=0.05) calculated from original photosynthetic rates.

[z] SDPH indicates the sum of differences of Pn (%) from pre-harvest values at each date. Positive numbers denote post-harvest rates higher than pre-harvest.

Table 3. Correlation coefficients (r) between changes in Pn and g_s calculated from time course and diurnal measurements in plum.

Type of experiment	Date	r			
		Defruited		Control	
		Morning	Afternoon	Morning	Afternoon
Time course	June 1987	0.39	0.58	0.73	0.56
Diurnal	6/16/86	0.64		0.95	
Diurnal	6/20/86	0.81		0.60	
Time course	Sept. 1986 Exper. I	0.72		0.90	
Time course	Sept. 1986 Exper. II	0.98		0.97	
Time course	Sept. 1987 Exper. I	0.34		0.57	

development under California conditions. The "fruit effect" on Pn in plum is evident when fruits are removed during stage II, but not at fruit maturity. At stage II the fruit is an active sink since it accumulates about 45 mg dry wt/day, whereas at the end of stage III the rate of accumulation is only 27 mg dry wt/day. Physiological factors, namely the growth rate of the fruit, reflecting its demand for assimilates, and the leaf/ fruit ratio are the most critical factors in observing the "fruit effect" in apple (Fujii and Kennedy 1985), peach (DeJong 1986), and grapevine (Downton et al. 1987). Studies that have failed to detect a fruit effect on Pn were conducted by removing the reproductive sink at early stages (blossoms, flowers, or fruitlets at stage I) (Sams and Flore 1983; Roper et al. 1988) or on immature trees with a high leaf/fruit ratio (Rom and Ferree 1986). However, endogenous parameters alone do not seem to be sufficient to cause a significant decline in Pn after fruit removal. Interaction with the environment is strong and can become prevalent in determining plant response. Thus, removal of mature fruits had different effects in 1986 and 1987, and suboptimal environmental conditions may have contributed significantly to these differences. Light and temperature in particular seemed to be the most critical factors, as also shown in studies on growth and photosynthesis of Fragaria vesca (Chabot 1978). Diurnal effects on Pn and related processes have been described for several woody species (Downton et al. 1987;

Raschke and Resemann 1986). In Arbutus unedo midday depression of Pn was dependent on changes in g_s driven primarily by VPD (Raschke and Resemann 1986). Changes in Pn and g_s in Arbutus occurred simultaneously (Raschke and Resemann 1986), whereas in plum the decline in Pn apparently precedes a decrease in g_s. In order to document more precisely such trends it is necessary to use controlled environments and response curves of CO_2 assimilation to CO_2 (A/Ci) to partition stomatal from mesophyll effects. In general, diurnal changes in Pn and g_s were closely coupled before and after harvest in plum (Table 3).

Girdling fruiting shoots produced a short term decrease of Pn and g_s decrease despite the presence of the fruit. Leaves from girdled shoots showed midday depression of Pn similarly to that observed after fruit removal, whereas no midday depression of Pn was observed in fruiting trees. Although Dann et al. (1983) hypothesized that an imbalance in growth regulators above and below the girdle was responsible for girdling effects no evidence was apparent from the present crude girdling experiment for a direct action of growth regulators on Pn inhibition after harvest. Obviously the question of the role of phytohormones in Pn regulation has to be addressed with more refined techniques and experiments to be answered properly.

Leaf senescence occurred prematurely on girdled shoots, but not in defruited trees. Pn decrease after fruit removal not associated with early leaf senescence has also been

reported in soybean (Mondal et al. 1978). In contrast, fruit removal or branch girdling reportedly reduces chlorosis in pecan (Wood 1988) and citrus leaves due to rapid accumulation of starch and consequent disruption of thylakoid membranes (Schaffer et al. 1986).

Plum responded to defruiting at stage II by increased vegetative growth of the canopy in the early summer. This confirming observations on other fruit crops (Chalmers et al. 1975; DeJong et al. 1987; Hansen 1970; Lenz 1967; Maggs 1963; Roper et al. 1988; Schaffer et al. 1986). Vegetative growth in the late summer is probably not affected because of incipient terminal bud set and the onset of shorter days. A close relationship between demand and supply of assimilates has been hypothesized by Chalmers et al. (1975) in peach. Sink strength seems therefore the most crucial factor regulating tree Pn in the field, when no other limitations are present. The "fruit effect" appeared to be confined mainly to leaves closest to fruits, in agreement with a local sink effect observed in wheat (Cook and Evans 1976) and peach (Chalmers et al. 1975). In addition to a local influence a quantitative relationship appears to exist between total sink strength and Pn inhibition following fruit removal in plum. This hypothesis, however appealing, needs further documentation because of high variability in the data.

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Keywords: Photosynthesis inhibition, gas exchange, Prunus domestica, sink activity, environment, total fruit harvest, partial fruit harvest.

Abbreviations: Pn, net photosynthesis; g_s, stomatal conductance; Ci, intercellular CO_2; PAR, photosynthetic active radiation; VPD, vapor pressure deficit; T, air temperature; IC, cloudiness index; CSA, cross sectional area.

Section IV

DIURNAL AND SEASONAL CHANGES IN LEAF NET PHOTOSYNTHESIS FOLLOWING FRUIT REMOVAL IN PLUM. II. WATER RELATIONS, PHOTORESPIRATION, PARTITIONING OF NON-STRUCTURAL CARBOHYDRATES IN THE LEAF

ABSTRACT

Decrease of photosynthesis (Pn) and stomatal conductance (g_s) occurred for Prunus domestica L. following removal of fruits at the pit-hardening stage of development. Water relations, photorespiration, non-structural carbohydrates of leaves from field-grown trees were investigated to determine possible stomatal and nonstomatal mechanisms of Pn inhibition. Stomatal contribution to the decrease in net photosynthesis calculated from assimilation/internal CO_2 (A/Ci) curves ranged from 31 to 46%. Defruiting did not significantly affect water potential, but defruited trees had lower osmotic potentials, especially in the afternoon. Loss of leaf turgor did not appear to be related with stomatal closure and lower osmotic potentials. Starch content increased 80% (from 0.35 to 0.62% dry wt) in leaves of defruited trees within 24 h from fruit removal, whereas levels of sorbitol, sucrose, glucose, and fructose in the leaf were not affected. Therefore, lower osmotic potentials in leaves of defruited trees probably reflected the increase of nonosmotic volume as a result of starch accumulation in the chloroplast. Photorespiration rates were similar in fruiting and defruited trees (6-7 μmols m^{-2} s^{-1}). Under field conditions Pn rates at 0.5% O_2 were 50% greater than those at 21% O_2. The results support the hypothesis that the non-stomatal component of Pn inhibition after fruit removal may be controlled by levels

of non-structural carbohydrates in the leaf. The exact mechanism of this feedback regulation remains unknown.

INTRODUCTION

Changes in leaf photosynthetic rate occur in response to alterations of source-sink relationships or environmental conditions in several species. Increased rates of Pn were found in soybean plants with a high sink demand (Clough et al. 1981), in unshaded leaves of soybean when the rest of the plant had been shaded (Peet and Kramer 1980), in partially defoliated grapevines (Flore and Gucci 1988), or in pepper plants transferred from low to high irradiance (Grange 1987). On the contrary, lower Pn rates have been measured when sink strength was limiting (Clough et al. 1981), i.e. when the rate of assimilate export had been reduced by leaf detachment (Claussen et al. 1985), fruit excision (Claussen et al. 1988), petiole girdling (Setter et al. 1980), or when the source of assimilates had been increased by CO_2 enrichment (DeLucia et al. 1985). However, Pn changes due to these types of manipulations are not universal, since similar experiments conducted on different plant systems or under different environmental conditions have failed to show such response (Little and Loach 1973; Rom and Ferree 1986; Roper et al. 1988).

Stomatal closure, which limits availability of CO_2, has been associated with reduced photosynthesis in soybean leaves after depodding or petiole girdling (Setter et al. 1980), and in grapevine , pepper, and apple after fruit removal (Hansen 1971; Kriedemann et al. 1976). Water stress caused stomatal closure in apricot (Loveys et al. 1987) and

hazelnut (Schulze and Kuppers 1979). Nonstomatal limitations to CO_2 fixation were also associated with Pn inhibition during water stress in several species (Ackerson and Hebert 1981; Bunce 1977; Bunce 1982; Briggs et al. 1986), in CO_2-enriched cotton plants (DeLucia et al. 1985), and after fruit excision in pepper (Hall and Milthorpe 1978). Photorespiration was involved in Pn changes following fruit removal in citrus (Lenz 1979), but not following defruiting of pepper (Hall and Milthorpe 1978) or shading of soybean plants (Peet and Kramer 1980).

The hypothesis of feedback inhibition by assimilation products was formulated over 100 years ago, and since then periodically exhumed to try to explain the mechanism of non-stomatal regulation of Pn (Neales and Incoll 1968; Guinn and Mauney 1980). However, since accumulation of non-structural carbohydrates in the leaf is also a general type of response to many environmental stresses and source-sink manipulations, it is difficult to demonstrate unequivocally a causal relationship between carbohydrate accumulation and inhibition of photosynthesis. For instance, obstructed translocation, water stress, CO_2 enrichment, continuous illumination, fruit removal, leaf detachment, shoot girdling, and low sink strength can all result in increased levels of soluble sugars and/or starch in the leaf (Ackerson 1981; Azcon-Bieto 1983; Bunce 1982; DeLucia et al. 1985; Hall and Milthorpe 1978; Mayoral et al. 1985; Schaffer et al. 1986). Evidence for a strong positive correlation

between levels of non-structural carbohydrates and Pn inhibition can be found in the literature. End-product inhibition of photosynthesis has been supported by several studies on wheat (Azcon-Bieto 1983), cotton (Ackerson 1981; DeLucia et al 1985), soybean (Clough et al. 1981), eggplant (Claussen et al 1985), cucumber (Mayoral et al. 1985), and citrus (Schaffer et al 1986), but not by studies on soybean and sunflower (Bunce 1982), balsam fir (Little and Loach 1973), and pepper (Hall and Milthorpe 1978).

In a previous section I reported the decline in Pn after fruit removal at stage II in plum trees, the simultaneous changes in g_s, and the near constancy of Ci. However, the roles of stomatal action vs non-stomatal components remain to be established. were also involved.

The objective of this study was to determine the mechanism of inhibition by fruit removal in plum, with particular emphasis on carbon and water status of the leaf.

MATERIALS AND METHODS

Plant material. Six 10-year old plum trees (Prunus domestica cv. Stanley) were chosen at the Horticultural Research Center of Michigan State University, East Lansing, Michigan in 1986. Trees were spaced 3.3 x 8.2 m and trained to an open center. Pesticides were applied according to commercial recommendations (Mich. Ext. Bul. E154, Fruit Pesticide Handbook). Treatments were randomly assigned using a randomized block design. Dates of fruit removal

were: June 15, 1986 and June 16, 1987. Fruiting shoots from 2 trees were girdled mechanically by removing a 0.5 cm wide strip of bark. Other details about plant material, location, and experimental conditions are as described in Section III.

Leaf potentials. Leaf water potentials were measured on 3-4 leaves per tree (2 trees per treatment 1n 1986, 3 in 1987) with a portable pressure bomb (PMS Instrument Company, Corvallis, Oregon). Two procedures for water potential measurement were employed: that of Turner and Long (1980) in 1987, whereby the leaf was enclosed in a zip-seal plastic bag prior to collection before placing it in the pressure bomb; in 1986 the leaf was harvested harvesting without the use of the zip-lock bag. Differences in WP between harvested and fruiting trees were not affected by the change in method. The time between detachment of the leaf and placing it in the pressure chamber never exceeded 30 seconds in both years.

Leaf osmotic potential was measured on frozen leaves (5-10). Major veins were excised, the thawed tissue placed in a 5 cc syringe, and 10 ul of cell sap was extracted. Solute concentration was measured at 37°C with a vapor pressure osmometer Wescor 5500 M2448 (Wescor Inc., Logan, Utah). Solute concentration (mmol/kg) was converted into osmotic potential using the Van't Hoff equation (OP = R x T x J), where R is the gas constant, T equals 37° C, and J indicates the molar concentration of the cell sap. Osmotic

potentials were determined in triplicate for each tree at each date.

Leaves sampled for determination of water and osmotic potentials were chosen to be similar in size, position, exposure, and distance from fruits to those measured for gas exchange parameters. Turgor pressure (TP) was calculated as difference between water and osmotic potentials.

Gas exchange parameters Gas exchange parameters were measured with an ADC LCA-2 portable open gas exchange system equipped with a Parkinson broad leaf chamber (Analytical Development Co., Hoddesdon, England) as explained in Section I. Photosynthesis, transpiration, and water use efficiency were calculated using the method described by Moon and Flore (1986).

In the field photorespiration was estimated and CO_2 response curves were measured on 3 leaves per tree (same as those measured for Pn in the time course experiment) from two trees per treatment following the method described in Section II. Measurements in 1987 were taken from 1130 to 1430 hr , 3 days after harvest. Environmental conditions were: PAR > 1600 µmols m^{-2} s^{-1}, air temperature 30-34° C, cuvette temperature 34-38 C, ambient relative humidity 48-50%, ambient CO_2 345 µl l^{-1}, flow rate 0.6 ml/min. Photorespiration was estimated by comparison of Pn rates at 21 and 0.5% O_2.

Excised shoots were used for estimation of photorespiration in the laboratory where environmental conditions could be held constant at 1000 μmols m^{-2} s^{-1} PAR, ambient temperature 24-25° C, cuvette temperature 28.1-28.5°C, flow rate ranging from 1.7 to 2.2 l/min depending on age and photosynthetic rate of the leaf. Photorespiration was measured by comparing Pn rates at 2.5 vs. rates at 21% O_2. CO_2-response curves were measured by increasing external CO_2 concentration from 0 to 345 μl l^{-1}. Field and laboratory measurements were made on material from the same trees on the same day. Stomatal contributions to changes in Pn were estimated from A/Ci curves according to the methods of Jones (1985) and Farquhar & Sharkey (1982) as explained in Section II.

Non structural carbohydrates.

Six to ten leaves were collected from each tree at each time of measurement, immediately frozen in liquid nitrogen or dry ice, transported frozen to the laboratory, and there lyophilized to constant weight (36-48 hours) in a Virtis 10-010 automatic freeze-drier (Virtis Inc., Gardiner, New York). Leaves were deveined, and weighed with an analytical balance (Mettler AE 163 Mettler Instruments AG, Greifensee, Switzerland). Leaf area of the dried sample was measured with a portable area meter LI-COR 3000 (LI-COR, Lincoln, Nebraska). Samples were ground in a Wiley mill, passed through a 40 mesh screen, and 50 mg of each sample was

extracted 4 times in 2 ml each of 80% ethanol. The homogenates were centrifuged at 1500 rpm (Glc, Sorvall, Connecticut) for 5 minutes after each extraction. The supernatant was used for analysis of soluble sugars, the pellet for starch determination.

For determination of the soluble sugars (sorbitol, fructose, glucose, inositol, and sucrose) the supernatants were poured into 50 ml round bottom flasks, evaporated to dryness using a rotary vacuum evaporator in a water bath at 40° C, and the dried samples stored overnight in a desiccator. The samples were converted into oximes (Roper et al. 1988) and derivatized to tri-methylsilyl ethers (Sweeley et al. 1963). Standards for sorbitol, fructose, glucose, sucrose, and inositol were prepared by dissolving 0.25 g of the first four compounds in 50 ml of 80% ethanol and 0.1 g of inositol in 25 ml of 50% ethanol, then combining the 2 solutions and drying 0.5 ml aliquots in a stream of nitrogen at room temperature. Standards were derivatized in the same way as samples. Analyses were performed using a dual column, temperature programmed Varian 3700 gas chromatograph (Varian Associates Inc, Sunnyvale, California) with flame ionization detectors and 3% OV-17 on 80/100 mesh chromosorb WHP in 2 mm x 2 m glass column. Temperature was programmed from 150 C to 250 C at a rate of 5 C/min. Quantity was calculated using the internal standards with a Spectra Physics SP4100 integrator (Spectra Physics, San Jose', California) and the resulting areas

checked at random by cutting the peaks and weighing them with an analytical balance (Mettler Instruments AG, Greifensee, Switzerland). Injections (1 ul) were repeated twice for each sample and results averaged. Soluble sugars were expressed as mg g^{-1} dry weight.

Starch was measured in the pellet remaining after extraction with 80% ethanol using the method of Roper et al. (1988) modified as follows. Samples resulting from the incubation at 55 C for 16 h with amyloglucosidase were diluted with distilled water to 15 ml volumes and three 0.25 ml aliquots from each sample were assayed colorimetrically using glucose oxidase (Sigma Tech. Bull. 510; EC 1.1.3.4). Absorbances were read at 440 nm with a Shimadzu UV-Vis 260 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

RESULTS

Transpiration rates of leaves from defruited trees were about 10% and 25% lower than the controls respectively 24 h and 48 h after fruit removal. In the morning there were no consistent differences between the two treatments (Figure 1c). Defruiting did not affect WUE significantly (Figures 1e and 1f). Transpiration rates of fruiting trees were usually higher in the morning than in the afternoon (Figure 1c and 1d). Contrary to what was observed in the time-course response, average diurnal WUE was 37% higher in fruiting trees (Figure 2c). No change of WP occurred after fruit

harvest (Figure 3b), but OP decreased, although such decrease was not associated with loss of turgor (Figure 3a). No diurnal differences in WP were found between the two treatments on the dates studied (Figure 4), but defruiting reduced OP starting from about 1130 hr (Figure 4).

In order to estimate the stomatal contribution to the decrease of Pn after harvest two methods were used both based on CO_2-response curves of photosynthesis (Farquhar and Sharkey 1982; Jones 1985) (Figure 5). Stomatal contribution to the decrease in Pn was 31 or 46% when calculated, respectively, by resistance (Farquhar and Sharkey 1982) or sensitivity analysis (Jones 1985) at ambient CO_2 (330 µl l^{-1} corresponding approximately to 180 µl l^{-1} Ci). Stomatal conductance did not vary significantly over the range of Ci tested, but was significantly higher (P=0.05) for fruiting trees (150 vs. 112 mmols m^{-2} s^{-1}; Figure 5). Stomatal limitations (Lg) to Pn were 53 and 59% respectively for fruiting and defruited trees (Farquhar and Sharkey 1982). (Pn of fruiting trees was 24.2 umols m^{-2} s^{-1}, while Pn of defruited trees was 17.3 µmols m^{-2} s^{-1} at 180 µl l^{-1} Ci). Calculated Lg overestimated the actual stomatal limitations because they were based on linear A/Ci curves (Farquhar and Sharkey 1982).

Photorespiration rates did not differ between defruited and fruiting trees (Table 1), Pn rate was 35% greater at low O_2 than at atmospheric O_2 concentration for both

Figure 1. Changes in photosynthetic rate (Pn), transpiration (Tr), and water use efficiency (WUE) following removal of plum fruits at stage II during the morning and afternoon in 1987. Arrows indicate date of fruit removal. Vertical bars ± SE (n=16-20).

Figure 1

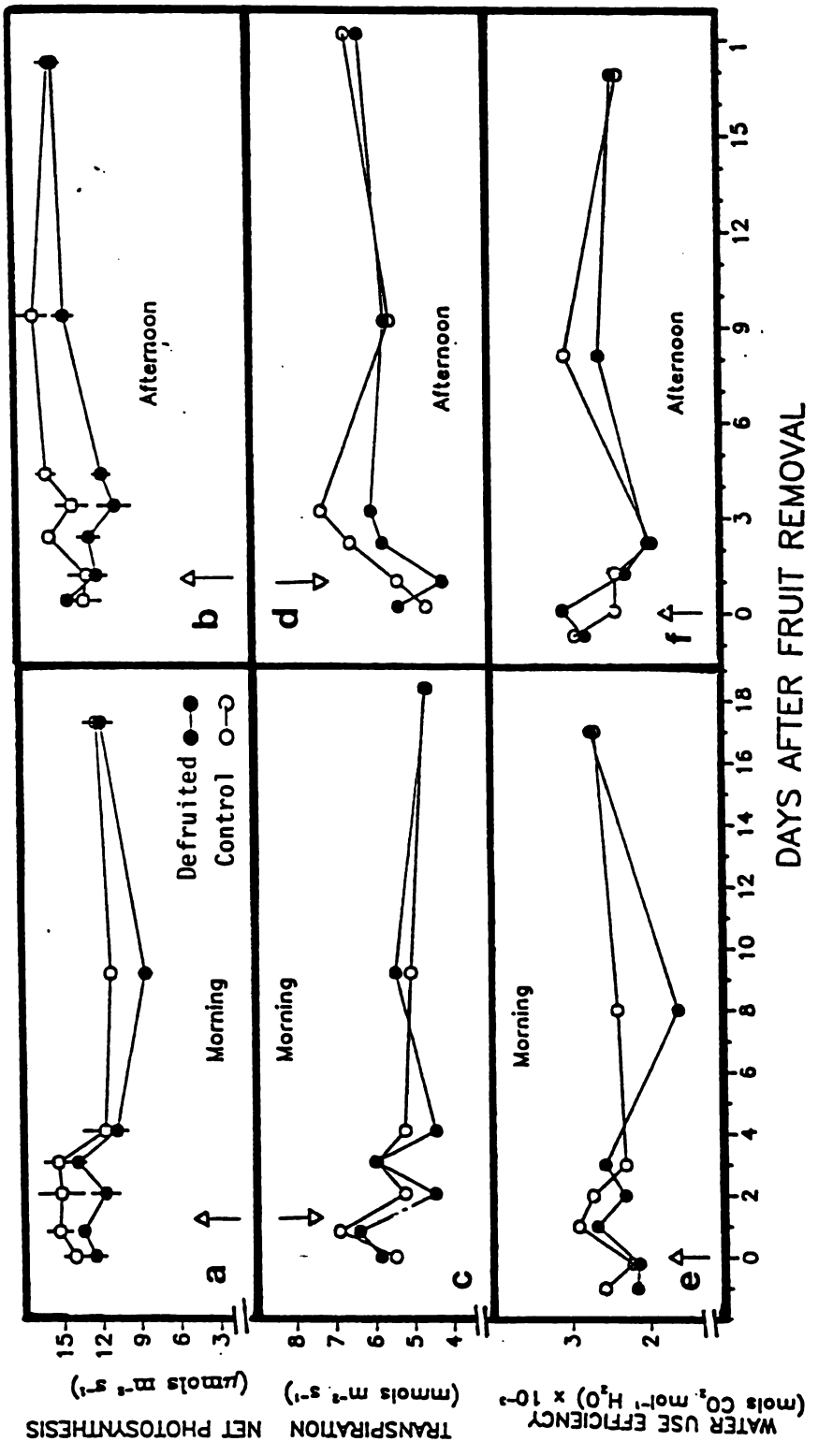

Defruited
Control
Morning
Afternoon
a
b
c
d
e
f
NET PHOTOSYNTHESIS (μmols m⁻² s⁻¹)
TRANSPIRATION (mmols m⁻² s⁻¹)
WATER USE EFFICIENCY (mols CO₂ mol⁻¹ H₂O) x 10⁻³
DAYS AFTER FRUIT REMOVAL

Figure 2. Diurnal changes in net photosynthesis (Pn), transpiration (Tr), and water use efficiency (WUE) of leaves from fruiting and defruited plum trees 1 day after fruit removal. Fruits were harvested during stage II of development (June 15, 1986). Vertical bars ± SE (n=6-8).

Figure 2

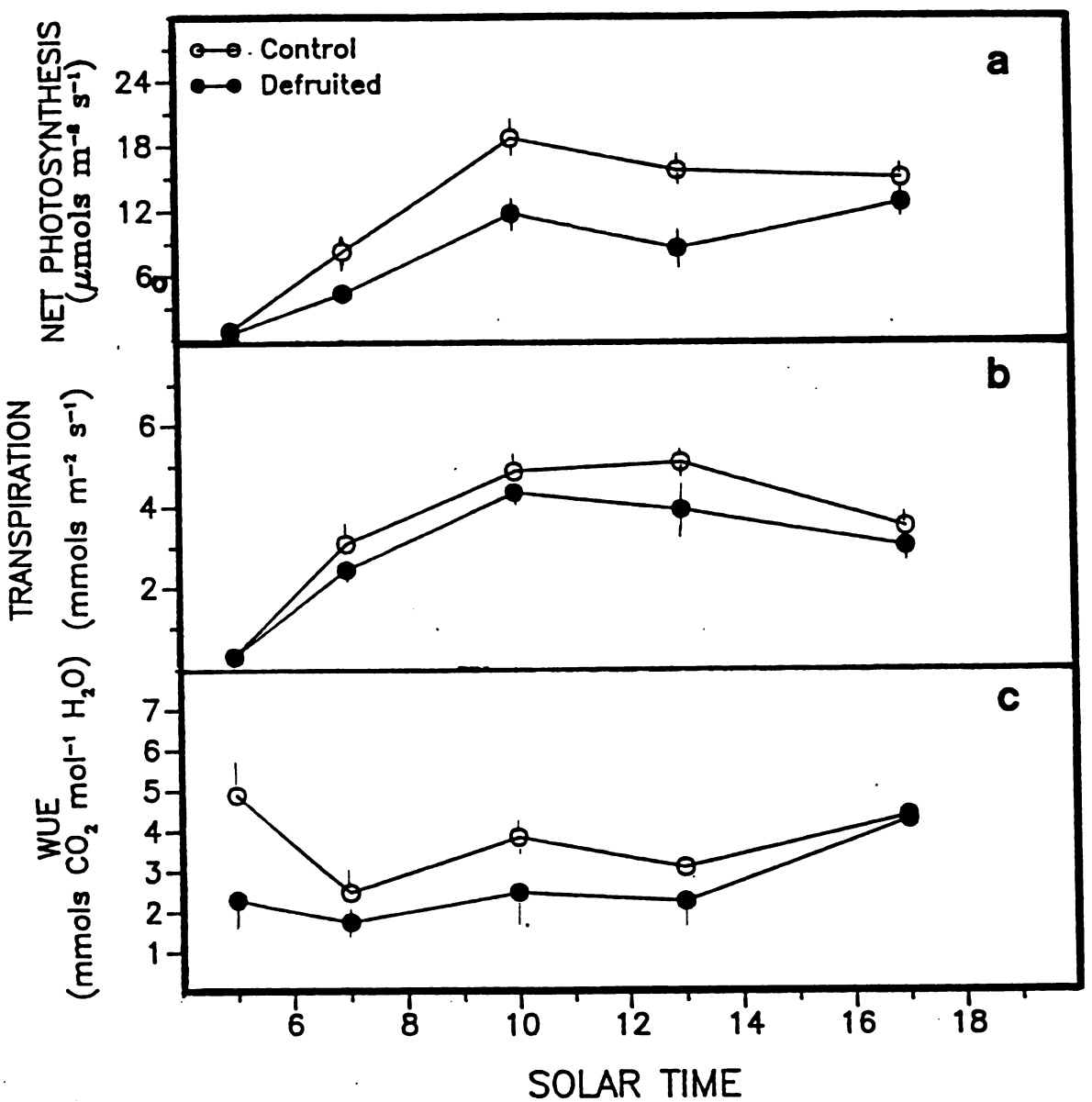

Control
Defruited
a
b
c
NET PHOTOSYNTHESIS (μmols m⁻² s⁻¹)
TRANSPIRATION (mmols m⁻² s⁻¹)
WUE (mmols CO₂ mol⁻¹ H₂O)
24
18
12
6
6
4
2
7
6
5
4
3
2
1
6
8
10
12
14
16
18
SOLAR TIME

Figure 3. Effect of defruiting on leaf water potentials (solid line), osmotic potential (dashed line), and turgor pressure (TP) in plum trees. Arrows indicate date of fruit removal. Vertical bars ± SE (n = 4-9).

Figure 3

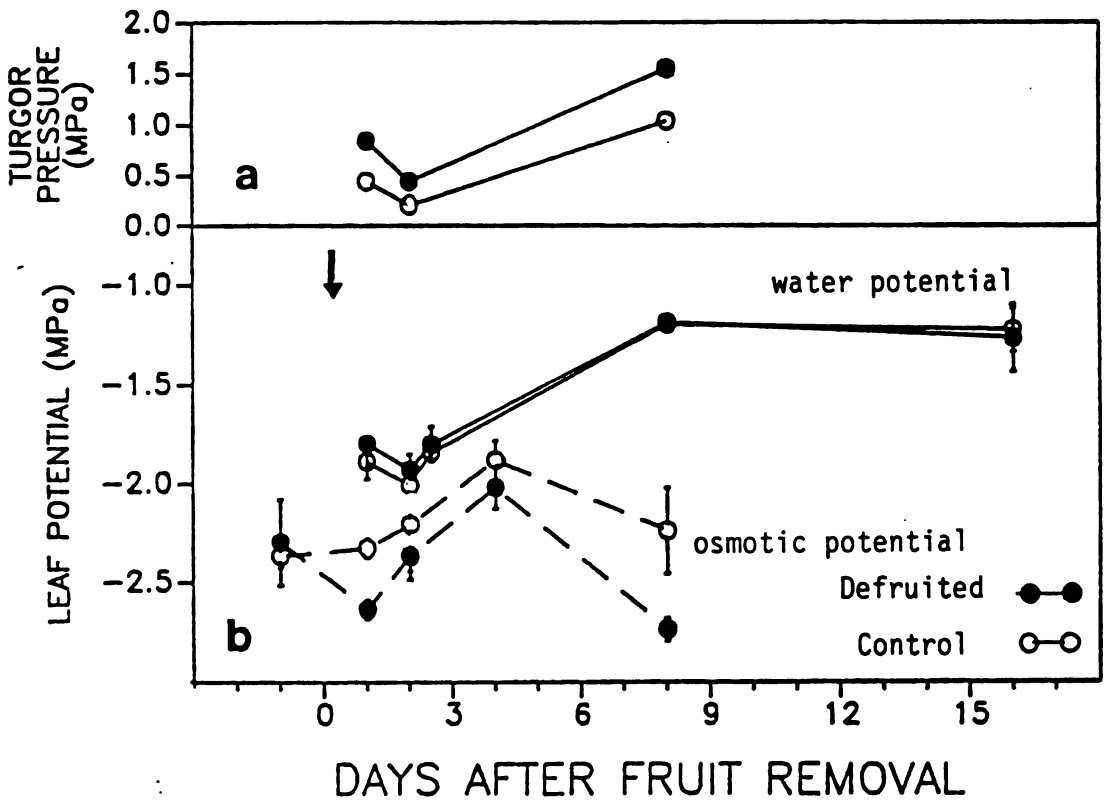

TURGOR PRESSURE (MPa)
2.0
1.5
1.0
0.5
0.0
a
LEAF POTENTIAL (MPa)
-1.0
-1.5
-2.0
-2.5
b
water potential
osmotic potential
Defruited
Control
0
3
6
9
12
15
DAYS AFTER FRUIT REMOVAL

Figure 4. Diurnal changes in leaf water potential (solid line), osmotic potential (dashed line), and turgor pressure of fruiting and defruited plum trees 1 day after fruit removal. Fruits removed on June 17, 1987. Vertical bars ± SE (n = 4-9).

Figure 4

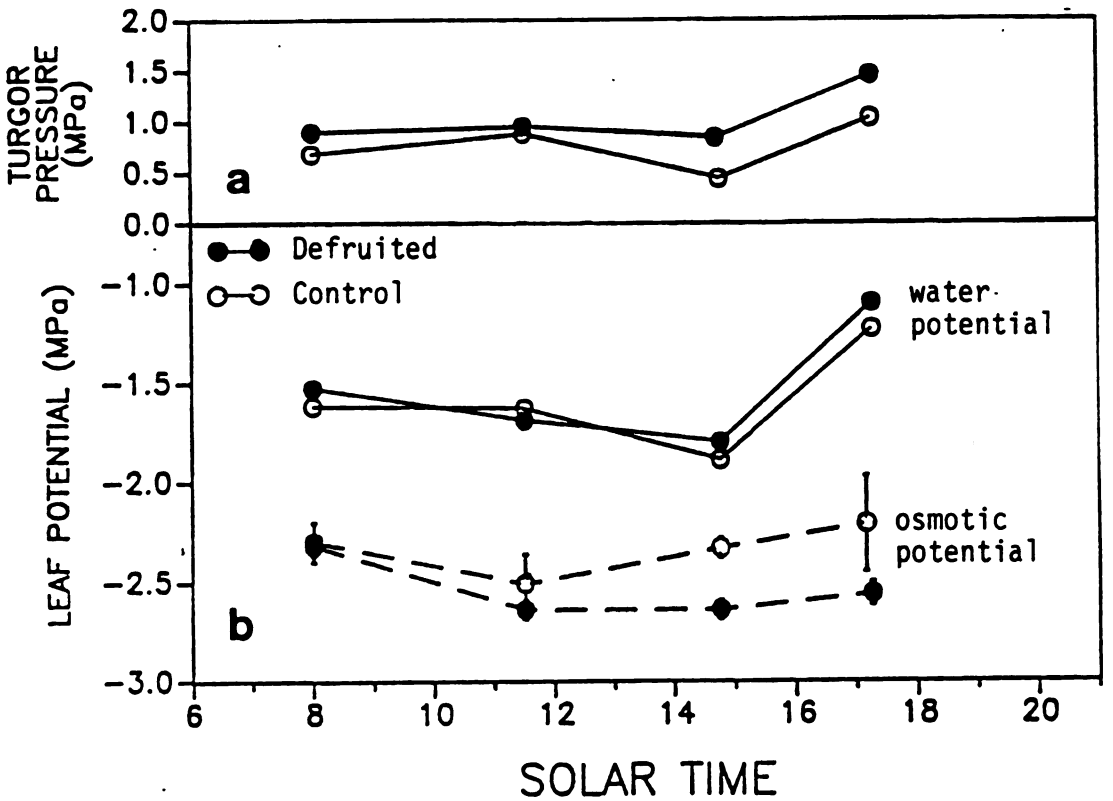
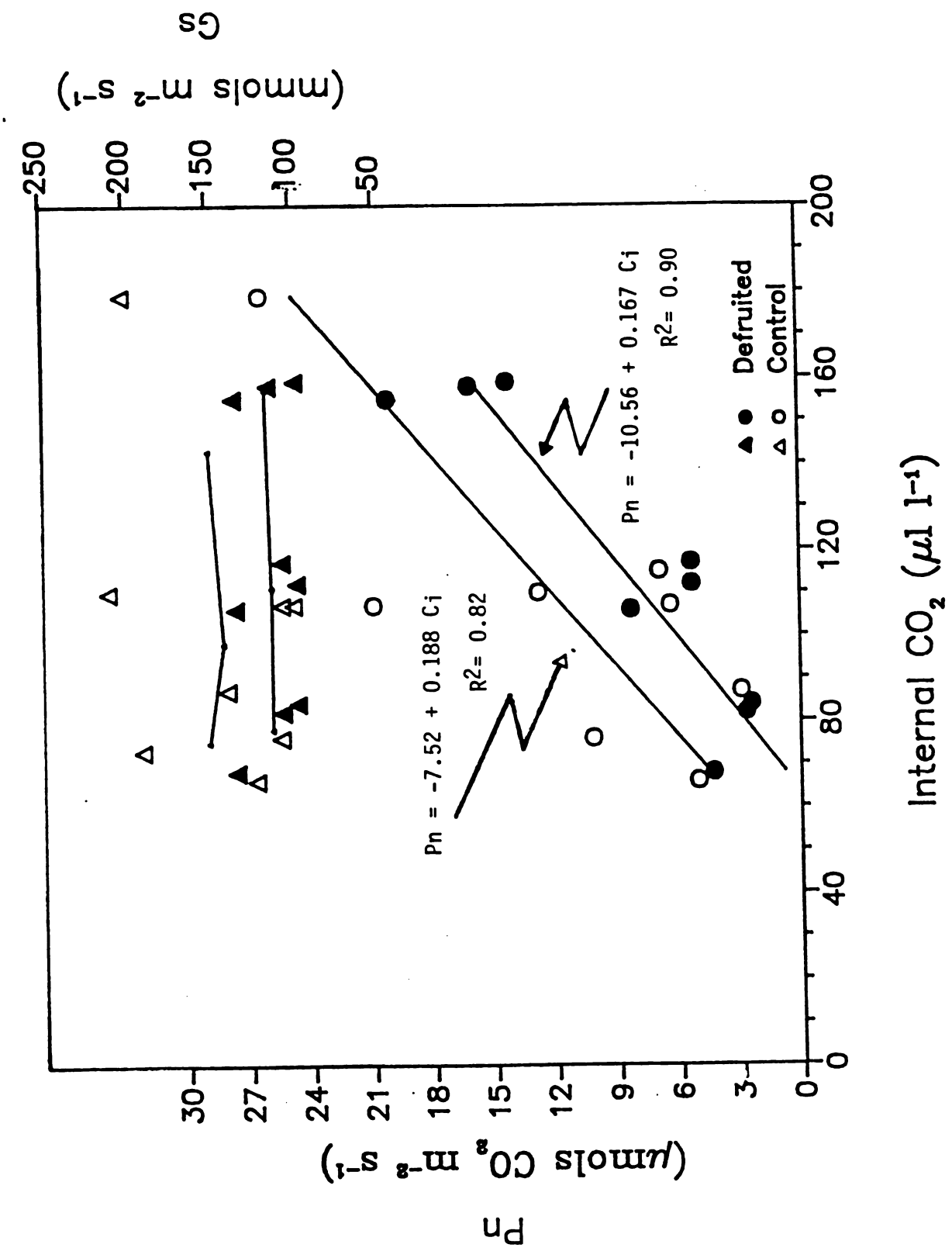

TURGOR PRESSURE (MPa)
2.0
1.5
1.0
0.5
0.0
a
Defruited
Control
LEAF POTENTIAL (MPa)
-1.0
-1.5
-2.0
-2.5
-3.0
water potential
osmotic potential
b
6
8
10
12
14
16
18
20
SOLAR TIME

Figure 5. Changes in net photosynthesis (circles) and stomatal conductance (triangles) in response to calculated levels of intercellular CO_2 (Ci) for leaves of fruiting (open symbols) and defruited (solid symbols) plum trees 1 day after fruit removal in 1987. Measurements made in the field at ambient CO_2 ranging from 100 to 345 $\mu l\ l^{-1}$ and O_2 concentration equal to 0.5%.

Figure 5


Pn
(μmols CO2 m-2 s-1)
Gs
(mmols m-2 s-1)
Internal CO2 (μl l-1)
Pn = -7.52 + 0.188 Ci
R2= 0.82
Pn = -10.56 + 0.167 Ci
R2= 0.90
Defruited
Control

treatments. Stomatal conductance was different between the treatments but did not vary at low O_2 (g_s of defruited trees was 115 at 0.5% O_2 and 120 mmols m^{-2} s^{-1} at 21% O_2; g_s of fruiting trees was 131 at 0.5% O_2 and 159 mmols m^{-2} s^{-1} at 21% O_2). The magnitude of post-harvest inhibition (78%) did not change at low O_2 (Table 1).

Sorbitol was the most abundant soluble sugar in plum leaves, its content ranging from 6 to 12% of dry weight, equal to 50-78% of the total sugar composition of the leaf. Glucose and sucrose were present at about 1-2.5% of dry wt., corresponding to 13-25% of total soluble sugars for sucrose, and to 11-25% for glucose. Sucrose and glucose amounted on average to 15% each, while fructose never exceeded 11% of the total sugar composition. Inositol was only present in traces (less than 0.1%)(Figure 6). Figures 7 and 8 report time-course and diurnal trends of total soluble sugars, translocatable sugars (sorbitol and sucrose), and hexoses (fructose and glucose). Defruiting did not modify the sugar composition of the leaf.

Starch content of leaves increased from 0.35% prior to treatment to 0.62% dry wt 24 h after harvest (measurement at 15:35), corresponding to a 79% increase and continued to increase for about 1 week (Figure 9). Since the levels of starch in the fruiting treatment also increased in this time interval (about 28%) the increase of starch content due to fruit removal amounted to about 51%. Average levels of starch in the morning were respectively 0.31% and 0.64%

Table 1. CO_2 assimilation rates of fruiting and defruited plum trees measured at ambient (21%) or low (0.5% or 2.5%) O_2 concentration in the field or in the laboratory.

O_2 (%)	Field CO_2 Assimilation (μmols m^{-2} s^{-1})		a/b (%)	Laboratory CO_2 Assimilation (μmols m^{-2} s^{-1})		a/b (%)
	Defruited a	Control b		Defruited a	Control b	
21	11.3 + 1.57	15.5 + 2.92	73	7.6 + 0.59	9.3 + 1.10	82
2.5[z]	16.8 + 1.76	23.4 + 2.13	72	16.3 + 1.51	16.8 + 2.08	97
Difference	5.5	7.9		8.7	7.5	
High/ Low O_2 (%)	67	66		46	55	

[z] Field measurements at 0.5% O_2 concentration. Fruits removed on June 16, 1987, measurements made between 12:00 and 15:00, values are means and standard errors of 3 replications. Environmental conditions in the field : PAR > 1400 μmols m^{-2} s^{-1}; ambient CO_2: 320-340 μl l^{-1}; cuvette temperature: 34-38 C; air relative humidity= 48-50%. Stomatal conductances did not vary significantly for both treatments at low O_2. Environmental conditions in the lab: ambient temperature= 23-25 C; cuvette temperature= 27.6-28.6 ^{o}C; PAR= 1000 μmols m^{-2} s^{-1}.

Figure 6. Gas chromatographic analysis of soluble sugars from fully expanded leaves of field-grown plum trees and β-phenyl-D-glucopyranoside (internal standard) using trimethylsilyl derivatives. a. Fructose. b. Sorbitol. c. Glucose. d. Inositol. e. β-phenyl-D-glucopyranoside. f. Sucrose.

Figure 6

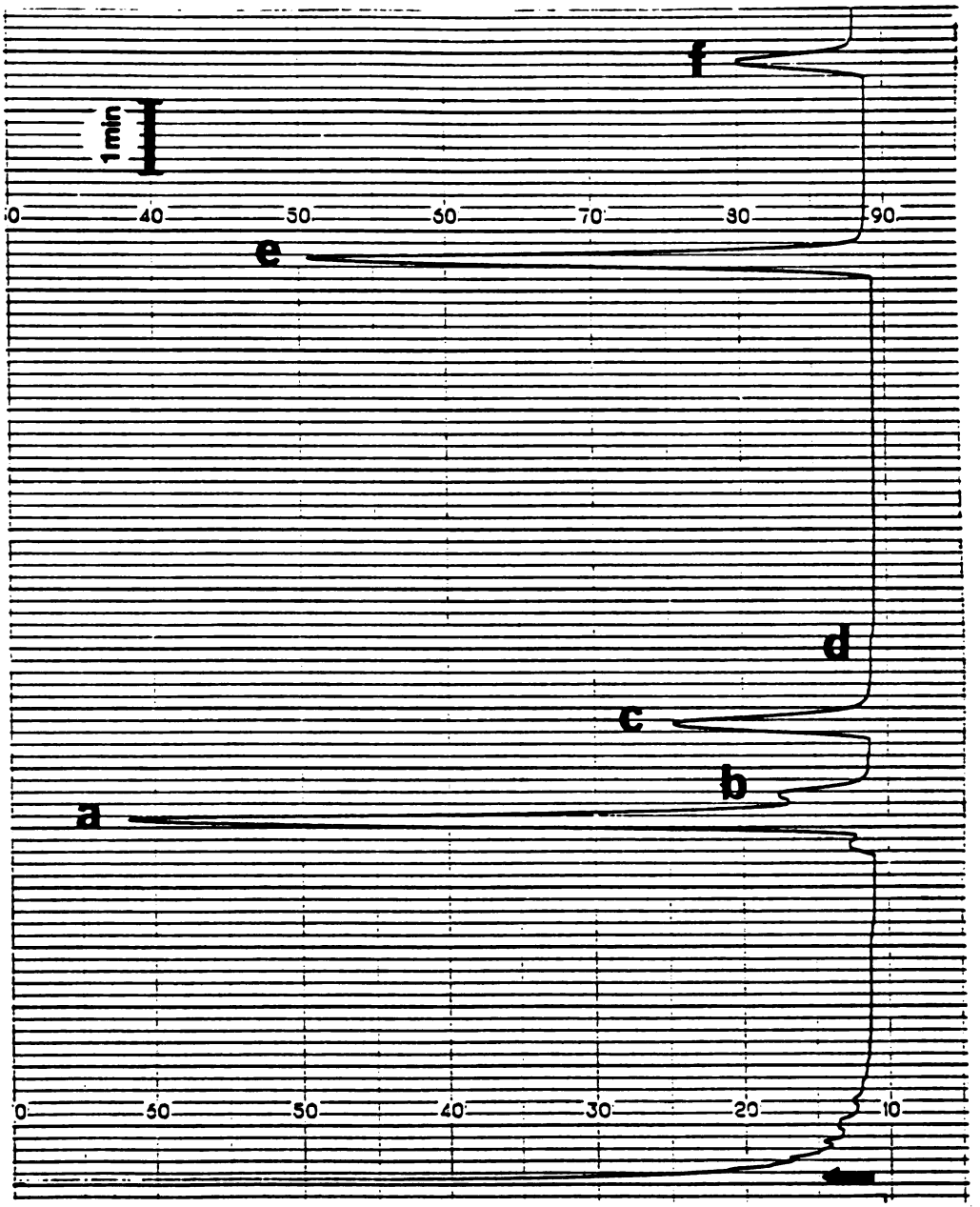


Figure 7. Content of hexoses (fructose, glucose), translocatable (sorbitol, sucrose), and total soluble sugars of leaves from fruiting (open circles) and defruited (solid circles) plum trees during the morning (a) and afternoon (b). Fruits removed on June 16, 1987 (date indicated by arrow). Vertical bars ± SE.

Figure 7

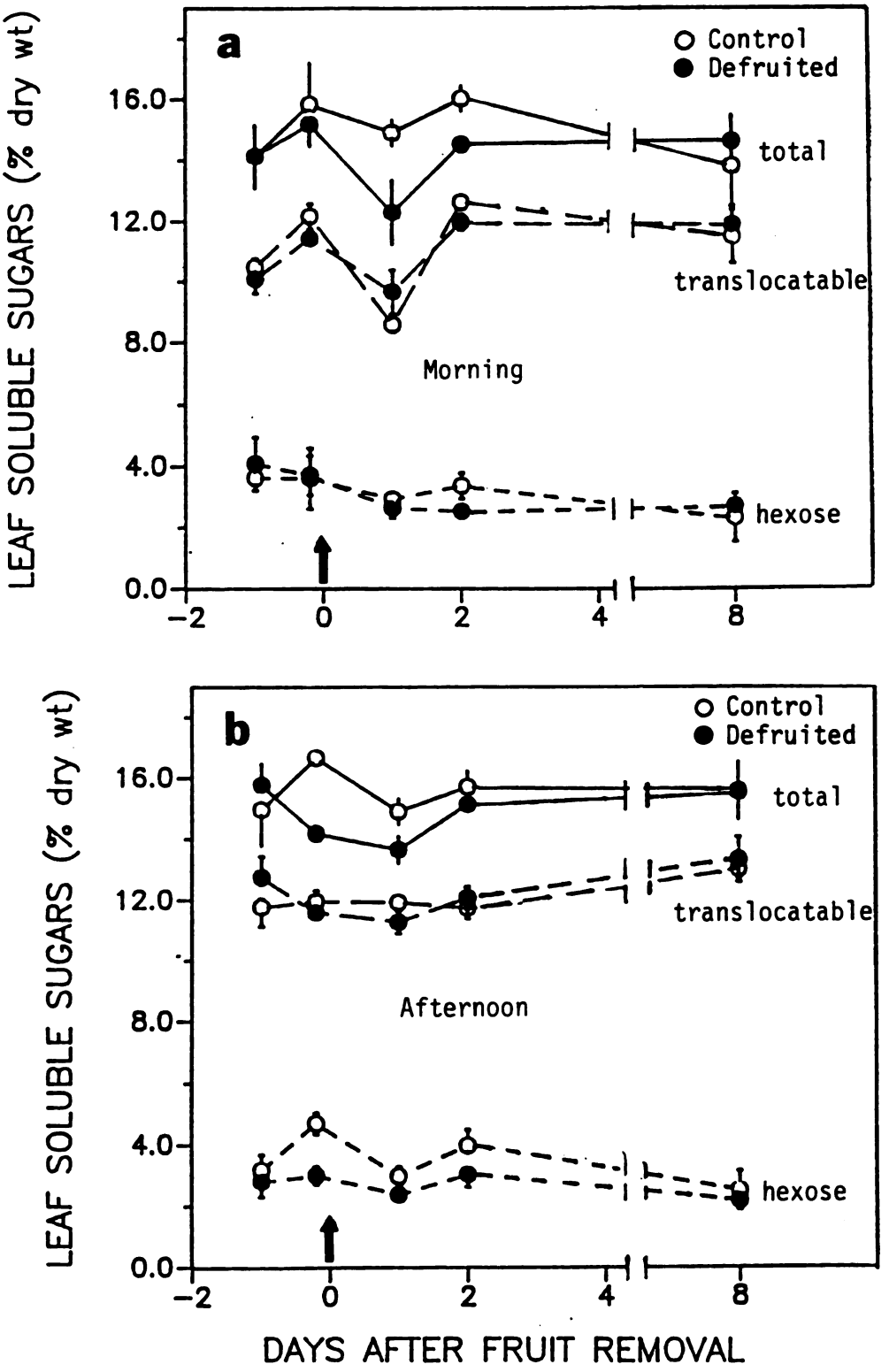

a
Control
Defruited
total
translocatable
Morning
hexose
b
Control
Defruited
total
translocatable
Afternoon
hexose
LEAF SOLUBLE SUGARS (% dry wt)
16.0
12.0
8.0
4.0
0.0
−2
0
2
4
8
DAYS AFTER FRUIT REMOVAL

Figure 8. Diurnal changes in content of soluble sugars in leaves from fruiting (open circles) and defruited (solid circles) plum trees 1 day before (a) and 1 day after (b) fruit removal. Fruits removed on June 16, 1987. Hexose sugars are fructose and glucose, translocatable are sorbitol and sucrose; total sugars also include inositol and raffinose. Vertical bars ± SE.

Figure 8

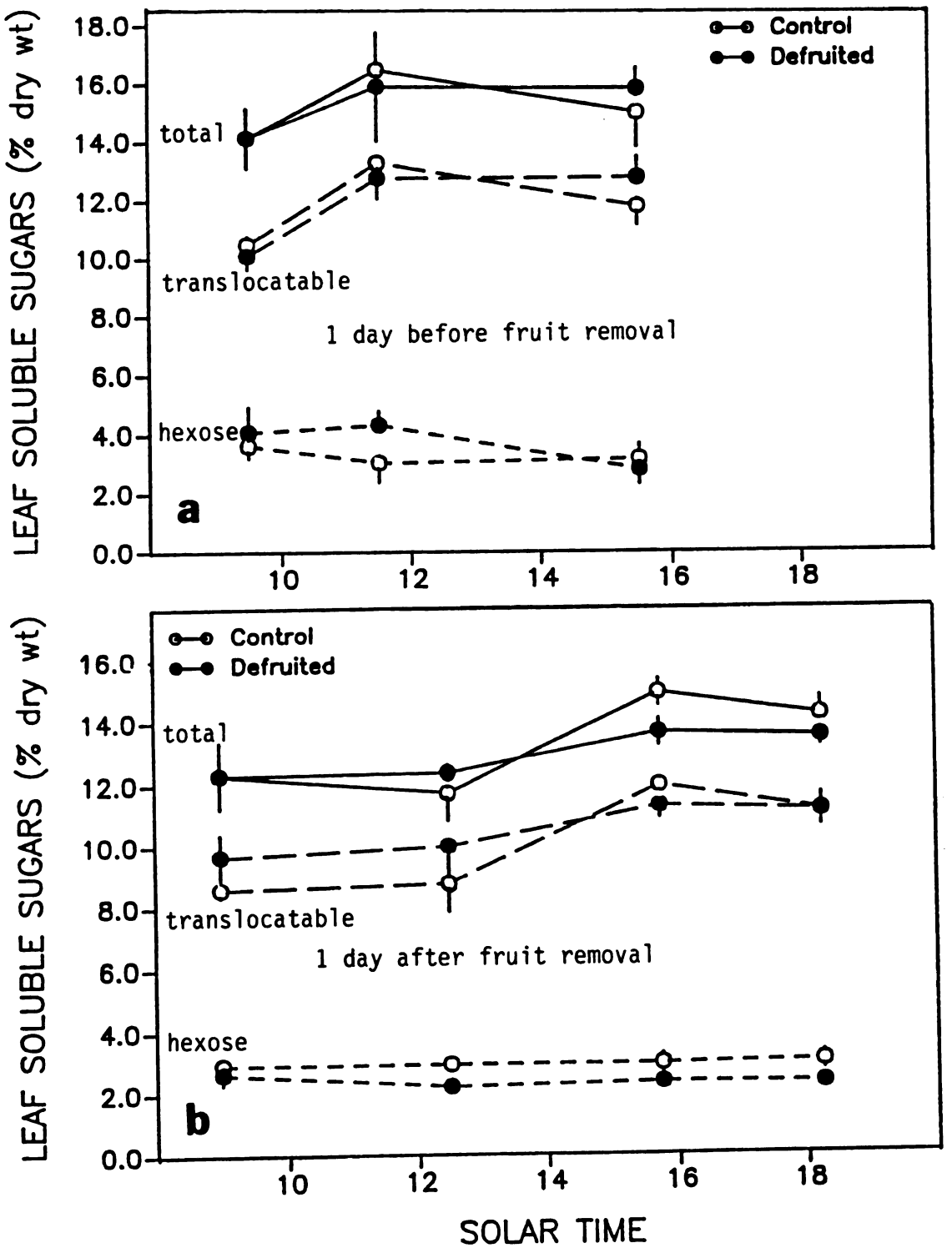

LEAF SOLUBLE SUGARS (% dry wt)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Control
Defruited
total
translocatable
1 day before fruit removal
hexose
a
10
12
14
16
18
LEAF SOLUBLE SUGARS (% dry wt)
Control
Defruited
total
translocatable
1 day after fruit removal
hexose
b
SOLAR TIME

Figure 9 . Changes in leaf starch content of fruiting and defruited plum trees in the morning and afternoon, 1987. Arrows indicate time of fruit removal. Vertical bars ± SE (n= 3-6).

Figure 9

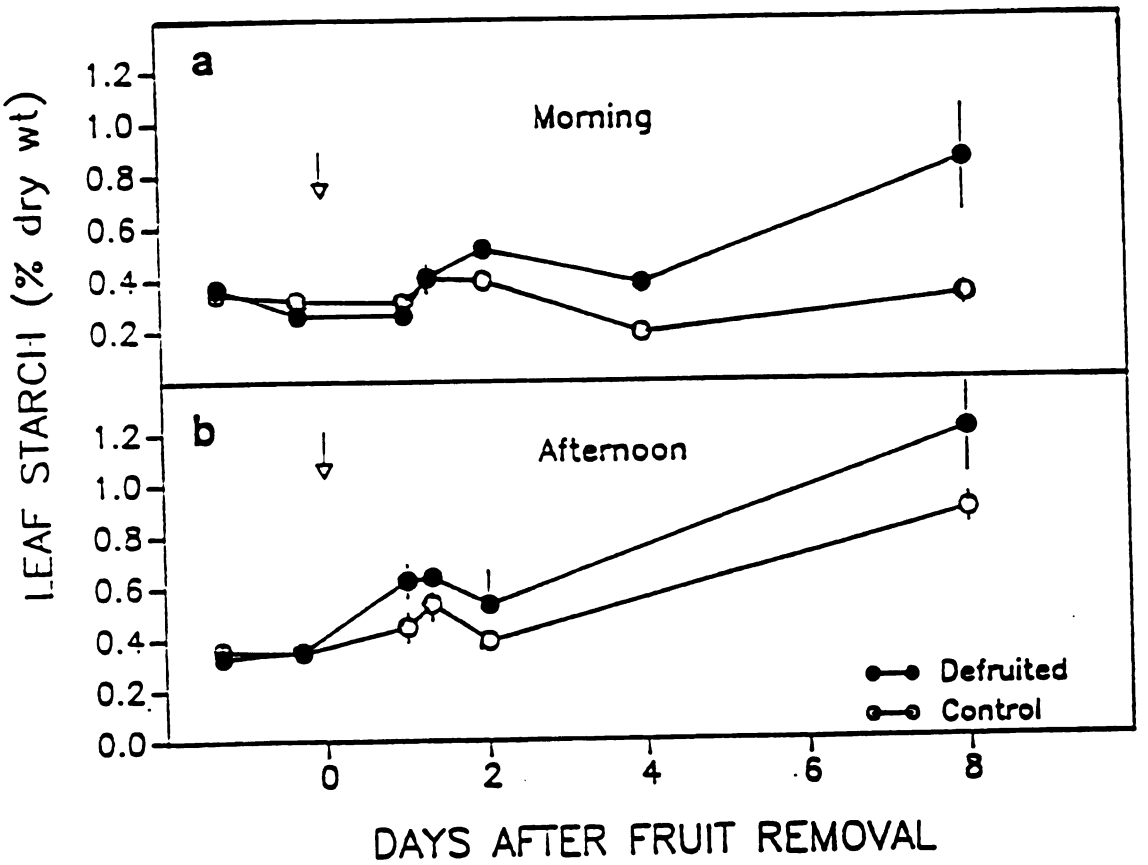

a
Morning
b
Afternoon
LEAF STARCH (% dry wt)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
DAYS AFTER FRUIT REMOVAL
Defruited
Control

Figure 10. Diurnal changes in leaf starch content of fruiting and defruited plum trees 1 day before (a) and 1 day (b) after fruit removal. Fruits removed on June 16, 1987. Vertical bars ± SE.

Figure 10

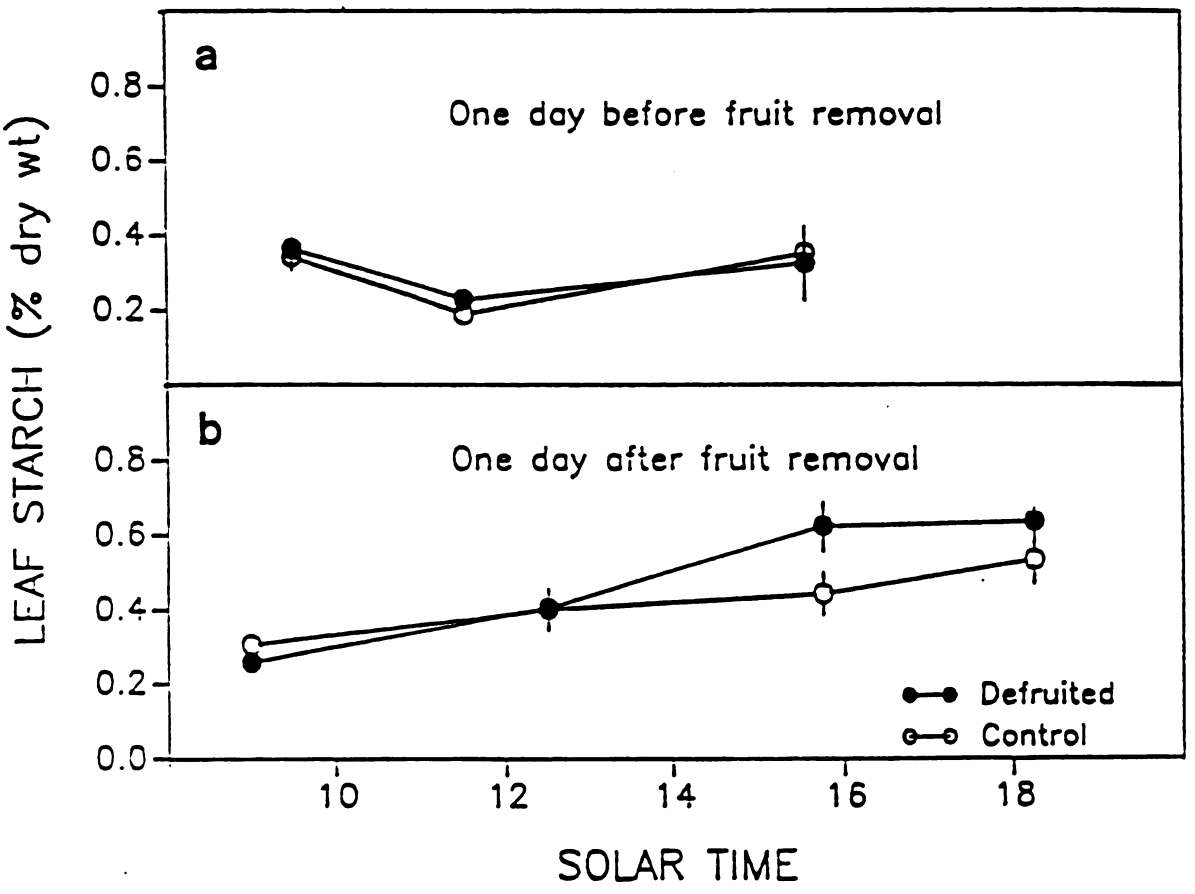
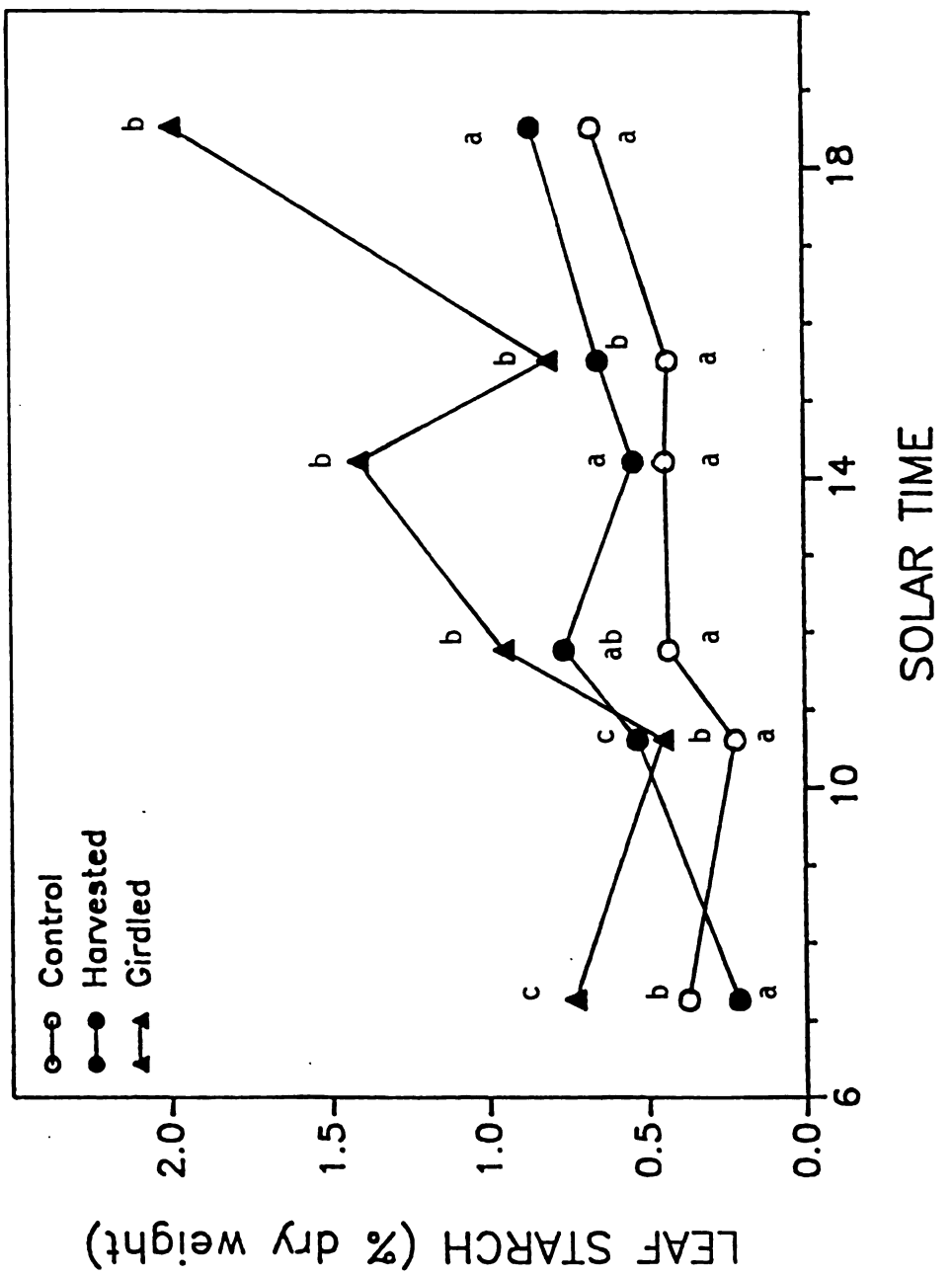

a
One day before fruit removal
b
One day after fruit removal
0.8
0.6
0.4
0.2
0.0
LEAF STARCH (% dry wt)
10
12
14
16
18
SOLAR TIME
Defruited
Control

Figure 11. Diurnal changes in starch content of leaves from fruiting, defruited, and girdled shoots of field-grown plum trees 4 days after fruit removal. Fruits were removed on June 16, 1986. Different letters indicate significant differences between treatments at each single date (P= 0.05).

Figure 11


LEAF STARCH (% dry weight)
2.0
1.5
1.0
0.5
0.0
6
10
14
18
SOLAR TIME
Control
Harvested
Girdled

before and after harvest, in the afternoon 0.34% and 0.78% respectively. The post-harvest increase in starch also showed a clear diurnal trend (Figure 10). The difference in starch levels was particularly evident after 1200 hr. Starch increased from 0.26% to 0.63% in leaves of defruited trees (from 900 to 1815 hr) and from 0.3% to 0.53% in leaves of fruiting shoots (Figure 10). The highest levels of starch were found in leaves of fruiting shoots that had been girdled at the same time the fruit removal treatment had been imposed on other trees (Figure 11). Leaves from fruiting shoots contained only 0.2-0.7% starch, whereas leaves from girdled shoots contained up to 1.6% at 1800 hr) (Figure 11). Similar diurnal trends were observed by measurements on two other dates.

DISCUSSION

Calculated stomatal contribution to the post-harvest decrease of Pn did not exceed 46% indicating that non-stomatal components were largely implicated in Pn inhibition. Among possible non-stomatal limitations, photorespiration was not responsible for the decrease of Pn (Table 1). O_2 inhibition in air amounted to about 34% in both fruiting and defruited trees, a value intermediate between that reported by Sharkey (1985) as indicative of RuBP limitation (40%) or RuBP regeneration limitation (30%). No evidence for involvement of photorespiration in Pn changes consequent to source-sink manipulations was found

for soybean (Peet and Kramer 1980) and pepper (Hall and Milthorpe 1978). Linearity of A/Ci curves and their estimating from measurements made at 0.5% O_2 do not allow to speculate about inhibition of carboxylation parameters. However, limitations of the carboxylation reaction, rather than inhibition within the carbon reduction cycle have been claimed to be responsible for the midday depression of Pn in Arbutus (Loske and Raschke 1987). This phenomenon in many aspects resembles the post-harvest inhibition of Pn in plum, especially with regard to its occurrence and dependence on environmental variables (Section III). Decreased carboxylation efficiency has also been reported for various herbaceous and woody species in response to water stress (Bunce 1977; Bunce 1982 ; Briggs et al. 1986), and has been related to lower translocation rates and diminished pressure flow between sources and sinks that results in accumulation of carbohydrates (Bunce 1982). Sharkey (1984) has also hypothesized that high VPD could decrease water potentials and transpiration rates, which in turn would induce changes in the photosynthetic capacity of leaves. However, in the case of fruit removal in plum, I did not find significant differences in the leaf water status and water potential between fruiting and defruited trees in the two years of study (Figures 3 and 4). Lower transpiration rates of defruited trees appeared essentially regulated by g_s and the higher turgor pressure of defruited trees was probably the result of lower transpiration and osmotic potential.

Decreased or constant WUE after fruit removal also seemed to indicate that defruited trees were not experiencing any particular water stress.

Lower osmotic potential of defruited trees was evident in the afternoon (Figure 4) and most likely the result of increase in nonosmotic volume due to accumulation of starch rather than to any increase of soluble sugar concentration in the leaf. Osmoregulation resulting from accumulation of starch and/or soluble sugars has been correlated with turgor maintenance or reduced export rates out of the leaf (Acevedo et al 1979; Ackerson 1981).

Leaf starch content increased 2-fold within 48 h from fruit removal and was particularly evident in the afternoon (Figures 9 and 10) at a time when Pn inhibition appeared maximum (Section III). Maximum starch accumulation occurred in leaves of girdled fruiting shoots (Figure 11). Starch accumulation following CO_2 enrichment or shoot girdling and fruit removal produces leaf chlorosis and senescence in soybean and citrus as a result of physical damage to the thylakoid membranes (DeLucia et al. 1985; Schaffer et al. 1986). It is interesting to note that girdled shoots of plum also senesced early (Section III); although I did not examine chloroplast structure of mesophyll cells it is likely that chlorosis was caused by both starch accumulation and moisture stress (Little and Loach 1973; Schaffer et al. 1986).

On the contrary, senescence did not occur early in defruited trees despite the increase in starch (Figure 11). Accumulation of non-structural carbohydrates, occurring in response to source/sink alterations in many species, has been ascribed to Pn inhibition based on correlation analysis (Azcon-Bieto 1983; DeLucia et al 1985; Nafziger and Koller 1976; Peet and Kramer 1980; Schaffer et al. 1986; Thorne and Koller 1974). However, despite the fact that starch may reduce leaf assimilation by enlargement of grains and consequent physical damage to the chloroplast (Schaffer et al. 1986), starch accumulation is probably the result of changes occurring outside the chloroplast. Moreover, if and only if changes of Pn rates are direct responses to modifications of sink demand, fine regulation of Pn is unlikely to be provided by starch, since the hypothesized mechanism of action of starch is merely physical and thus not suitable for fine adjustments.

A likely mechanisms of Pn regulation by sink demand is through the supply of Pi. Pn inhibition by utilization of triose phosphates and Pi supply pool has been recently discussed by Sharkey (1985) and Sivak and Walker (1986). According to this hypothesis, in order to maintain a high rate of CO_2 assimilation, starch and sucrose synthesis must also increase, otherwise triose phosphates will build up and Pi availability will decline (Sharkey 1985). Once the Pi pool is low enough to limit photophosphorylation, CO_2 assimilation could be regulated by the rate at which starch

and sucrose synthesis can metabolize triose phosphates and recycle Pi. Therefore, a critical Pi concentration may exist at which both phosphorylation and triose phosphate utilization are sensitive to changes in the pool size of Pi (Sharkey 1985). Pi availability may also be limited in the cytosol or the chloroplast, even when total cellular Pi is abundant, because of its vacuolar compartmentation.

Is the Pi limitation a likely mechanism of Pn inhibition after fruit removal ? Pi limitations have been associated with O_2-insensitive photosynthesis (Azcon-Bieto 1983; Sharkey 1985), but O_2-insensitivity in the field after fruit removal was not observed. However, I measured responses to low O_2 only at temperatures > 25 C, which may have been higher than the threshold of O_2-insensitive photosynthesis (Sage and Sharkey 1987). Experiments in a controlled environment are needed to clarify this point. In turn, limitations of Pi availability have been related to reduced rates of translocation due to low sink demand (Grange 1987; Sivak and Walker 1986), and changes in activities of sucrose-synthesizing enzymes (Claussen et al. 1987; Hendrix and Huber 1986). Since the final step of sorbitol synthesis involves a phosphatase the mechanism of Pi sequestration may occur similarly to that seen for sucrose.

Pn inhibition due to fruit removal at the pit-hardening stage in plum seems to be a process controlled by several components. There are two pieces of evidence in favor of a

major role of nonstomatal limitations: a) the constancy of Ci despite the decrease of Pn and g_s (Section III); b) the partitioning of stomatal and non-stomatal components derived from A/Ci curves measured in the field.

The documented accumulation of starch is compatible with the hypothesis of end-product inhibition (Azcon-Bieto 1983; Neales and Incoll 1968) and Pi limitations (Sharkey 1985; Sivak and Walker 1986). On the other hand, I did not find changes in levels of soluble sugars, which again points towards a lack of Pi availability (Sivak and Walker 1986). However, since soluble sugars are a much more readily available metabolic currency, variability caused by the large plant system used and environmental parameters may have masked any definite trend.

Neither water relations nor photorespiration appeared to be clearly involved in the post-harvest Pn inhibition. The concomitant decrease in g_s seemed related to environmental and endogenous parameters (Section III) as described for other species (Raschke 1975; Tenhunen et al. 1984; Tenhunen et al. 1987). However, since we did not investigate changes of ABA levels, we cannot rule out <u>a priori</u> the possibility that accumulation of ABA in the leaf regulates stomatal movement after fruit removal (Kriedemann et al. 1975; Raschke 1975). A new perspective on the role of ABA on stomatal regulation was recently presented by Downton et al. (1988). By the use of fluorescence and autoradiography methods they have shown that the nonstomatal

inhibition of Pn induced by ABA is an artifact resulting from the techniques used in gas exchange studies and the type of leaf measured (amphistomatous).

The determination of the effect of fruit removal on ABA leaf levels and the necessity of further studies in controlled environments are the next two priorities.

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KEYWORDS: Photosynthesis inhibition, Prunus domestica, water relations, photorespiraton, starch, soluble sugars.

ABBREVIATIONS: Pn, net photosynthesis; g_s, stomatal conductance; Ci, intercellular CO_2; Tr, leaf transpiration; WUE, water use efficiency; VPD, vapor pressure deficit; R, gas constant; T, temperature; J, molar concentration; WP, leaf water potential; OP, leaf osmotic potential; TP, leaf turgor pressure; A/Ci, CO_2 assimilation response curve to intercellular CO_2; Pi, inorganic phosphate; PAR, photosynthetic active radiation; Lg, stomatal limitation; RuBP, Ribulose-1,5-bisphosphate.

SUMMARY AND CONCLUSIONS

The optimization of yield per unit of input has always been a major goal in horticulture. Since plant productivity depends largely on carbon assimilation (carbohydrates may represent 90-95% of plant dry weight), studies on the relationship between sources and sinks of assimilates may improve carbon efficiency (yield per unit of leaf area) of fruit crops.

This study was an attempt to characterize the relationship between leaves and fruits by measuring changes in gas exchange, water relations, and carbohydrate partitioning following fruit removal in sour cherry and plum under field conditions.

Defruiting at the beginning of the summer (June 15 to July 7) reduced Pn rapidly in both species, but the effect persisted longer in sour cherry than in plum, probably because of the different dynamics of development of alternative sinks in the two species. The magnitude of Pn inhibition was similar in both terminal and spur leaves, but greater in the afternoon than in the morning. No apparent change in Pn existed when light and/or temperature were suboptimal for assimilate production or when the leaf/fruit ratio exceeded certain threshold values. The reduction in

Pn caused by fruit removal was also less evident in plum when fruit growth rate was slower (end of stage III vs. stage II). However, environmental conditions at the two different times of fruit removal were also different and may have influenced the response of the tree to defruiting. Experiments in controlled environments are needed to partition precisely the influence of temperature, light, and ambient humidity on the Pn reduction following fruit removal.

Since turgor was maintained in leaves of defruited sour cherry trees, the apparent wilting of the foliage after fruit removal does not seem to be related to water stress. Therefore, normal scheduling of irrigation does not need to be modified after fruit harvest.

Defruiting also decreased stomatal conductance. Nevertheless, constancy of internal CO_2 values, lack of strong correlation between Pn and g_s, and analysis of A/Ci curves, derived both from field and laboratory measurements, indicate that nonstomatal factors contributed more than stomatal ones to the Pn change.

On the contrary, defruiting did not affect photorespiration, chlorophyll content, or concentration of soluble sugars in the leaf. Leaf starch content increased dramatically following fruit removal, but did not cause disruption of chloroplast membranes. Starch accumulation may have been responsible for the decrease of leaf osmotic potential in defruited trees by reducing the nonosmotic

volume of the cell. Based on similarities between the results presented in this study and reported cases of Pn inhibition from the literature, feedback inhibition may have limited Pn rate because of reduced carboxylation efficiency, RuBP regeneration rate, or triose phosphate utilization. Evidence and implications for each of these mechanisms in relation to fruit removal have been extensively discussed in this study. Experiments aimed at determining activity of enzymes responsible for synthesis and degradation of translocatable sugars, and Pi availability in the cytosol following fruit removal are necessary to clarify the molecular mechanisms of Pn decline.

Another question that needs to be addressed is whether the regulation of stomatal behavior after fruit removal is driven mainly by environmental (VPD, T) or endogenous factors (Ci, ABA).

The major limitation of this study is that gas exchange parameters were measured only on 1-year-old shoots, and that water relations and carbohydrate partitioning were analyzed only within the leaf, without considering, for example the rate of carbon export to other tissues and organs. In order to use these results for a model of the whole tree, experiments should be carried out to determine carbon partitioning to stems, trunk, and roots. Rates of translocation will also have to be considered to attempt to identify the organ location at which inhibition occurs first. Moreover, this may also help to determine whether

levels of leaf soluble sugars do not change following fruit removal because they are readily exported out of the leaf or because defruiting has no effect.

Finally, more refined techniques (fluorescence) to measure gas exchange may allow to determine whether the electron transport pathway of photosynthesis is also inhibited after fruit removal.

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APPENDIX

Figure 1. Gas chromatographic analysis of soluble sugars from fully expanded leaves of field-grown sour cherry trees and β-phenyl-D-glucopyranoside (internal standard) using trimethylsilyl derivatives. a. Fructose. b. Sorbitol. c. Glucose. d. Inositol. e. β-phenyl-D-glucopyranoside. f. Sucrose. Bar indicates retention time. (Section II)

Figure 1

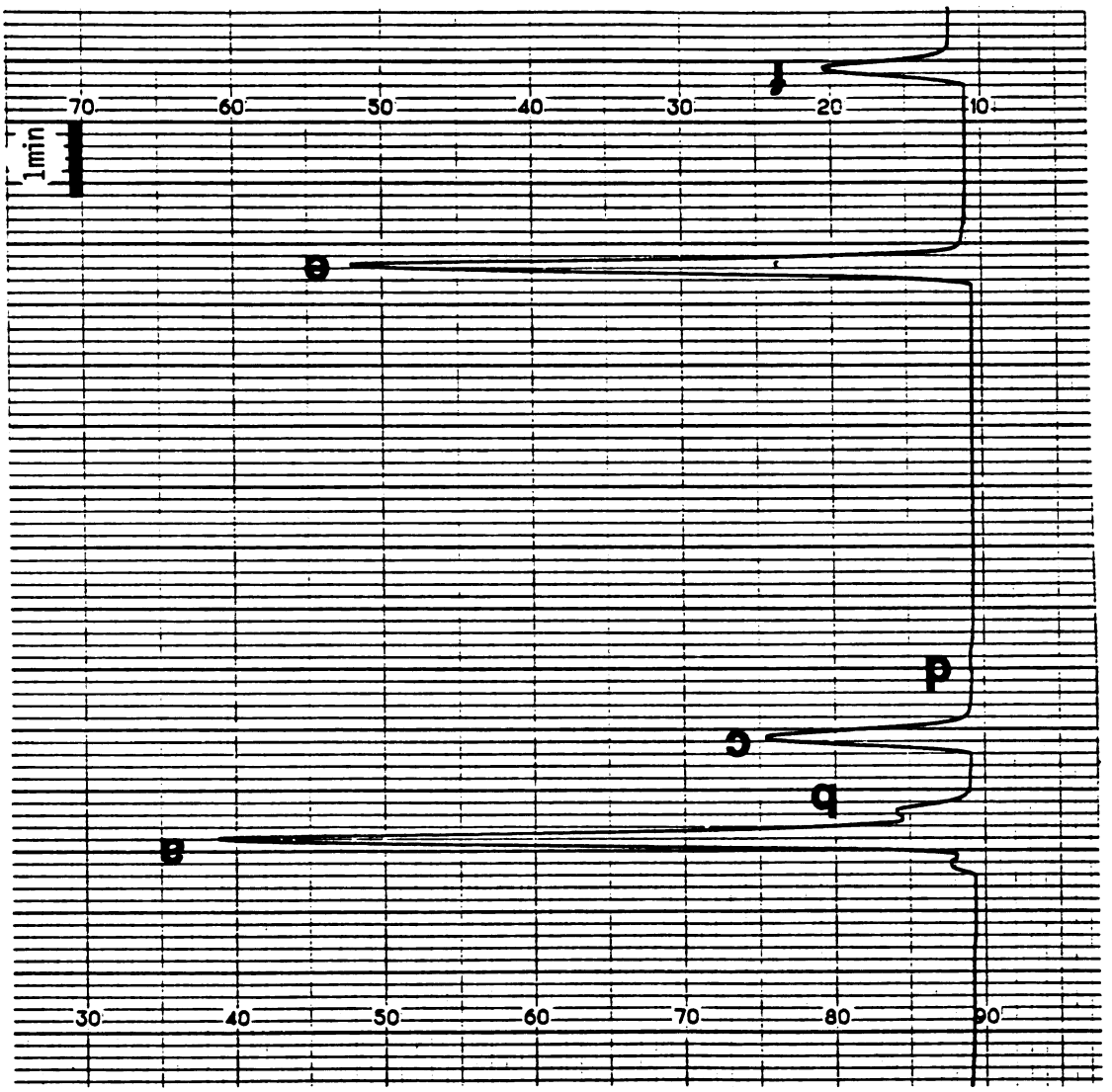


Table 1. Changes in chlorophyll content of leaves from fruiting and defruited plum trees at HRC, East Lansing. Fruits removed on June 16, 1987.

Date	Solar time	DAFR[z]	Defruited			Control		
			chl [a/b]	µg.chl/ mg leaf fr. wt.	mg chl./ dm^2 LA	chl [a/b]	µg chl/ mg leaf fr. wt.	mg chl/ dm^2 LA
June 15	1430	-1	4.1	2.28	4.6	3.6	2.20	5.3
June 17	1545	1	3.5	2.25	5.4	3.5	2.01	5.3
June 26	1400	8	3.7	2.72	6.5	3.6	2.75	6.6
July 2	1400	16	3.9	2.22*	5.2	3.9	2.55*	5.9

* Values significantly different at 5% level (LSD= 0.32); each value is a mean of 3 replications. Statistical analysis not performed for ratios of chlorophyll [a/b].

[z] DAFR = Days after fruit removal; LA = Leaf area; fr. wt. = Fresh weight; chl. = Chlorophyll.

Table 2. Diurnal changes of chlorophyll content in leaves from fruiting and defruited plum trees 1 day after fruit removal. Fruits removed on June 16, 1987.

Solar time	Defruited[z]			Control		
	chl. [a/b]	µg.chl/ mg.leaf fr.wt.	mg.chl/ dm^2 LA	chl. [a/b]	µg.chl./ mg.leaf fr.wt.	mg chl/ dm^2 LA
900	3.0	2.12	5.8	3.4	2.13	6.1
1545	3.5	2.25	5.4	3.5	2.01	5.3
1815	3.7	2.19	5.8	3.6	2.06	5.4

[z] Values are means of 3 replications; no differences are statistically significant. Symbols are same as in Table 1 of Appendix.

Figure 2. Diurnal changes of Pn and g_s of leaves from fruiting and defruited shoots of field-grown plum trees 1 day after fruit removal. Branches defruited on June 16, 1987. Vertical bars ± SE. (Section III)

Figure 2

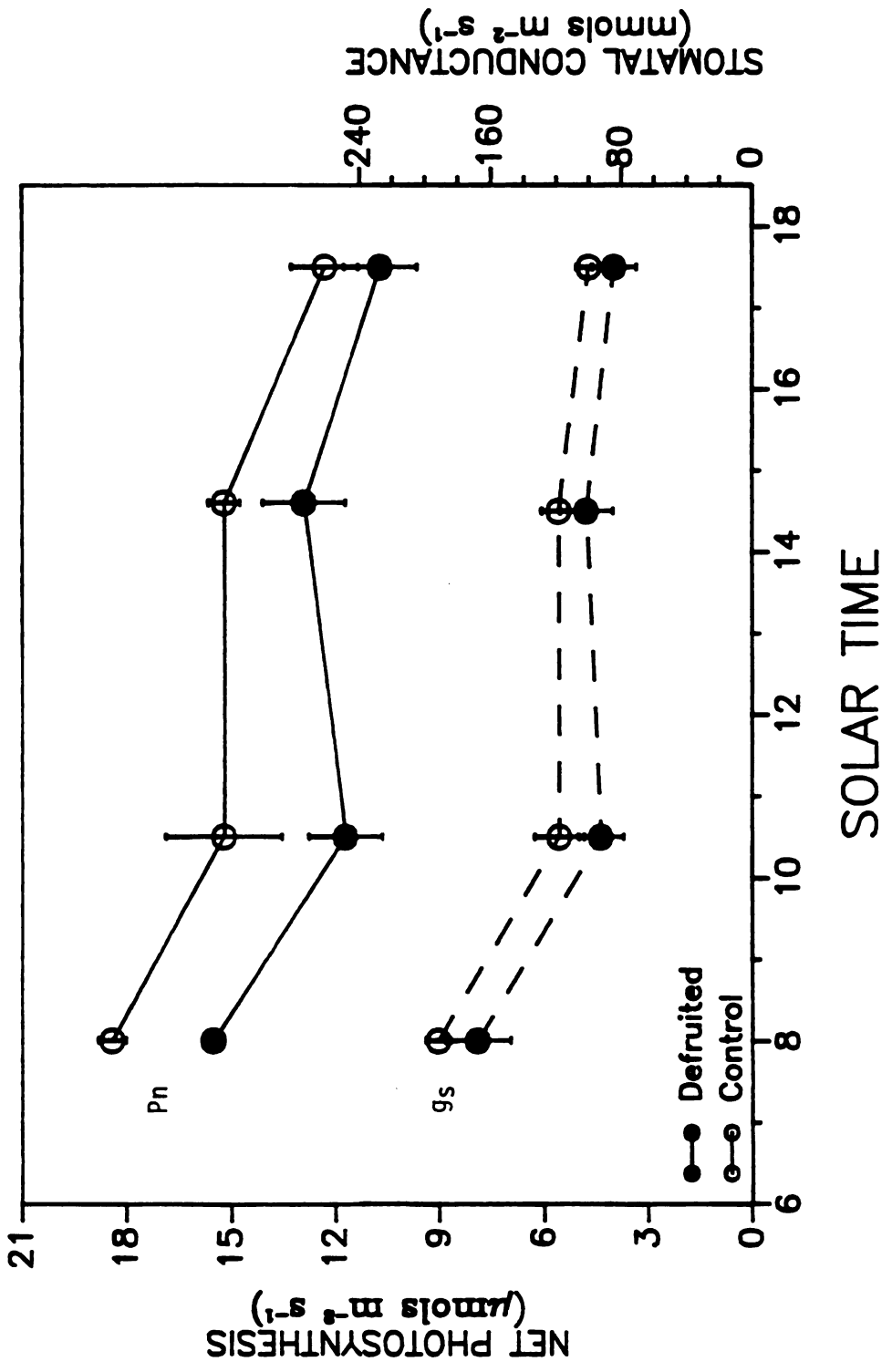

NET PHOTOSYNTHESIS
(μmols m⁻² s⁻¹)
STOMATAL CONDUCTANCE
(mmols m⁻² s⁻¹)
SOLAR TIME
Pn
gs
Defruited
Control
0
3
6
9
12
15
18
21
0
80
160
240
6
8
10
12
14
16
18

Figure 3. Sectors of the canopy defruited and measured for photosynthesis during Experiment 1 of partial defruiting of plum trees in 1986 (schematic). (Section III)

Figure 3

Experiment 1

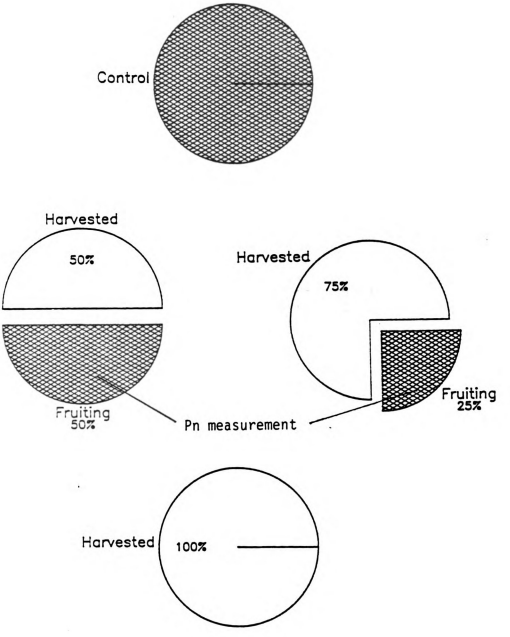


Figure 4. Sectors of the canopy defruited and measured for photosynthesis during Experiment 2 of partial defruiting of plum trees in 1986 and 1987 (values shown refer to percentages in 1986; schematic). (Section III).

Figure 4

Experiment 2

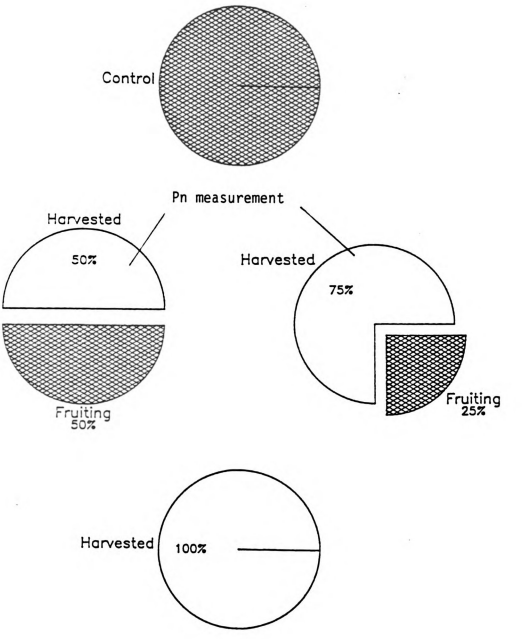


Figure 5. Changes in Pn, Tr, and WUE following removal of plum fruits during stage II in the morning and afternoon in 1987 expressed as percentage of pre-harvest values. Arrows indicate date of fruit removal. (Section IV)

Figure 5

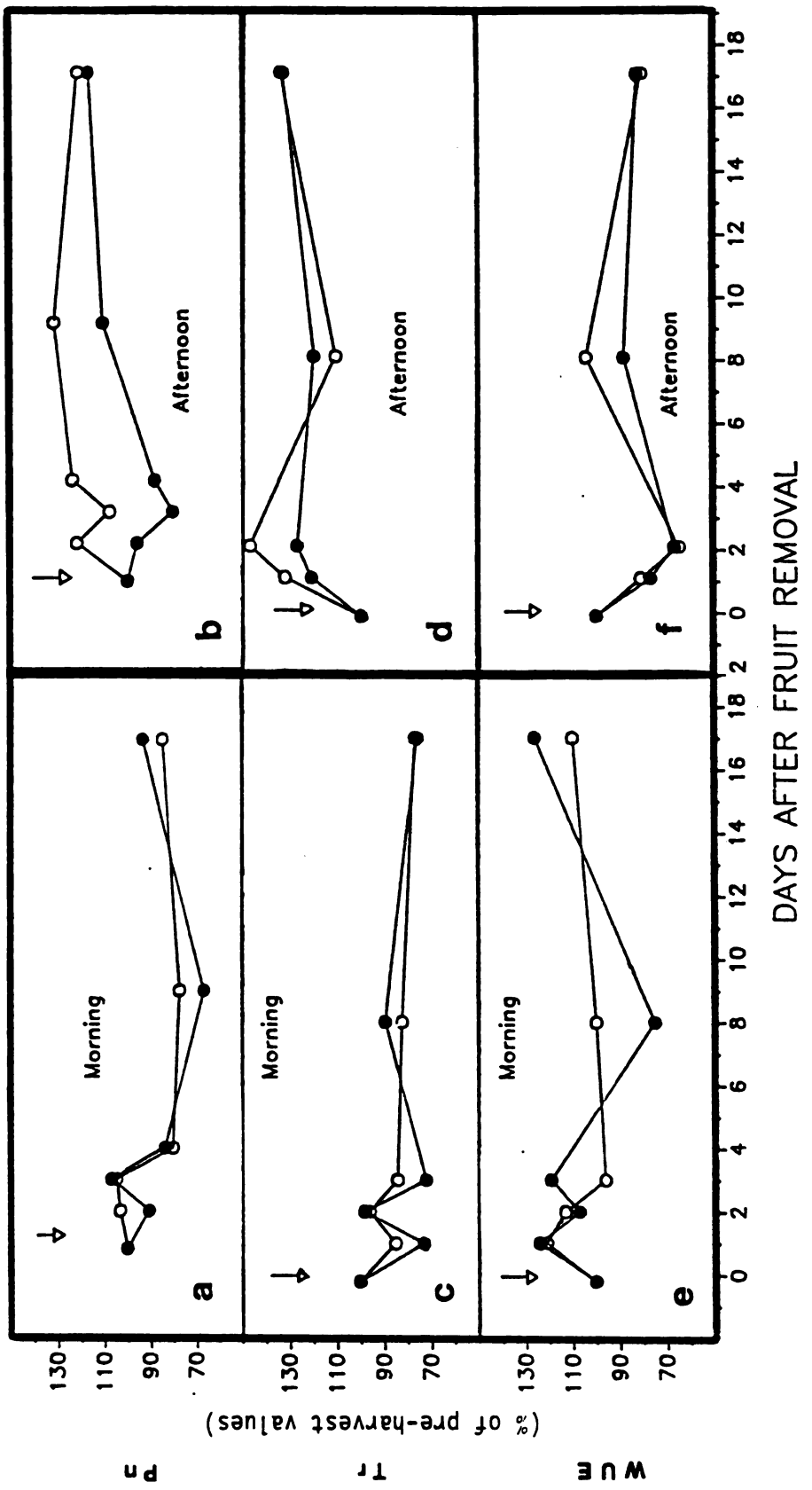

Pn
Tr
WUE
(% of pre-harvest values)
Morning
Afternoon
a
b
c
d
e
f
130
110
90
70
0 2 4 6 8 10 12 14 16 18 2
DAYS AFTER FRUIT REMOVAL

Figure 6. Diurnal changes in Tr, and WUE of leaves from fruiting and defruited plum trees 4 days after fruit removal. Fruits removed on June 15, 1986. (Section IV)

Figure 6

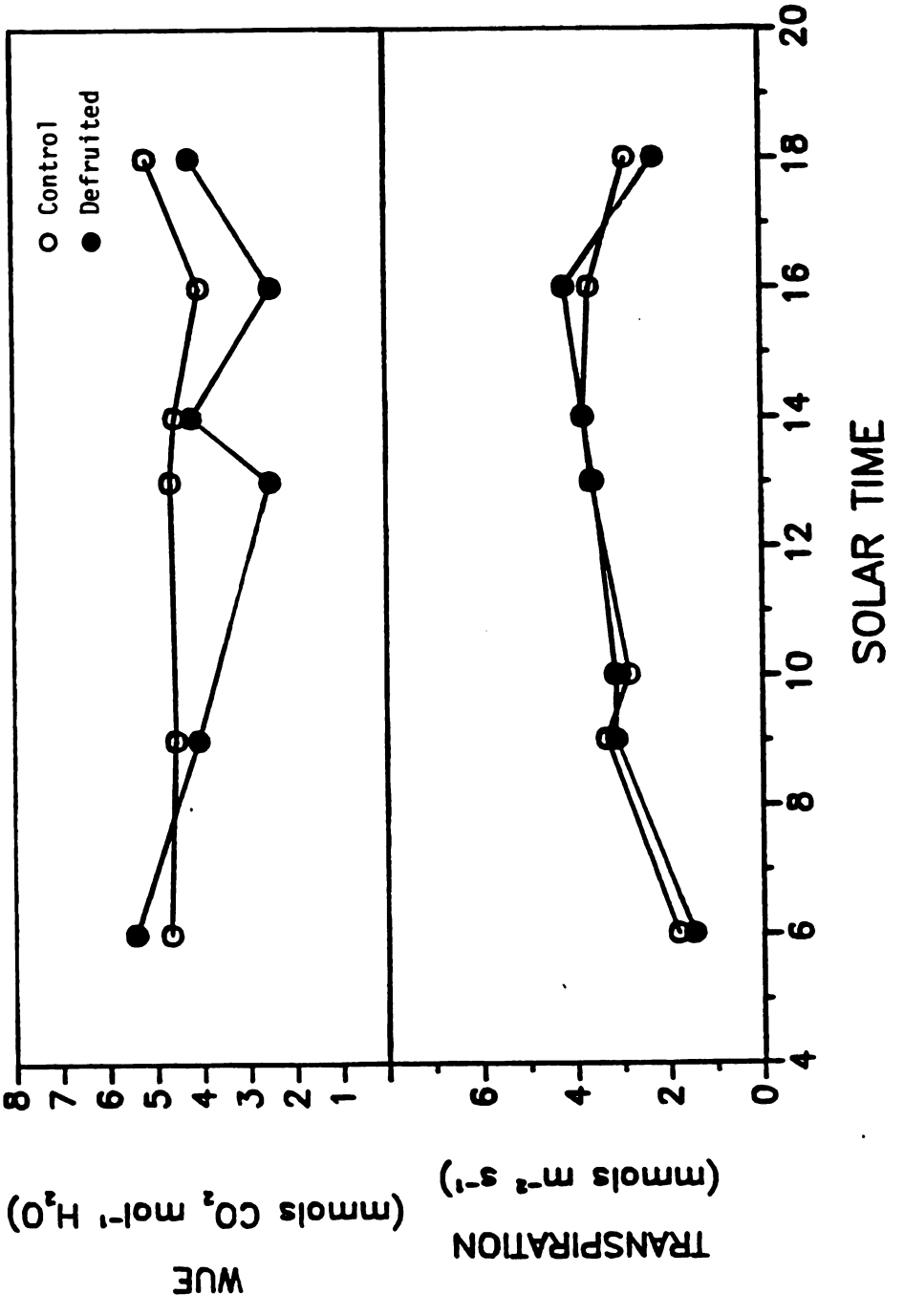
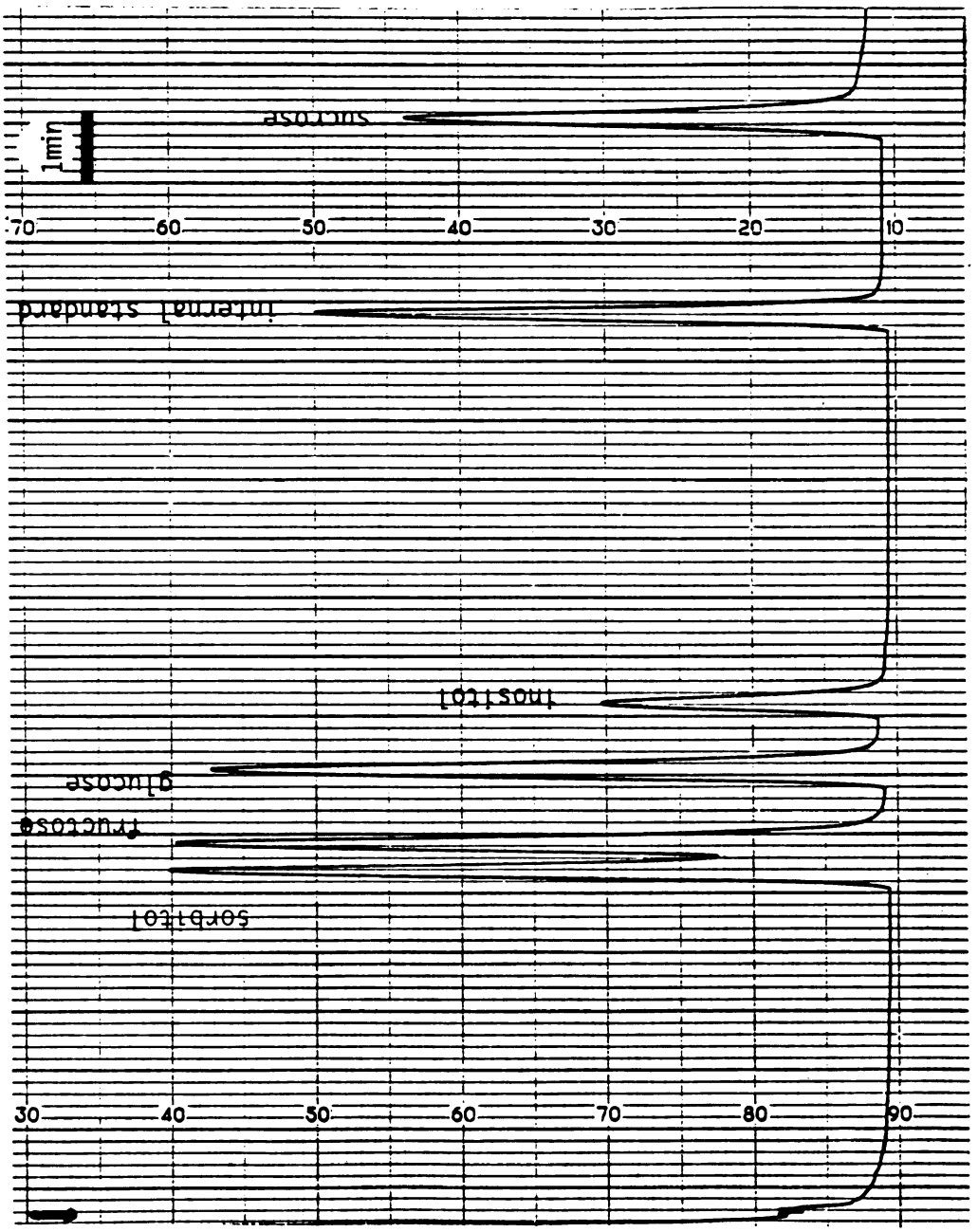

O Control
● Defruited
WUE
(mmols CO2 mol-1 H2O)
TRANSPIRATION
(mmols m-2 s-1)
SOLAR TIME

Figure 7. Gas chromatographic analysis of soluble sugars of standard solutions prepared as described in Section IV (Materials and Methods). Bar indicates retention time.

Figure 7


1min
sucrose
internal standard
inositol
glucose
fructose
sorbitol
70
60
50
40
30
20
10
30
40
50
60
70
80
90