KEEPING QUALITY AND ABSCISSION OF ILEX VERTICILLATA (L.) GRAY (AQUIFOLIACEAE) FRUIT

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
HUGH CHRISTOPHER BOYLAN
1975

This is to certify that the

thesis entitled

Keeping quality and abscission of Ilex verticillata (L.) Gray (aquifoliaceae) fruit

presented by

Hugh Christopher Boylan

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in **Horticulture**

Major professor

Date May 12, 1975

O-7639

ABSTRACT

KEEPING QUALITY AND ABSCISSION OF *ILEX VERTICILLATA* (L.) GRAY (AQUIFOLIACEAE) FRUIT

By

Hugh Christopher Boylan

SECTION I. KEEPING QUALITY, ABSCISSION AND LEVELS OF O_2, CO_2 AND ETHYLENE DURING STORAGE OF FRUITING BRANCHES OF *ILEX VERTICILLATA* (L.) GRAY

Fruiting branches of *Ilex verticillata* (L.) Gray were stored at 9 environment combinations of low, intermediate and high relative humidities (RH) in 0°; 8.8° or 10° and 20°C chambers for a period of 8 weeks in 1973 and repeated in 1974. In 1973, water loss as indexed by weight loss of fruit, twigs and leaves was minimized with 0°C/99% RH. Leaves present on these twigs at 20°C and high humidities turned brown and were shed within 3 weeks. Fruit stored in high RH at 0° and 8.8°C remained in good condition for the entire experiment. The moisture content of fruit, in both 1973 and 1974, was only marginally reduced when stored in high RH at 0°C whereas fruit quality deteriorated before week 2 at all other combinations of temperature and low or intermediate RH. By week 5 fruit stored at 20°C/94% RH had shrivelled. It was observed that red fruit when dried, in an oven for moisture determinations, failed to turn brown if the moisture was 20% or less.

Abscission, indexed by reduction in fruit removal force (FRF), was correlated with vapor pressure deficits (VPD) during storage.

Usually the fruit separated from the pedicel (distal type) with 60% frequency, pedicel separation from the stem (proximal type) was 30% and breakage at any point along the pedicel (intermedial type) was 10%. Frequencies of the distal type were least and that of the proximal type were largest at 20°C and high humidities. Ethylene concentration (due to fungal contamination) in chambers at high humidity and 20°C reached 315 ppb at the end of week 4. Reduced values of FRF were recorded for fruit in these chambers. Carbon dioxide concentrations remained below 0.5% in all chambers and the minimum levels of oxygen were 20%. A study on ethylene production showed that green, red-green and red fruit produced peaks of 5.8, 4.6 and 1.1 µl/kg/hr at 5½, 6½ and 9½ days after harvest respectively.

SECTION II. THE EFFECT OF ANTITRANSPIRANTS ON KEEPING QUALITY OF FRUITING BRANCHES OF ILEX VERTICILLATA (L.) GRAY

Studies on the effect of wax and plastic antitranspirant materials at concentrations of 2.5 and 5% were conducted on fruiting branches of Ilex verticillata (L.) Gray. Vapor Gard treatments reduced loss in fresh weight, water uptake and transpiration. Water uptake for 5% Vapor Gard treatments was less than those at 2.5%. Twigs treated with Primafresh 23 at both concentrations, showed an increase in water uptake. Fruit treated with Vapor Gard remained in excellent condition until day 23 when shrivelling occurred, all other treatments shrivelled by day 17. Respiration rates of fruit treated with Vapor Gard and Concord Alcoat SE-12 did not differ from that of the control.

Cross sectional views of the epicarp (exocarp) under plane polarised light identified the cuticle as being isotropic. Scanning electron micrographs of Vapor Gard treatments confirmed its presence on the surface of I. verticillata fruit. Surfaces of young I. opaca fruit were heavily coated with a smooth wax which was covered with another wax in the form of spheres. These spheres were absent from old fruit of I. opaca and the continuity of the underlying surface wax was broken.

SECTION III. PHYSIOLOGY OF FRUIT ABSCISSION IN ILEX VERTICILLATA (L.) GRAY

Field and laboratory experiments were conducted to determine the fruit abscission process of Ilex verticillata (L.) Gray. Fruit removal force (FRF) of fruit from branches treated with naphthaleneacetic acid (NAA), cycloheximide (CH), gibberellic acid A3 (GA), sodium azide and (2-chloroethyl) phosphonic acid (ethephon) and non treated branches were similar. Fruit and leaf abscission occurred on branches treated with ethephon and CH. Histological and histochemical studies revealed the presence of sclereids along the entire length of the pedicel with no structural or staining differences indicative of abscission layer formation.

FRF increased from July to September, then decreased to July values. Approximately 50% of fruit with pedicels attached abscised just before leaf fall.

Proximal applications of CH, NAA, sodium azide, calcium chloride, sucrose, 8 hydroxyquinoline sulfate (8 HQS) and alar; and distal applications of CH, NAA, sodium azide, calcium chloride, kinetins and benzyladenine caused loss in fresh weight, for detached fruiting branches,

which was similar to or greater than the control. Loss in fresh weight was increased when polyvinylpyrrolidone (a phenol inhibitor) was included with proximal treatments. And antitranspirant applications reduced loss in fresh weight.

SECTION IV. THE EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND WIND ON FRUIT ABSCISSION OF ILEX VERTICILLATA (L.) GRAY

Killing temperatures for fruit and pedicels of Ilex verticillata (L.) Gray were between -10°C and -11°C and -12°C and -13°C respectively. Fruit removal force (FRF) of fruiting branches exposed to -18°C were less than those exposed to -9°C. FRF for branches subsequently stored at 20°C was lower than 0°C. FRF was significant for the interaction between storage temperature and high and low relative humidities (RH) after 16 days. Fruiting branches when subjected to a maximum wind force of 24.4 meters per second retained 100% of their fruit. It was considered that Ilex verticillata fruit and pedicels are killed by low temperature; abscise when temperatures fluctuate above 0°C and heavy winter storms occur.

KEEPING QUALITY AND ABSCISSION OF *ILEX VERTICILLATA* (L.) GRAY (AQUIFOLIACEAE) FRUIT

By

Hugh Christopher Boylan

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1975

Dedicated to my daughter Michelle

ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Dr. Harold Davidson for valuable suggestions and direction during the course of my graduate program. Gratitude is extended to Drs. M.J. Bukovac, R. Herner, I.W. Knobloch, R.A. Mecklenburg and H.P. Rasmussen for counsel and assistance on my guidance committee. For suggestions, help and use of laboratory facilities I am indebted to Drs. A. De Hertogh, D.H. Dewey, D.R. Dilley, J.F. Foss, G.R. Hooper, H.P. Rasmussen and Mr. Philip Mott.

Appreciation is also extended to Hidden Lake Gardens Trust Fund for financial aid, without which this study would not have been possible.

A special thanks is extended to my wife Mairead for her patience, encouragement and assistance during this program of study.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION	1
LITERATURE REVIEW	3
SECTION I KEEPING QUALITY, ABSCISSION AND LEVELS OF O_2, CO_2 AND ETHYLENE DURING STORAGE OF FRUITING BRANCHES OF ILEX VERTICILLATA (L.) GRAY	5
MATERIALS AND METHODS	8
RESULTS	11
DISCUSSION	29
SECTION II THE EFFECTS OF ANTITRANSPIRANTS ON KEEPING QUALITY OF FRUITING BRANCHES OF ILEX VERTICILLATA (L.) GRAY	36
MATERIALS AND METHODS	39
RESULTS	41
DISCUSSION	61
SECTION III PHYSIOLOGY OF FRUIT ABSCISSION IN ILEX VERTICILLATA (L.) GRAY	63
MATERIALS AND METHODS	66
RESULTS	70
DISCUSSION	88
SECTION IV THE EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND WIND ON FRUIT ABSCISSION OF ILEX VERTICILLATA (L.) GRAY	91

Page

MATERIALS AND METHODS 93

RESULTS 95

DISCUSSION 103

SUMMARY AND CONCLUSIONS 105

LIST OF REFERENCES 108

LIST OF TABLES

Table — Page

SECTION I

1. Loss in fresh weight (%) of fruiting branches at weeks 3, 4½ and 6 as affected by temperature, relative humidity and vapor pressure deficit 12

2. Moisture content (%) of fruit at weeks 1, 2 and 8 of storage as affected by temperature, relative humidity and vapor pressure deficit 12

3. Fruit removal force as affected by vapor pressure deficit, temperature and relative humidity 16

4. Moisture content (%) of fruit as affected by vapor pressure deficit, temperature and relative humidity . . . 23

SECTION III

1. Fruit removal force as affected by growth regulators in a field experiment 71

2. Loss in fresh weight (%) of fruiting branches as affected by various chemical media 80

3. Loss in fresh weight (%) of fruiting branches as affected by addition of polyvinylpyrrolidone to the media . 81

4. Loss in fresh weight (%) of fruiting branches as affected by Vapor Gard, Geon Latex and control 81

5. Loss in fresh weight (%) of fruiting branches for the interaction between polyvinylpyrrolidone and various media. 83

SECTION IV

1. Freezing and killing temperatures °C of fruit when super cooled at 10°C/hour 96

2. Number of pedicels alive after subfreezing temperature treatments 96

LIST OF FIGURES

Figure — Page

SECTION I

1. Loss in fresh weight (%) of fruiting branches and moisture content (%) of fruit in 1973 13

2. Loss in fresh weight (%) of fruiting branches after 4½ weeks and moisture content (%) of fruit at weeks 2 and 8 as affected by temperature and relative humidity . 17

3. Fruit removal force (g) and frequency (%) of distal separation as affected by temperature and relative humidity 19

4. Moisture content (%) of fruit and twigs in 1974 as affected by temperature and relative humidity 21

5. Fruit removal force (g) at weeks 1 and 6 and moisture content (%) of fruit at weeks 2 and 5 as affected by temperature and relative humidity 24

6. Ethylene concentrations (ppb) at 20°C; fruit removal force (g) and CO_2 (%) concentrations at high relative humidity as affected by temperature 26

7. Ethylene production rate of fruit 30

8. The pedicel viewed under the dissecting microscope . . 33

SECTION II

1. Loss in fresh weight (g) and water uptake (ml) of fruiting branches as affected by Vapor Gard, Concord Alcoat and control 43

2. Loss in fresh weight (g), water uptake (ml) and transpiration (ml) of fruiting branches as affected by Vapor Gard, Concord Alcoat, other treatments and control. 45

Figure | Page

3. Respiration rate of fruit as affected by Vapor Gard, Concord Alcoat and control 47

4. Cross sectional views of fruit epicarp 50

5. Asphondylia illicifolia Foote 52

6. Morphology of fruit surface (SEM) 53

7. Morphology of fruit surface (SEM) 55

8. Morphology of fruit surface (SEM) 57

9. Morphology of fruit surface (SEM) 59

SECTION III

1. Longitudinal sections of the pedicel 73

2. Longitudinal sections of the pedicel 75

3. Frequency (%) of abscission types and fruit removal force (g) from July 8 through October 13, 1974 . . . 77

4. Loss in fresh weight (%) of fruiting branches as affected by the interaction between polyvinylpyrrolidone and chemical media; Vapor Gard and control . . 84

5. Loss in fresh weight (%) of fruiting branches for the interaction between Vapor Gard and chemical media on day 10 and day 17 86

SECTION IV

1. Fruit removal force as affected by subfreezing and storage temperatures 97

2. Fruit removal force for the interaction between storage temperature and relative humidity on day 16 . . 99

3. Effect of subfreezing temperatures on pedicel and fruit. 101

Guidance Committee:

The Paper-Format for this dissertation was adopted in accordance with departmental and university regulations. The dissertation body was separated into 4 sections. Each section was styled for publication in the Journal of the American Society for Horticultural Science.

INTRODUCTION

Ilex, commonly called holly, is a genus within the plant family Aquifoliaceae. Some evergreen species (Ilex aquifolium L. English holly and Ilex opaca Ait. American holly) are commercially produced for the use of cut branches in Christmas decorations. This utilization of holly is not restricted to evergreen types, many deciduous species can be used for floral arrangements. One such species- Ilex verticillata (L.) Gray (common winterberry) is common in low lying areas throughout the state of Michigan where it fruits abundantly.

In the past, fruiting branches were obtained by destructive cutting of the plants in their natural habitat. Land owners then prohibited trespassers and the popularity of this shrub declined. Some people (among them Mr. Harry A. Fee, former owner of Hidden Lake Gardens in the Irish Hills near Tipton, Michigan) transplanted several plants into landscapes and others attempted to cultivate specimens as a row crop.

The most recent local entrepreneur in this area is Mr. Philip Mott of Kalamazoo, a veteran of the old holly harvesting system. He has successfully propagated over 25,000 plants and has plans for further expansion. Very little scientific information on the propagation of this species and post harvest physiology of the fruit was available. The need for research in this area was sensed by Dr. Harold Davidson who had been interested in Michigan holly for some years when he became familiar with Mr. Mott's efforts.

An M.S. program by the present author was devoted to propagation methods and keeping quality. The present Ph. D. dissertation

deals with keeping quality and fruit abscission of detached branches. When harvested and placed in floral arrangements, the fruit on cut branches dried and shrivelled within 7 to 10 days. Moreover, the fruits abscised easily during handling. The following experiments were designed to determine the optimum storage environment and to increase the period of keeping quality at room conditions. Experiments were also conducted to discover the physiology of fruit abscission in the hope that abscission could be delayed by use of growth regulators. In the wild, the majority of I. verticillata plants remain heavily fruited until mid winter. Experiments were conducted to evaluate the influence of environmental factors on abscission in mid-winter.

LITERATURE REVIEW

Harvesting of fruiting branches of Ilex verticillata (L.) Gray (common winterberry) in the wild was common practice in the early part of this century. Although Ricker in 1924 (86) urged the cultivation of this species as a row crop, it was not until 1948 that attempts were made by Fee (34), Hamblin (44), Neal (72) and Reinsmith (85) to make this a business similar to any other fruit crop.

Research on post harvest utilization of fruiting branches of common winterberry was never conducted because their use in Christmas decorations declined due to unavailability of plant material. In recent years attention has again been directed toward cultivation of common winterberry. Thus information from research on keeping quality and abscission of fruit is necessary for the grower, florist and consumer. Production of Ilex aquifolium L. (English holly) on the northwest coast of the U.S.A. and Ilex opaca Ait. (American holly) on the eastern coast is a commercial venture for use of holly branches in Christmas floral arrangements. These hollies are popular mainly because of their glossy green evergreen leaves. Discoloration of the leaves followed by abscission occurred during storage at high temperatures and high relative humidities (RH) (87, 114). This was overcome by partial drying before packing or by cold storage and application of α naphthaleneacetic acid at 37 to 40 ppm (27, 35, 68, 69, 87). Storage recommendations for fruiting branches were 0°C and 85 to 90% RH

(12, 82, 114).

The above studies dealt with maintaining keeping quality of evergreen leaves but no information was available on quality and abscission of fruit. Since I. verticillata is a deciduous holly, the fruit is of major impertance. The objective of the following experiments was to determine storage requirements of fruiting branches of common winterberry; evaluate the influence of antitranspirants on maintenance of keeping quality of the fruit and determine the process of fruit abscission by use of growth regulators and environmental stresses.

SECTION I

KEEPING QUALITY, ABSCISSION AND LEVELS OF O_2, CO_2 AND ETHYLENE DURING STORAGE OF FRUITING BRANCHES OF *ILEX VERTICILLATA* (L.) GRAY

Most horticultural products are stored at low temperature and high relative humidity (RH) whereby moisture loss is reduced to a minimum because the vapor pressure deficit (VPD) is small. In addition, plant material stored at low temperature has a low rate of respiration; aging and ripening processes are slowed down (58). Additionally, spoilage and changes in texture and color are minimized.

At a constant temperature, evaporation of water from plants increases with decreasing RH, resulting in wilting or shrivelling of the product (108). To prevent this, high RH is desirable but control of pathogenic organisms becomes necessary (58). As RH decreased from 100 to 65%, water loss from apples increased and then reached a plateau (97). In grapes, this plateau occurred at 40% RH. The drying of the outer fruit layers was considered as being the explanation of this phenomenon (9).

Most fruits and vegetables contain 80 to 95% water by weight (24, 58). During storage, loss in fresh weight results principally from transpiration (109). Most fruits shrivel when they lose 5 to 7% of their weight (22). In addition to factors already discussed, the extent of this loss is influenced by a number of factors including product type, size, composition and structure. When environmental conditions exist which cause plants to lose moisture, the initial loss is rapid but eventually stabilizes (97). At constant RH (excluding low values) within a temperature range of 3 to 15°C, water loss from plants is directly related to VPD (24, 97).

Recommendations for storage of ornamental plant material are contained in handbooks prepared by the American Association of Nurserymen (60) and the U. S. Department of Agriculture (58). Rooted

cuttings and seedlings of many plant species can be stored at temperatures slightly above 0°C for 4 to 6 months (58). To prevent injury to florist greens, bulbous plants and some nursery stock they should not be stored with ripening fruit or other sources of ethylene (58). Since evergreens are in full leaf, particular care is essential to prevent dessication (60). Cut branches of Ilex aquifolium L. (English holly) and Ilex opaca Ait. (American holly) can be stored up to 4 weeks at 0°C and 85 to 90% RH (12, 82, 114). Discoloration of the leaves, followed by abscission, was found to occur at high temperatures and high humidities (87, 114). This was overcome by partial drying before packing or by cold storage and application of α naphthaleneacetic acid (27, 35, 68, 69, 87). Treated leaves should not be packed with untreated holly to avoid the adverse effect on the untreated leaves of increased ethylene production resulting from auxin treatments (27).

Holly fruit were reported to produce very small amounts of ethylene but the quantity was not specified (68). Defoliation can occur in 3 to 4 days in response to ethylene levels as low as 5 ppm (68, 69, 82, 87). Despite the presence of ethylene provided by ripening apples, defoliation was prevented for 30 days at 0°C and high RH storage but similar storage at 20°C resulted in 25% leaf drop.

The objective of this study was to determine how much ethylene is produced by the fruit and to evaluate the effects of temperature, RH and VPD during storage on levels of O_2, CO_2, ethylene and keeping quality of fruiting branches of Ilex verticillata (L.) Gray (common winterberry).

MATERIALS AND METHODS

Moisture loss 1973. Fruiting branches with leaves were stored in 9 environment combinations, 3 temperatures:(0°C, 10°C and 20°C) and within each temperature 3 RH (low, intermediate and high) which varied with temperature (Table 1). Low RH (20 to 35%) were achieved utilizing air previously passed through a calcium chloride filter. Intermediate RH (30 to 45%) were obtained using air and high RH (89 to 98%) by first passing air through water. Three replications of each RH were obtained by using 7.5 liter plastic containers within each temperature chamber. The lid was equipped with 2 air-line connectors for an inlet and an outlet. Calculations of RH were obtained with a potentiometer equipped with wet and dry thermocouples. On September 27 branches of Ilex verticillata were harvested from a single plant. In each container, 3 uniformly fruited twigs, approximately 15 cm in length, were inserted in 3 bottles (2.3 cm diameter) containing approximately 75 ml of water. Each twig was weighed initially and again at 3, 4½ and 6 weeks to determine loss in fresh weight. The condition of the leaves and fruit were rated each time.

Moisture content of the fruit 1973. Experimental conditions were identical as previously reported. Defoliated plant material was collected on November 23. Five twigs, supported by a wire mesh frame, were placed in each dry container. Data consisted of fresh and dry weight measurements for which moisture content of fruit in % was calculated at time zero and at 1, 2, 4, 6 and 8 weeks of storage. For dry weight determinations, fruit were placed in an oven at 65°C for 2 days.

Fruit removal force (FRF), moisture content of fruit and twigs and gas analysis 1974. Treatment combinations and replications were similar to the 1973 experiments. Environmental replications were enclosed within cardboard boxes, approximately 0.04 m^3 in volume, encased in 10 µm opaque polyethylene bags. These were fitted with a serum cap for withdrawl of gas samples. Low RH within each temperature were obtained by placement of dry calcium chloride inside the boxes. Saturated salt solutions of this material at 0° and 8.8°C and lithium chloride at 20°C were used for establishment of intermediate RH. High humidities occurred in boxes containing distilled water. RH and temperature parameters recorded with thermohydrographs are shown in Table 3.

Samples of air were extracted weekly from each container and ethylene concentrations determined by injection of 1 cc into a Varian Aeregraph 1700 gas chromatograph. The stainless steel column (0.32 X 46 cm) contained activated alumina at 50°C. The carrier gas was nitrogen and detection was by flame ionization. Oxygen and CO_2 levels were determined by similar methods on a vapor fractometer using a parallel column system; a molecular sieve for oxygen and silica gel for CO_2 with He as the carrier gas.

Observation of the separation point and FRF were measured for 20 fruit on a single twig from each replication with a Hunter Mechanical Force Gauge (Hunter Springs, Hatfield, Pa.). Abscission at the point of contact between the fruit and the pedicel was termed distal; between the pedicel and the twig proximal and between any parts of the pedicel intermedial. Moisture content of fruit and twigs was determined as previously stated as was a subjective rating of fruit quality. At the

end of week 8, December 7, the experiment was terminated. VPD calculated for treatment combinations were correlated with data on FRF. Statistical analysis employed was a split plot design with temperature being the main split and 3 replications of RH. Significant differences of means were determined by Tukey's HSD test, (98).

Determination of ethylene production rate. On September 22 samples of 2 to 4 green, red-green and red fruit were placed in 10 ml syringes, at room temperature. Treatments, including controls, were replicated 3 times. Filter paper soaked in 10% NaOH (for absorption of CO_2) was attached to the plunger with a pin. An oxygen supply (drawn in by negative partial pressure) was provided from a reservoir and passed through ammonium sulfate. Samples of gas (1 cc) were withdrawn at intervals of 3, 6 or 12 hours and analyzed by gas chromatography as described above. The system was flushed with ethylene free air for 15 minutes after each sample was taken and the data plotted on semi-logarithmic paper in µl/kg/hr over a time period from September 23 to October 13.

RESULTS

Moisture loss 1973. Fruiting branches stored at 0°C/98% RH showed an increase in fresh weight (by an average of 4.9%) at the end of week 3, all other treatment combinations lost weight (Table 1). Treatments at high temperature or low RH resulted in greater weight losses than those at low or intermediate temperatures and intermediate or high RH (Figs. 1A and 1B). The effect of RH at the 3 temperatures for week 4½ is graphically presented in Fig. 2A. Leaves were blackened and loosely attached in high humidity chambers at 10° and 20°C on week 3. In

contrast, similar symptoms were not apparent under 0°C and high humidity until 4½ weeks. At 0° and 10°C and high RH, fruit remained in excellent condition throughout the experiment. The 20°C/89% RH treatments developed fungal growth and were discontinued after 4½ weeks.

In intermediate and low humidity chambers at 10° and 20°C the fruits were shrivelled and dehydrated and the twigs had lost their leaves by week 3. At 0°C some leaves turned brown but the majority remained green and held well. Fruit quality deteriorated by week 6 in intermediate and low RH at all temperatures.

Moisture content of the fruit 1973. Fruit at 0°C retained more moisture than those at 10° or 20°C (Fig. 1C, Table 2). Dehydration at 20°C was rapid and the fruit were brittle with the result that routine handling of the containers on week 2 caused fruit abscission. Low and intermediate humidity effects were similar but differed from those of high humidity throughout the experiment (Fig. 1D). The combination of 0°C/98% RH consistently maintained more moisture than other combinations such as 35.6% on week 8. The effect of RH at the 3 temperatures for weeks 2 and 8 is graphically presented in Figs. 2B and 2C.

During oven drying, fruit with a moisture content of 20% or less remained red, while fruit with higher moisture contents turned brown.

FRF, moisture content of the fruit and twigs, and gas analysis 1974. Values for proximal, distal and intermedial separation were similar, hence overall values were used in computations. Throughout the experimental period, FRF was inversely related to temperature: higher values were consistently recorded for the lowest temperature of 0°C (Fig. 3A).

Table 1. Loss in fresh weight (%) of fruiting branches of *Ilex verticillata* (L.) Gray at weeks 3, 4½ and 6 as affected by temperature (temp), relative humidity (RH) and vapor pressure deficit (VPD).

			Loss in fresh weight of fruiting branches (%)		
Temp °C	%RH	VPD mm Hg	wk.3	wk.4½	wk.6
0	98	0.01	s4.9	9.1	9.2
10	97	0.28	13.4	31.0	37.2
20	89	2.07	31.6	56.6	---
0	45	2.52	7.2	10.1	12.2
0	35	2.98	7.3	22.5	24.4
10	40	5.52	31.5	42.2	54.6
10	30	6.44	37.2	54.8	56.4
20	30	13.14	50.4	63.4	---
20	20	15.01	52.1	72.2	---

s= a positive value weight increase due to water uptake

--- = missing data

Table 2. Moisture content (%) of fruit of *Ilex verticillata* (L.) Gray at weeks 1, 2 and 8 of storage as affected by temperature (temp), relative humidity (RH) and vapor pressure deficit (VPD).

			Moisture content of the fruit (%)		
Temp °C	%RH	VPD mm Hg	wk.1	wk.2	wk.8
0	98	0.01	61.9	61.3	35.6
10	97	0.28	60.2	58.5	25.1
20	89	2.07	31.9	11.2	---
0	45	2.52	59.7	53.7	19.1
0	35	2.98	60.3	58.2	20.8
10	40	5.52	52.6	39.5	1.5
10	30	6.44	52.3	38.4	1.7
20	30	13.14	22.8	3.8	---
20	20	15.01	25.8	4.8	---

--- = missing data

Figure 1 A-D Loss in fresh weight (%); moisture content (%) of fruiting branches of Ilex verticillata (L.) Gray in 1973, as affected by temperature (temp) and relative humidity (RH).

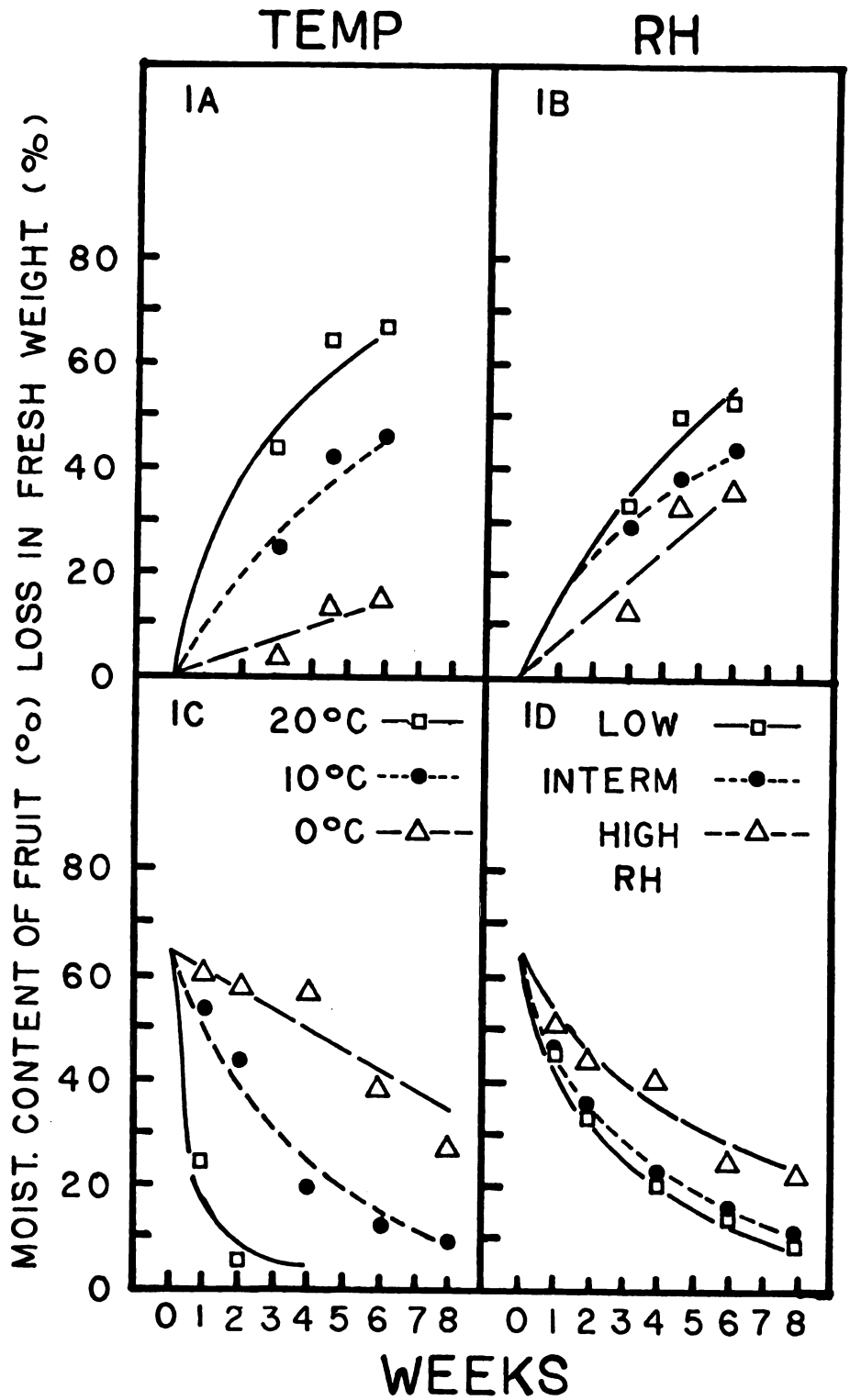
A-- loss in fresh weight as affected by temperature

zB-- loss in fresh weight as affected by RH

C-- moisture content of fruit as affected by temperature

zD-- moisture content of fruit as affected by RH

z = means of low and high RH were significantly different at the 0.01 level


TEMP
RH
IA
IB
IC
ID
20°C
10°C
0°C
LOW
INTERM
HIGH
RH
MOIST. CONTENT OF FRUIT (%) LOSS IN FRESH WEIGHT (%)
0
20
40
60
80
0 1 2 3 4 5 6 7 8
WEEKS

At the end of weeks 1 and 2 (Fig. 3B) FRF at high RH were greater than those at low RH. For the following 3 weeks FRF for intermediate RH treatments decreased to levels similar to those of low RH (150 g) and the FRF for high RH remained above 200 g.

The interaction of humidity and temperature at weeks 1, 5 and 6 was significant (Table 3). At 0°C/98% RH (Fig. 6B) there was no reduction in FRF and while at 20°C/94% RH there was an initial increase, it later was followed by a rapid decrease. RH effects at the 3 temperatures for weeks 1 and 6 are shown in Figs. 5A and 5B. Estimated VPD correlated with the mean of FRF are shown in Table 3.

In general over 60% of the fruits separated from the pedicel (distal), 30% were removed with the pedicel attached (proximal) and the remaining 10% were separated by a break in the pedicel (intermedial). Frequencies of the intermedial type did not fluctuate as much as those of the distal and proximal. Minimum distal frequencies at 0°, 8.8° and 20°C were 69.2, 61.1 and 49.8% respectively (Fig. 3C). Similar frequencies for low, intermediate and high humidities were 62.5, 68.3 and 53.9% respectively (Fig. 3D).

Moisture content of the fruit was highest for 0°C treatments (Fig. 4A, Table 4). The levels at week 7 fell to 35.0, 26.0 and 21.9% for 0°, 8.8° and 20°C respectively. Minimum values for high, intermediate and low humidities were 53.8, 16.0 and 9.4% respectively (Fig. 4B) and each level from week 2 onward was highly significant. Interactions occurred on weeks 2, 3, 4 and 5. While intermediate values of VPD did not demonstrate a consistent trend the combinations of 0°/98% RH contained 61.9 and 58.5% moisture and 20°C/25% RH treatments retained only 13.2 and 4.6% moisture after 2 and 5 weeks respectively (Figs. 5C and 5D, Table 4).

Table 3. Fruit removal force for Ilex verticillata (L.) Gray fruit, stored for a period of 7 weeks, as affected by vapor pressure deficit (VPD), temperature (temp) and relative humidity (RH).

			Fruit removal force (g)						
VPD mm Hg	Temp °C	%RH	wk.1	wk.2	wk.3	wk.4	wk.5	wk.6	wk.7
0.09	0.0	98	256bc	261	259	272	284b	270b	256
0.34	8.8	96	240abc	283	235	222	255b	241a	202
1.05	20.0	94	287c	239	252	214	x174a	x139a	x160
2.06	0.0	55	261bc	243	211	203	190a	174a	176
2.98	0.0	35	242abc	226	196	185	158a	174a	162
4.27	8.8	50	268bc	253	221	171	173a	160a	157
5.98	8.8	30	247abc	194	179	176	188a	168a	168
9.64	20.0	45	228abc	226	153	169	153a	161a	160
13.15	20.0	25	196a	202	192	182	145a	165a	---
r			0.67	0.56	0.55	0.56	0.64	0.41	
r^2			0.45	0.32	0.30	0.31	0.41	0.17	

Means within a column followed by a different letter are significantly different at the 0.01 level.

Initial FRF = 265

x = contaminated with fungi

--- = missing data

r = coefficient of linear correlation

r^2 = variance accounted for by VPD

Figure 2 A-C A-- Loss in fresh weight (%) of fruiting branches after 4½ weeks; B-- moisture content (%) of fruit at week 2; C-- and week 8 of Ilex verticillata (L.) Gray as affected by temperature (temp) and relative humidity (RH). Vertical lines indicate standard deviations above the mean.

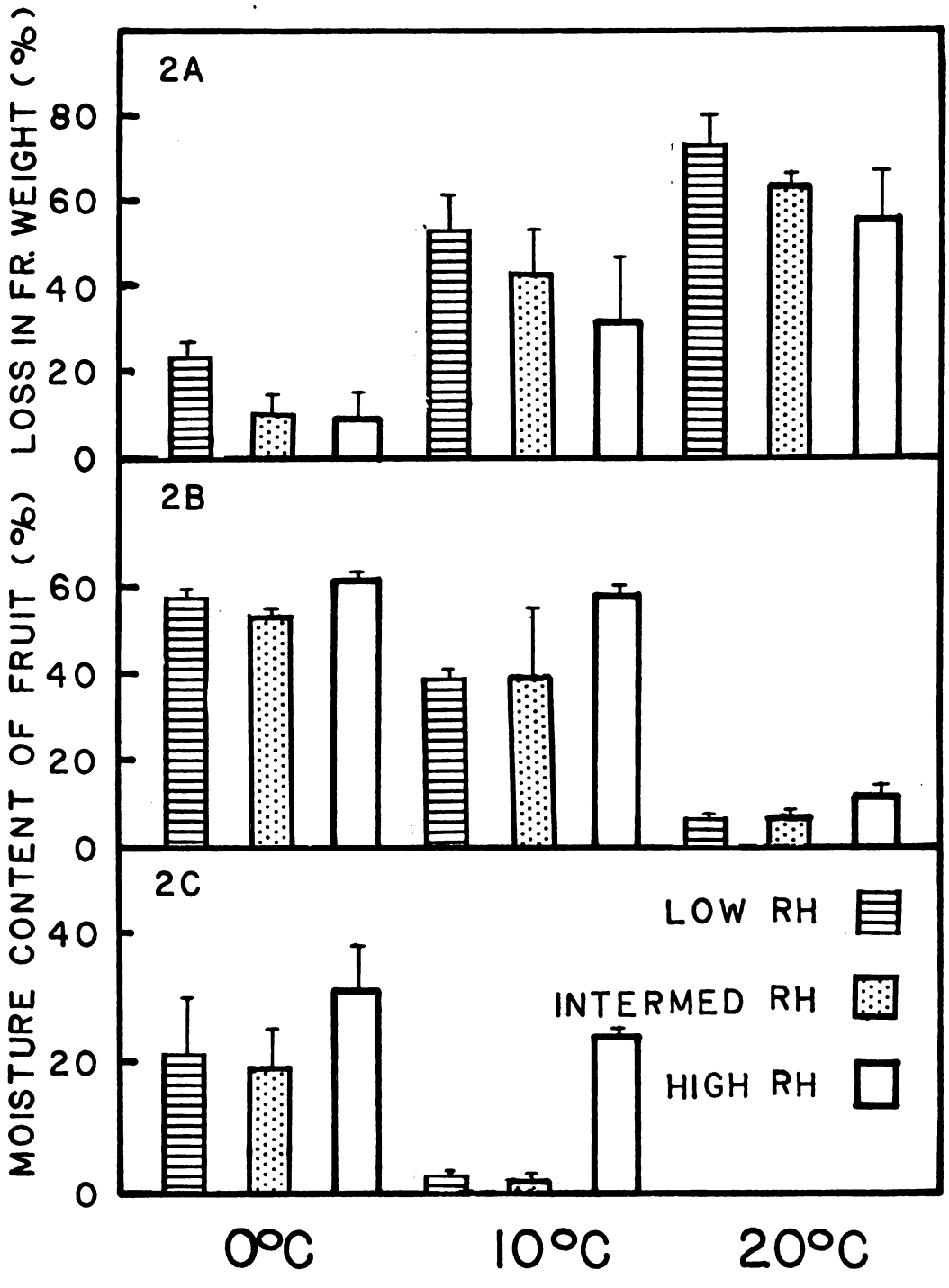

MOISTURE CONTENT OF FRUIT (%) LOSS IN FR. WEIGHT (%)
2A
2B
2C
0
20
40
60
80
LOW RH
INTERMED RH
HIGH RH
0°C
10°C
20°C

Figure 3 A-D Fruit removal force (FRF) (g) and frequency (%) of distal separation of Ilex verticillata (L.) Gray as affected by temperature (temp) and relative humidity (RH).

A-- FRF as affected by temp

zB-- FRF as affected by RH

C-D-- frequency as affected by temp and RH respectively

z = means of low and high RH were significantly different at the 0.01 level

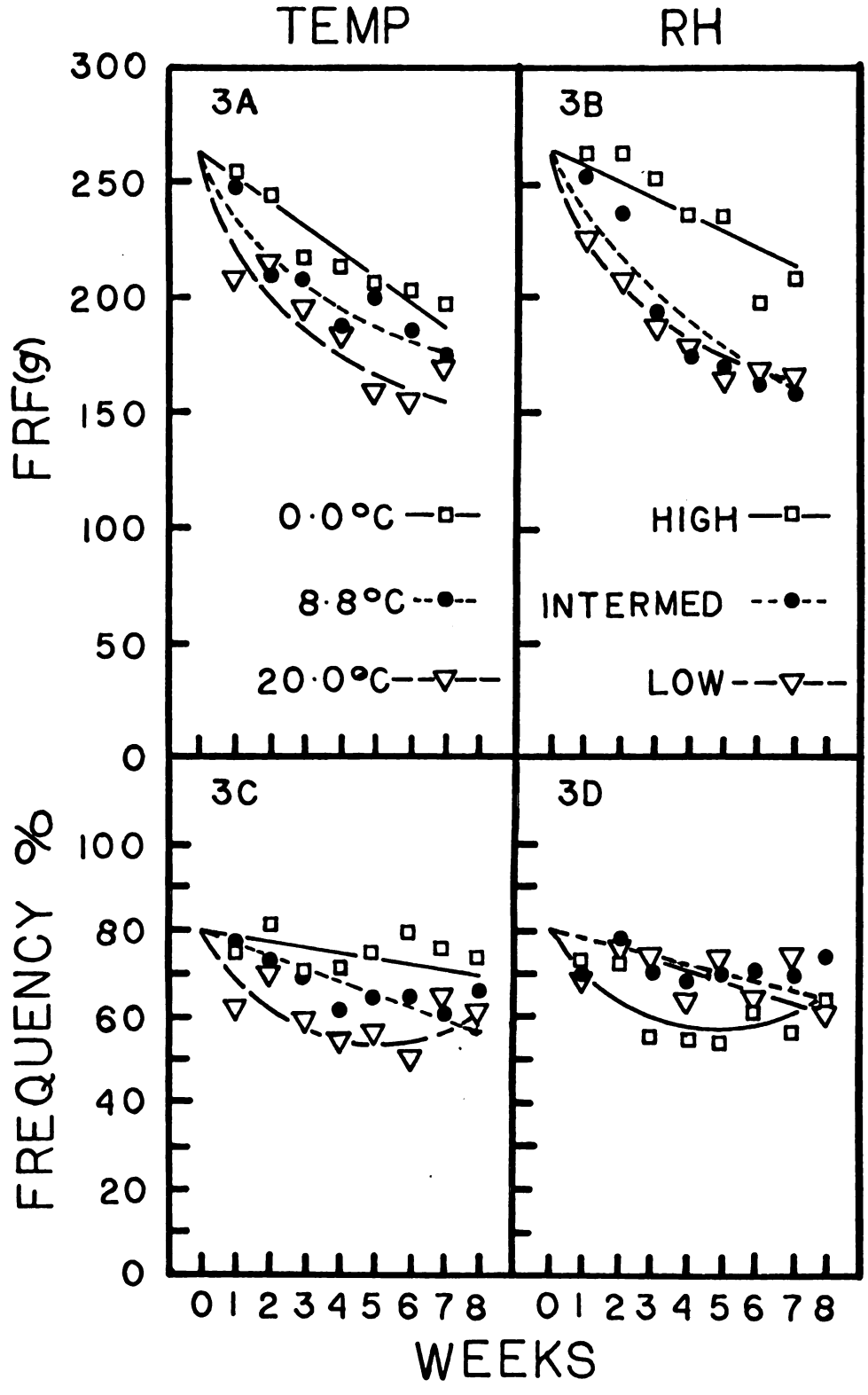

TEMP
RH
3A
3B
3C
3D
FRF(g)
FREQUENCY %
300
250
200
150
100
50
0
100
80
60
40
20
0
0·0°C
8·8°C
20·0°C
HIGH
INTERMED
LOW
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
WEEKS

Figure 4 A-D Moisture content (%) of fruit and twigs of *Ilex verticillata* (L.) Gray as affected by temperature (temp) and relative humidity (RH) in 1974 experiment.

A-- moisture content of fruit as affected by temp

zB-- moisture content of fruit as affected by RH

C-- moisture content of twig as affected by temp

yD-- moisture content of twig as affected by RH

z = means of low and high RH were significantly different at the 0.01 level

y = means of all RH were significantly different at the 0.01 level

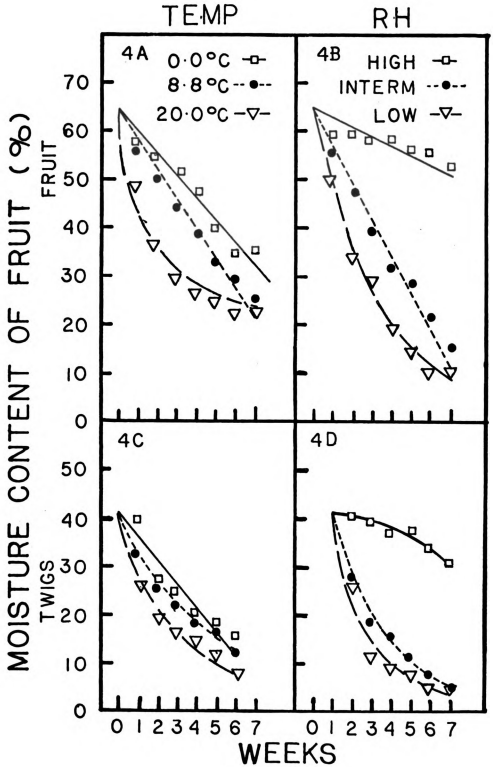

TEMP
RH
4A
0·0°C
8·8°C
20·0°C
4B
HIGH
INTERM
LOW
4C
4D
MOISTURE CONTENT OF FRUIT (%)
FRUIT
TWIGS
0
10
20
30
40
50
60
70
0 1 2 3 4 5 6 7
WEEKS

Table 4. Moisture content (%) of Ilex verticillata (L.) Gray fruit, stored for a period of 7 weeks, as affected by vapor pressure deficit (VPD), temperature (temp) and relative humidity (RH).

			Moisture content of fruit (%)						
VPD mm Hg	Temp °C	RH%	wk.1	wk.2	wk.3	wk.4	wk.5	wk.6	wk.7
0.09	0.0	98	62.2	61.9e	60.2d	60.9d	58.5d	58.1	59.9
0.34	8.8	96	61.4	59.5d	59.8d	60.1d	58.2d	57.5	52.5
1.05	20.0	94	62.2	57.0d	55.2d	54.0d	x51.8d	50.9	49.0
2.06	0.0	55	58.9	x52.8d	49.4cd	43.8c	38.8c	29.5	27.3
2.98	0.0	35	57.0	x52.1d	40.1bc	37.7c	26.8bc	y20.5	17.7
4.27	8.8	50	57.6	x50.3cd	45.3cd	35.3c	31.5c	24.5	y16.3
5.98	8.8	30	x52.8	38.9b	29.9b	y19.1b	11.9a	7.6	7.9
9.64	20.0	45	x50.7	40.1bc	26.3b	y19.7b	15.8ab	12.8	12.1
13.15	20.0	25	x42.4	y13.2a	7.3a	5.3a	4.6a	2.9	4.7

Means within a column followed by a different letter are significantly different at the 0.01 level.

Initial moisture content mean = 65%

x = first appearance of dehydrated fruit

y = first appearance of red color of fruit when dried, for moisture determination.

Figure 5 A-D A-- Fruit removal force (FRF) (g) at weeks 1; B-- week 6; C-- moisture content (%) of fruit at week 2; D-- week 5 of Ilex verticillata (L.) Gray as affected by temperature (temp) and relative humidity (RH). Vertical lines indicate standard deviations above the mean.

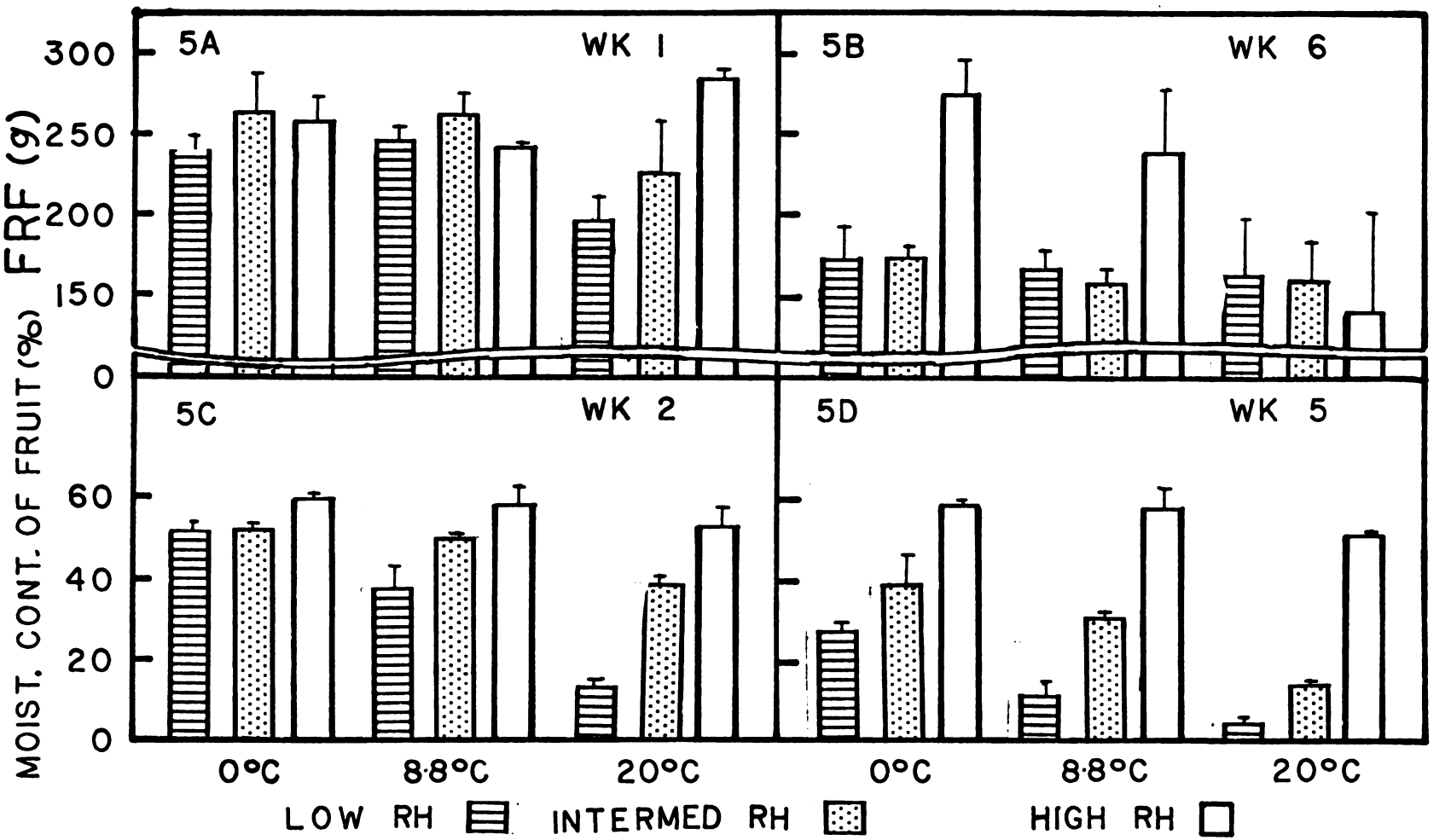
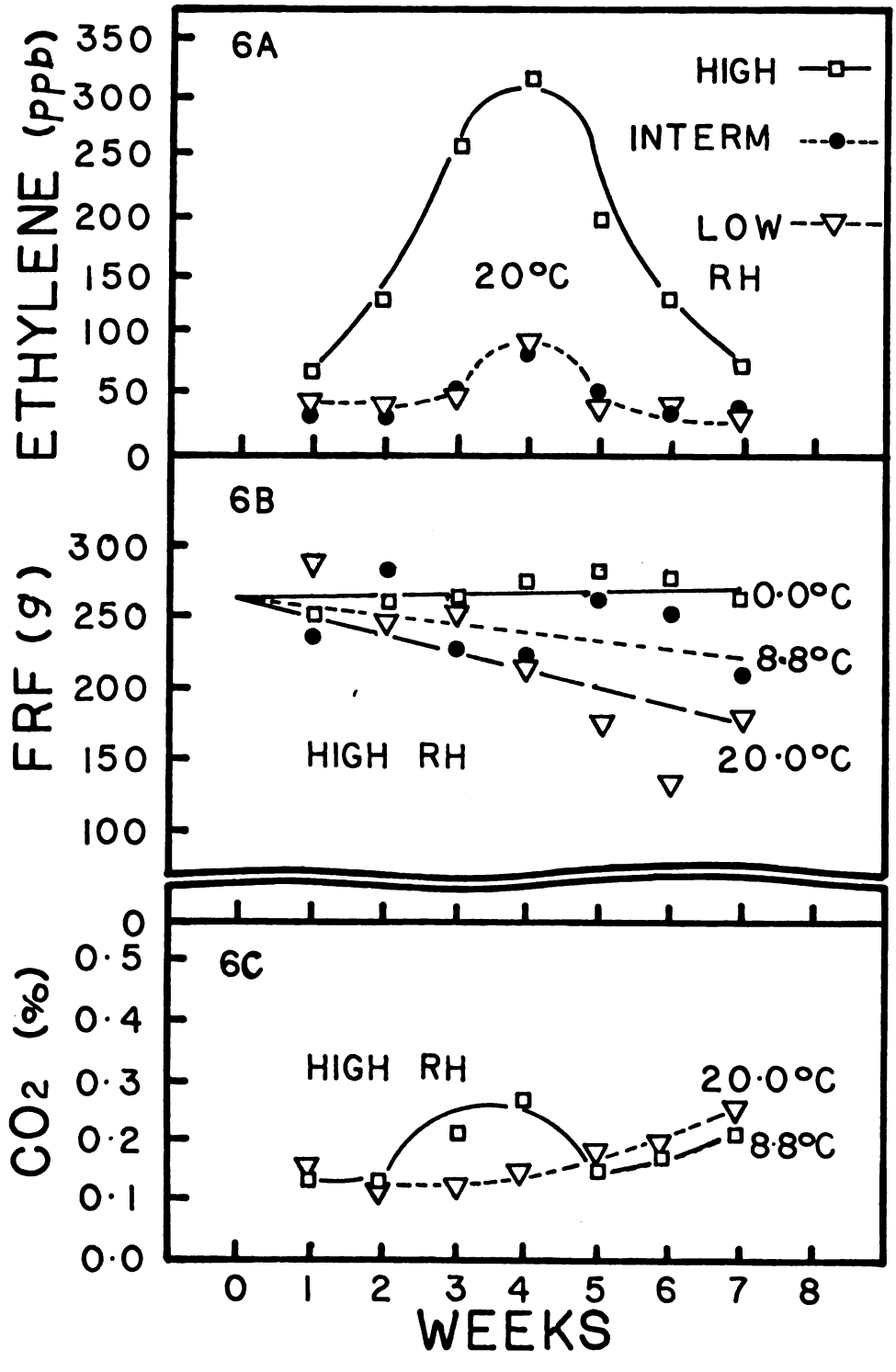

5A
WK 1
5B
WK 6
5C
WK 2
5D
WK 5
300
250
200
150
0
60
40
20
0
MOIST. CONT. OF FRUIT (%) FRF (g)
0°C
8·8°C
20°C
0°C
8·8°C
20°C
LOW RH
INTERMED RH
HIGH RH

Figure 6 A-C A-- Concentrations (ppb) of ethylene during storage of Ilex verticillata (L.) Gray fruiting branches at 20°C as affected by relative humidity (RH)

B-- Fruit removal force (g) (FRF) of Ilex verticillata (L.) Gray at high relative humidity (RH) as affected by storage temperature.

C-- Concentration (%) of carbon dioxide (CO_2) during storage of Ilex verticillata (L.) Gray fruiting branches at high RH at 8.8°C and 20°C.


6A
ETHYLENE (ppb)
350
300
250
200
150
100
50
0
HIGH
INTERM
LOW
20°C
RH
6B
FRF (g)
300
250
200
150
100
0·0°C
8·8°C
20·0°C
HIGH RH
0
6C
CO2 (%)
0·5
0·4
0·3
0·2
0·1
0·0
HIGH RH
20·0°C
8·8°C
0 1 2 3 4 5 6 7 8
WEEKS

Red fruit with 20% or less moisture did not turn brown during oven drying (Table 4). Shrivelled dehydrated fruit had a moisture content of 52.8% or less. The lowest moisture content (with the exception of 20°C/94% RH in week 2) recorded for good quality fruit was 53.4%. Thus the threshold value for good quality fruit lies between these points. With the exception of material stored at 20°C/94% RH, which became contaminated with fungi in week 3, all fruit stored at high RH remained in excellent condition throughout the entire experiment. Shrivelling of fruit was observed following 2 weeks of low or intermediate RH storage in all 3 temperatures.

Temperature differences for twig moisture were small (Fig. 4C). By week 6, a mean between 8 and 16% moisture was recorded for all temperatures. Twigs stored at high RH had a mean value of 30.2% moisture at the end of the experiment (Fig. 4D). Moisture content of twigs at low and intermediate RH dropped to 2.1 and 4.6% respectively by week 6 when the last data was recorded.

Minimum levels of oxygen in all chambers were 20%. Ethylene concentrations in the chambers at 0° and 8.8°C fluctuated between 50 and 100 ppb. Similar values were recorded for 30 and 50% RH at 20°C, but fungal contamination in the 94% RH chamber was assumed to be responsible for mean levels of 317 ppb at week 4 (Fig. 6A). Concentrations of CO_2 were generally in the region of 0.2%. Values increased to 0.5% at week 4 in combinations of 20°C/94% RH and at week 7 in combinations of 8.8°C/96% RH (Fig. 6C).

Determination of ethylene production rate. The red fruit were larger than the red-green or green fruit. Consequently occasional injury occurred from contact with the alkaline NaOH. Such injury

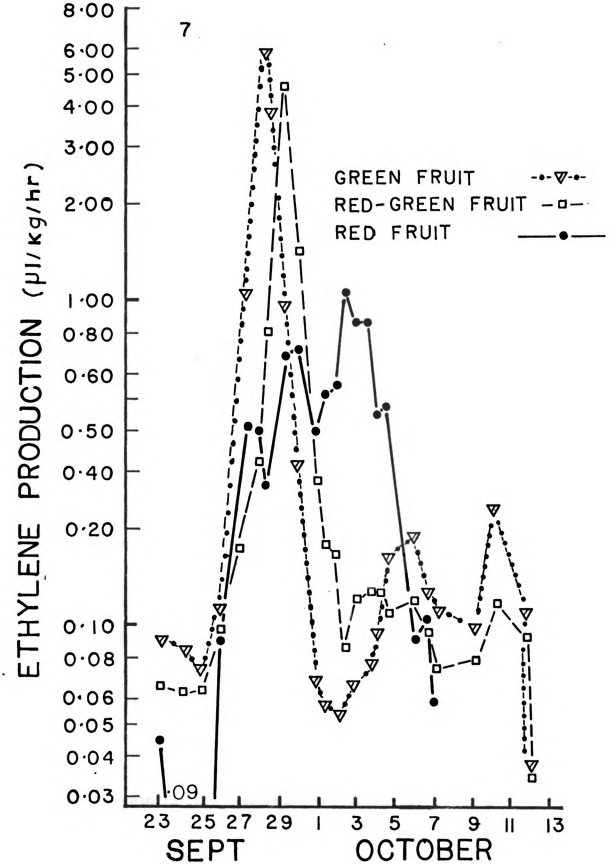
was more common with 4 fruit than with 2 fruit per syringe. Data from damaged fruit was excluded. Peaks of ethylene production were highest and occurred earlier for green fruit (5.8 µl/kg/hr at 5½ days after harvest)(Fig. 7). Red-green fruit climaxed with 4.6 µl/kg/hr at 6½ days from harvest. Red fruit began to increase ethylene production at the same time as green and red-green. The peak (1.1 µl/kg/hr) was much smaller and delayed until 9½ days from harvest.

DISCUSSION

In the 1973 experiments, RH control and measurement proved quite difficult to obtain. Wet bulb depressions at 0°C were very small compared with those at 20°C for equivalent RH. Additional technological variations spurred the development of an alternative system in 1974. Containers sufficiently large to contain thermohydrographs were found more satisfactory.

Abscission in this experiment, as measured by a reduction in FRF, was shown to be related to VPD of the environment (Table 3). Fruit stored in low and intermediate RH showed greater reduction in FRF than those in high RH (Fig. 3B). This difference could have been much greater if it were not for fungal contamination at 20°C/94% RH resulting in ethylene production values greater than 300 ppb (Fig. 6A). The consequences can be seen in Fig. 6B, where FRF with this combination declined from 251 g in week 3 to 140 g at the end of week 6. This occurrence was responsible for the low coefficient of linear correlation (0.41) on week 6. The best treatment combination was 0°C/98% RH where FRF did not significantly change throughout the experiment (Figs. 5A, 5B and 6B, Table 3) and moisture loss from fruit was least (Figs. 5C

Figure 7. Ethylene production rate in µl/kg/hr for *Ilex verticillata* (L.) Gray fruit.


7
8·00
6·00
5·00
4·00
3·00
2·00
1·00
0·80
0·60
0·50
0·40
0·20
0·10
0·08
0·06
0·05
0·04
0·03
ETHYLENE PRODUCTION (µl/kg/hr)
GREEN FRUIT
RED-GREEN FRUIT
RED FRUIT
·09
23 25 27 29 1 3 5 7 9 11 13
SEPT
OCTOBER

and 5D, Table 4). Moisture loss from fruit in the 1973 experiment was also minimised with the 0°C/98% RH combination (Figs. 2B and 2C). Differences due to temperature were much greater in 1973 (Fig. 1C) than in 1974 (Fig. 4A). Because of the previously mentioned variations in 1973 and the greater precision of variable control in 1974 this difference was assumed due to experimental error. This same error was present for the 1973 experiment on loss in fresh weight of fruiting branches with attached leaves.

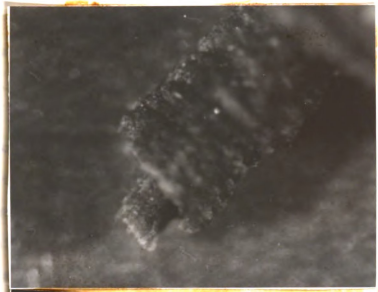
Abscission at the point of contact between the fruit and the pedicel (distal type) occurred 60% of the time. Separation of the pedicel from the twig (proximal) occurred with 30% frequency and the intermedial type made up the remaining 10%. Intermedial fruit abscission was characterised by separation of the central xylem core from the cortex tissue. Breakage occurred at any point along the pedicel with the fruit retaining the xylem core and leaving the tubular cortex on the twig (Figs. 8A-C). High temperatures and high humidities produced lowest frequencies of distal separation (Figs. 3C and 3D).

Interpretation of data corresponds with those who postulated that a lack of moisture stress results in stronger cell walls which act to delay abscission and loss of moisture causes shrinkage which aids separation (8). This shrinkage of fruit and branches was evident in low and intermediate RH treatments no later than week 2 (Table 3). From this period on, routine handling of such material resulted in fruit abscission. Good quality fruit had 53.4% moisture and greater, whereas shrunken fruit were recorded as having a maximum of 52.8% moisture. Assuming a threshold value of 53% in fruit containing 65% initial moisture the calculated point of shrinkage was 8% loss in fresh weight, similar to values for other fruits (9, 22). The

Figure 8 A-C *Ilex verticillata* (L.) Gray pedicels

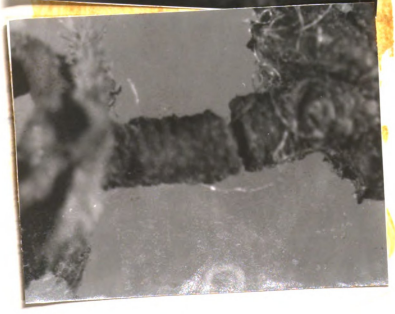
A and B-- Intermedial abscission; fracture of cortex

C-- shrivelled cortex exposing xylem core



8A

8B



8C

occurrence of brown leaves which abscised at high temperatures and high RH agrees with information available on evergreen holly (68, 69, 114).

Fruit of Ilex verticillata were reported to contain 3-glucoside and 3-xylosylglucoside pelargonidin types of anthocyanin pigments (90). Stability of this red color is decreased at high temperatures where browning occurs by enzymatic or non enzymatic (Maillard) reactions (53, 96). This reaction was prevented when the moisture content was decreased to approximately 20% where the water soluble anthocyanins at this level were not free for hydrolysis.

Fruit of this species (at room temperature) produced a definite peak of ethylene production (Figure 7). The highest peak (5.8 µl/kg/hr) occurred with green fruit after 5½ days. Red-green fruit reached 4.6 µl/kg/hr one day later. The peak for red fruit was approximately 20% the height of the previous peaks (1.1 µl/kg/hr) and was delayed until 9½ days from harvest. The cranberry Vaccinium macrocarpon Ait. has been reported to produce increased amounts of ethylene as the fruit matured (36). Respiration measured by CO_2 production did not show an increase, yet these fruit were classified as being climacteric. In absence of data on respiration and because a climacteric fruit is defined by an increase in this process the author prefers not to place the fruit of common winterberry in any category until more data is accumulated.

SECTION II

THE EFFECT OF ANTITRANSPIRANTS ON KEEPING QUALITY OF FRUITING BRANCHES OF *ILEX VERTICILLATA* (L.) GRAY

If water loss from plants (transpiration) is considerably reduced, some researchers suggest that cooling and nutrient uptake in plants is not significantly altered (10, 38, 80). Diffusion of water vapor occurs mainly through stomata and lenticels. Cuticular water loss becomes important when the stomata are closed (38). Therefore a critical area of water loss from plants is the leaf-air interface, and retardation proximal to this point will cause the development of moisture stress in the distal region (38). The prevention of this occurrence is possible by use of antitranspirants.

There are at least 4 approaches to artificial reduction of transpiration. These comprise A) use of materials which increase reflectance (primarily for leaves) (63, 80), B) windbreaks, C) enclosures surrounding the plant and D) by increasing resistance to vapor diffusion (80). This last method involves the use of film forming materials for example silicone, or compounds which cause stomatal closure such as abscisic acid (38, 63, 80, 105).

The list of film forming materials includes wax, latex, various plastics, silicones, polyterpenes and higher alcohols (77). Earlier work dealt with multimolecular layers of wax (23, 43, 50, 63, 79, 95, 101), which were difficult to use because they required heating before application and were inclined to chip when hard (79).

Modern antitranspirants form monomolecular films on plant surfaces by molecule coalescence after solvent evaporation (63). Permeation of liquids and gases through these polymers occurs by a process called activated diffusion (56). The 3 steps involved are sorption by the polymer and diffusion through it, followed by desorption on its other

side. An ideal film forming antitranspirant, not yet developed, would have convenient and inexpensive application, greater permeability to gases than to water, and complete coverage which should be effective for long periods of time (38, 63, 80). The selective permeability is sometimes said to be an attribute of polyethylene and some other polymers (38, 77). Evidence presented by Lebovits 1966 (56) and Wooley 1967 (113) has proven otherwise and such references are only relevant to other polymers. In all polymers studied, permeability to water was greater than to CO_2.

Reductions of 20 to 50% transpiration were obtained with antitranspirant applications during the growing season of vegetable crops and trees and prior to transplanting (29, 30, 41, 61, 62, 78), but because of impeded gas exchange counteractive effects of photosynthesis inhibition have also been reported (28, 31, 37, 49, 73, 105). Phytotoxicity was a result of incomplete cover and toxin accumulation.

In post harvest utilization of plant material, photosynthesis is rarely a concern. Thus antitranspirant applications have maintained keeping quality of leaves and fruit by decreasing moisture loss (30, 42, 67, 93, 94, 101). Reduction of water loss was greatest for fruit varieties such as Golden Delicious apples and European forcing cucumbers which have little natural wax and shrivel during long term storage. Effects on some other varieties have been mainly to impart a lustre and increase consumer acceptance. The study reported here was designed to determine the effectiveness of various antitranspirants on the keeping quality of fruiting branches of Ilex verticillata (L.) Gray.

MATERIALS AND METHODS

Loss in fresh weight, water uptake, transpiration and respiration. Fruiting branches of Ilex verticillata were harvested on October 17, 1974 from which uniformly fruiting twigs, approximately 15 cm in length were selected. Ten twigs were randomly selected for each of 9 treatments. These comprised 2 plastic formulations of antitranspirants: Vapor Gard (Miller Chemical and Fertilizer Corp., Hanover, Pa.) (polyterpene) and Polyethylene Emulsion (American Machinery Corp., Orlando, Florida), and 2 wax materials: Concord Alcoat SE-12 (Concord Chemical Company, Camden, New Jersey) (anionic wax emulsion) and Primafresh 23 (Johnson Wax, Racine, Wisc.) (a shellac base). Fruiting twigs were momentarily dipped in 0, 2.5 or 5% aqueous solutions of these materials. When the fruit surfaces appeared dry the stem bases were cut under water and transferred to test tubes which contained distilled water. Treatments were assigned to a completely randomized design under conditions of 22°C and 40% relative humidity to simulate room conditions.

The test tubes with and without the fruiting twigs were weighed at intervals of 3 to 4 days, from time zero to day 26. The loss in fresh weight of twigs was transformed to a per cent of the original weight and water uptake from the test tubes was determined. The latter was adjusted for evaporation by deduction of water loss from test tubes not containing twigs. The contribution of respiration was considered negligible and therefore water loss was assumed due to loss in fresh weight of twigs and fruit. Thus transpiration estimates were achieved by the sum of water uptake and loss in fresh weight of fruit and twigs

and both were standardized for a 10 g fruiting twig. In addition, ratings of fruit quality were made at each recording date.

Extra samples of control, Vapor Gard and Concord Alcoat SE-12 concentrations were used for respiration rate determinations. At intervals of 3 to 4 days samples of fruit were removed and placed in a Warburg Constant Volume Respirometer (Gibson Medical Electronics). Carbon dioxide evolved was absorbed with 10% KOH and oxygen uptake in µl/g/hr was determined at 25°C (103). At commencement of the experiment, parameters of moisture content in fruit expressed in per cent and fruit removal force were obtained as an indication of material quality. When treatments were significant the following orthogonal contrasts were conducted: control vs treatments; Vapor Gard vs other treatments; Concord Alcoat SE-12 vs Primafresh 23 and Polyethylene Emulsion; Primafresh 23 vs Polyethylene Emulsion; and 2.5% vs 5% concentrations of each material.

Fruit surface morphology. Epicarp samples of 5 untreated fruit of Ilex verticillata and Ilex opaca Ait. were prepared for observation with an Advanced Metals Research Model 900 scanning electron microscope (SEM). Samples were placed in liquid nitrogen and immediately transferred to a Virtis (model 10-010) automatic freeze-dryer where water was removed by sublimation. Five other samples were critical point dried using a graded series of ethanol, amyl acetate and liquid CO_2. The CO_2 was released as a gas in a Denton (DCP-1) critical point apparatus. In 1974, observations on the SEM were repeated for Ilex verticillata and 5 specimens from fruit treated with 5% Vapor Gard were included.

Fruit surface in cross section. Fruit epicarp specimens were embedded in Ames OCT compound at -20°C and sectioned at 2 µm on an International Equipment model CTD cryostat. When placed on slides these sections were stained with Sudan IV and IKI/H2SO4 for lipids and cellulose (54). A test for birefringence by polarized light was also carried out.

RESULTS

Holly fruit treated with Vapor Gard 5% and 2.5% was maintained in an excellent condition for 23 to 26 days. Whereas in all other treatments, including control, the quality deteriorated by day 17. The fruit and twigs contained 60 and 40% moisture respectively at commencement of the experiment. Fruit removal force was 240 g with 70% separation between the fruit and the pedicel, 20% between the pedicel and twig and the remaining 10% were breaks through the pedicel. Some fruit in a uniform distribution throughout the experiment were contaminated by a midge. These fruit turned black and a larvae emerged (Figure 5). Taxonomic research, by Mr. John Newman of the Entomology Department, Michigan State University, identified this insect as being Asphondylia illicicola Foote (the Holly Midge).

After day 7 treatment differences for loss in fresh weight were highly significant and orthogonal contrasts showed that fruiting twigs dipped in Vapor Gard were superior to all other treatments (Figures 1A and 2C). Effects of Primafresh 23 and Poltethylene Emulsion were similar to Concord Alcoat SE-12 (Figure 1B). The effects of Vapor Gard

reduced loss in fresh weight for example a mean of 3.7 g compared with 5.4 g for the control on day 23 (Figure 1A). Effects from 5% and 2.5% concentrations were similar.

Water uptake for all treatments was greater during the first 3 days than at any period later on but treatment differences were highly significant throughout the experiment. Uptake for twigs treated with Primafresh 23 was greater than control, but uptake for twigs of other treatments were less than or similar to the control. The least amount of water uptake was 6.4 ml for twigs treated with 5% Vapor Gard by day 20 (Figure 1C). The effects of Primafresh 23 and Polyethylene Emulsion were similar to Concord Alcoat SE-12 (Figure 1D). While the effects of Vapor Gard at 2.5% were similar to those of the control (Figure 1C), the combined mean of Vapor Gard treatments was significantly different from the mean of other antitranspirants (Figure 2D).

Transpiration differences were highly significant during the experiment. The effects of Primafresh 23 and Polyethylene Emulsion were similar to Concord Alcoat SE-12 (Figure 2B). The lowest cumulative values: 13.2 and 10.1 ml, were recorded for twigs treated with Vapor Gard 2.5 and 5% on day 20 (Figure 2A). These combined treatments were superior to all other antitranspirants in reducing transpiration. The largest amount of cumulative transpiration on day 20 was 15.4 ml recorded for 5% Primafresh 23 and the control was 14.7 ml.

In general an equal decline in the respiration rate was observed for the control and Vapor Gard treatments (Figures 3A and 3B). Fruit treated with Concord Alcoat SE-12 reached lower values earlier, but experienced an increase from day 17 to day 19 and then declined (Figure 3C).

Figure 1 A-D A-B loss in fresh weight (g)

C-D water uptake (ml)

of fruiting branches of Ilex verticillata (L.) Gray as affected by Vapor Gard, Concord Alcoat and control.

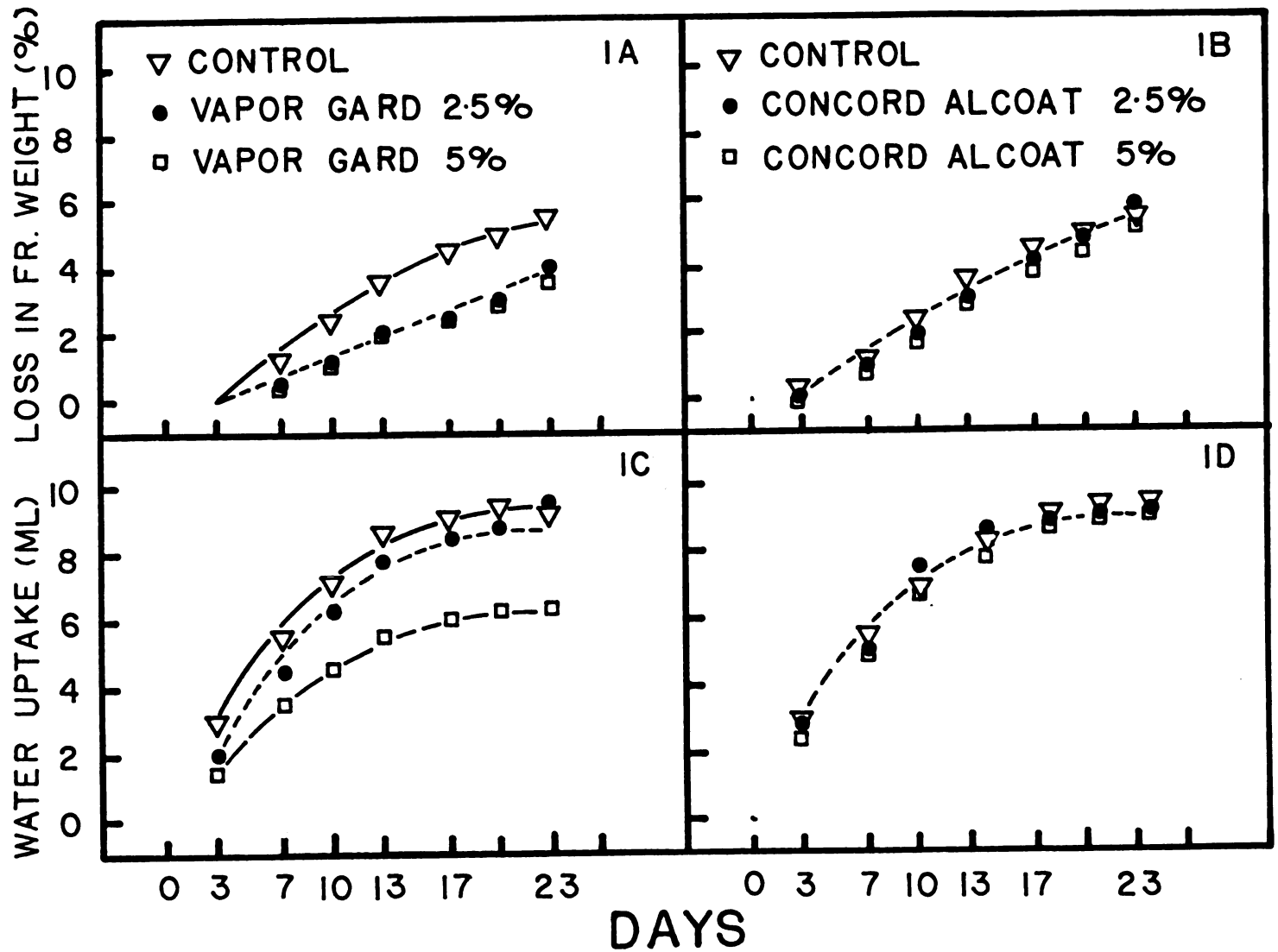

LOSS IN FR. WEIGHT (%)
WATER UPTAKE (ML)
0
2
4
6
8
10
1A
CONTROL
VAPOR GARD 2·5%
VAPOR GARD 5%
1B
CONTROL
CONCORD ALCOAT 2·5%
CONCORD ALCOAT 5%
1C
1D
0 3 7 10 13 17 23
DAYS

Figure 2 A-D Loss in fresh weight (g), water uptake (ml) and transpiration (ml) of fruiting branches of Ilex verticillata (L.) Gray.

A--transpiration as affected by control, Vapor Gard 5% and 2.5%

B--transpiration as affected by control, Concord Alcoat 5% and 2.5%

C--loss in fresh weight as affected by control, Vapor Gard, other treatments

D--water uptake as affected by control, Vapor Gard, other treatments

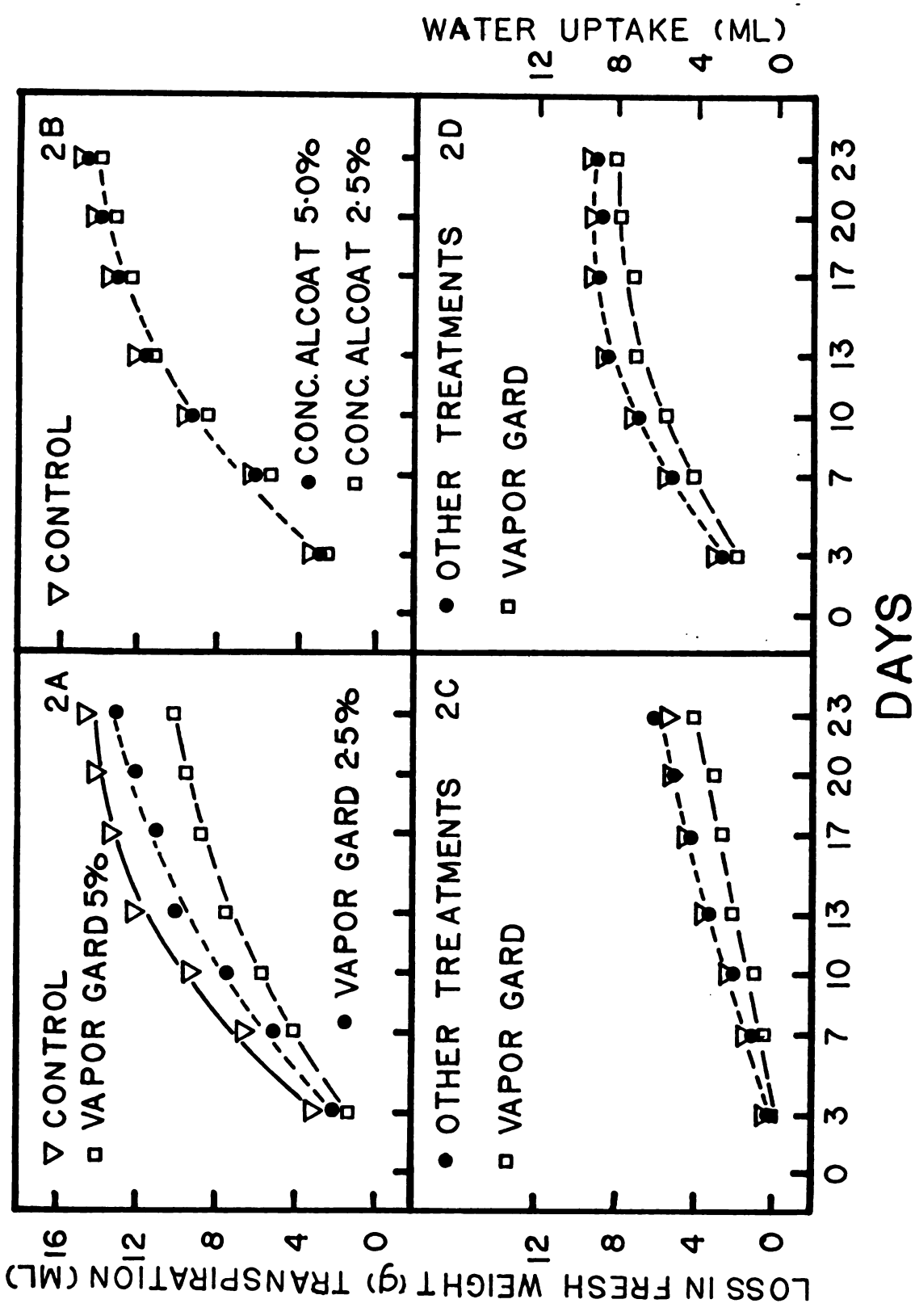
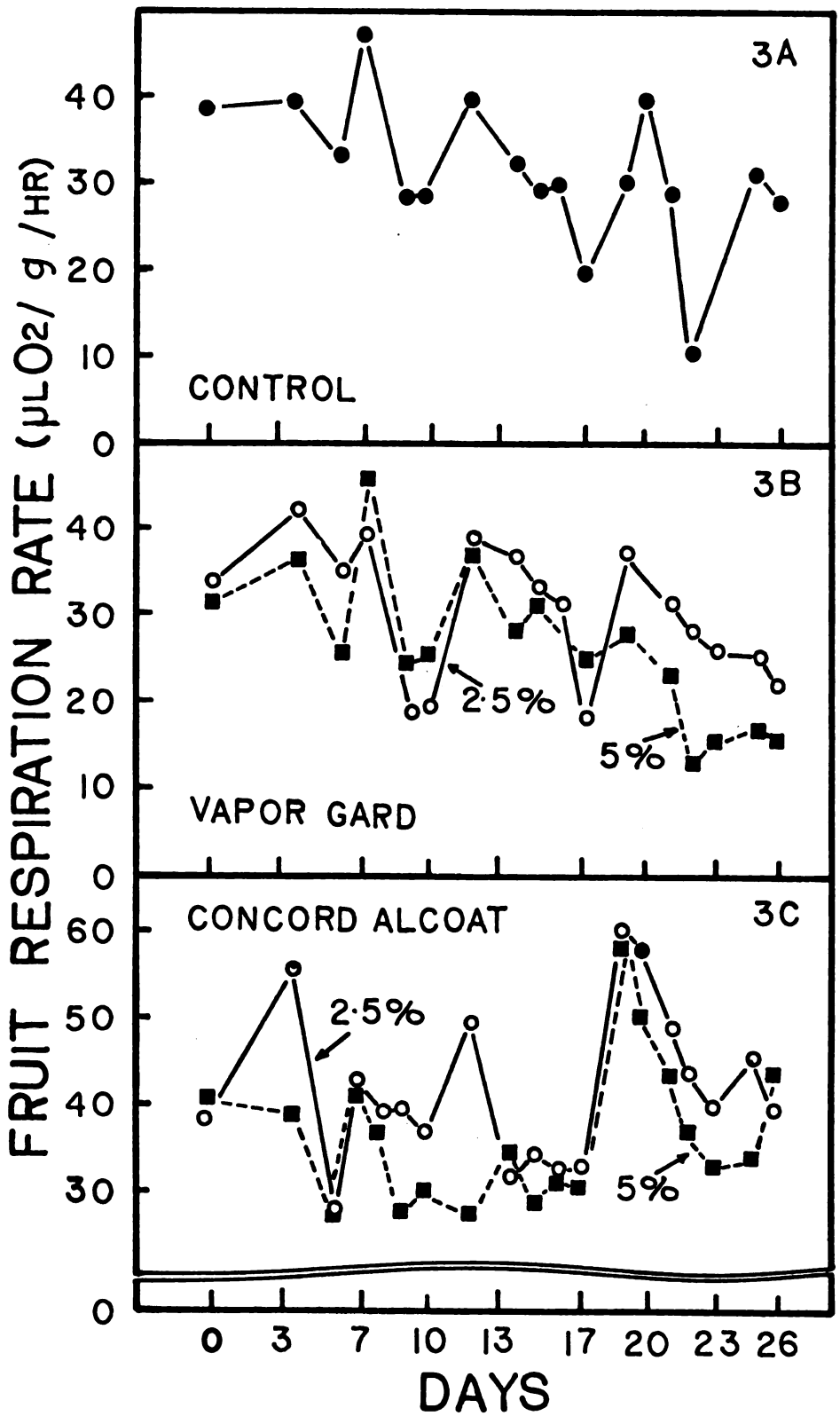

WATER UPTAKE (ML)
12
8
4
0
2B
▽ CONTROL
• CONC. ALCOAT 5·0%
▫ CONC. ALCOAT 2·5%
2D
• OTHER TREATMENTS
▫ VAPOR GARD
2A
▽ CONTROL
▫ VAPOR GARD 5%
• VAPOR GARD 2·5%
2C
• OTHER TREATMENTS
▫ VAPOR GARD
0 3 7 10 13 17 20 23
DAYS
LOSS IN FRESH WEIGHT (g) TRANSPIRATION (ML)
16
12
8
4
0

Figure 3 A-C Respiration rate in µl O_2/g/hr uptake of *Ilex verticillata* (L.) Gray fruit as affected by control, Vapor Gard and Concord Alcoat.


3A
CONTROL
3B
VAPOR GARD
2·5%
5%
CONCORD ALCOAT
3C
2·5%
5%
FRUIT RESPIRATION RATE (µLO2/ g /HR)
0
10
20
30
40
60
50
0 3 7 10 13 17 20 23 26
DAYS

Fruit surface morphology. The raised stylar end of Ilex verticillata fruit had surface waxes which were a mixture of irregular cylinders and spheres partially covered by a smoother wax (Figures 6A and 6B). In the 1973 specimens, fruit surface had few stomata and impressions of epidermal cells were evident (Figures 6C and 6D). Low magnification views of 1974 fruit showed similarities to 1973 material. Pictures at high magnification revealed smoother wax formations near the calyx end of the fruit (Figure 7A) compared with the stylar end (Figure 8A).

Specimens of fruit treated with Vapor Gard contained platelets of the antitranspirant at the calyx end (Figure 9B), and a tangled braided appearance toward the stylar end of the fruit (Figure 9D). Compared with the rest of the fruit surface the ciliated calyx lobes contained more wax which was abundant on the outer rim (Figures 7A and 7B). More epicuticular waxes were observed on the pedicel than on the fruit surface (Figure 7C).

Wax formations on the fruit of I. opaca contained spherical structures and epidermal cell impressions were not evident (Figure 7D). The calyx had many stomata (Figure 8B). Samples of 2 year old fruit had few spherical particles and there was evidence of a break in the continuity of the underlying surface wax (Figures 8C and 8D).

Fruit surface in cross section. The cuticle was identified as being a non cellular, colorless covering over red epidermal cells (Figure 4A) and was isotropic when viewed by plane-polarized light. When stained with Sudan IV, the cuticle was seen to project between epidermal, anticlinal cell walls (Figure 4C). By use of cellulose stains (Figures 4B and 4D) it was observed that the periclinal epidermal cell walls were cutinized.

Figure 4 A-D Cross sectional views of Ilex verticillata (L.) Gray fruit epicarp at 2 um

A--unstained epicarp

B, D-- IKI/H_2SO_4 stain

C--Sudan IV stain

4A
2 µm
4B
3 µm
4C
0.7 µm
4D
0.7 µm

Figure 5. Asphondylia illicifolia Foote (Holly Midge) larvae emergence from fruit.

Figure 6 A-D Morphology of fruit surface. Scanning electron micrographs of Ilex verticillata (L.) Gray.

A-B -- stylar end

C-D -- fruit surface

6A
8 µM
6B
16 µM
6C
8 µM
6D
0.4 µM

Figure 7 A-D Morphology of fruit surface. Scanning electron micrographs of fruit surfaces from

A-B--*Ilex verticillata* (L.) Gray calyx end

C--*Ilex verticillata* (L.) Gray pedicel

D--*Ilex opaca* Ait. fruit surface

B--critical point dried

7A
40 µm
7B
40 µm
7C
1.6 µm
7D
4 µm

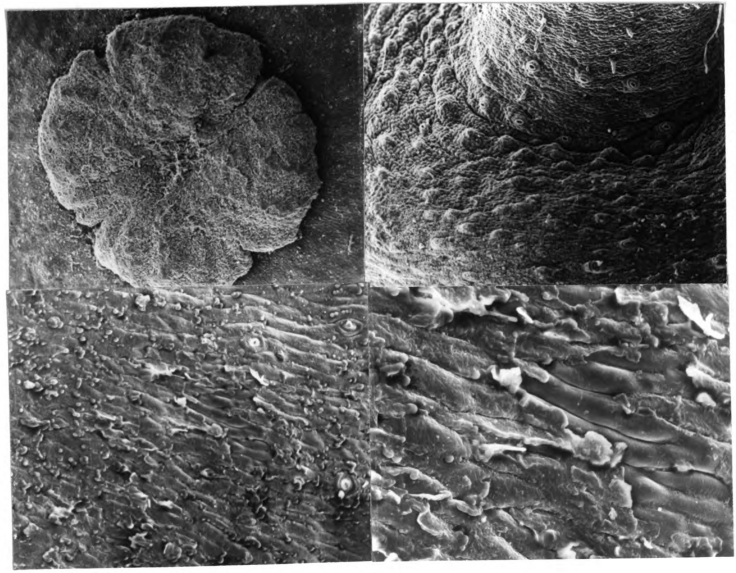
Figure 8 A-D Morphology of fruit surface. Scanning electron micrographs of Ilex opaca Ait.

yA--stylar end

yB--calyx end

zC-D--fruit surface

y = 2 year old fruit z = 1 year old fruit



8A
16 µm

8B
8 µm

8C
4 µm

8D
1.6 µm

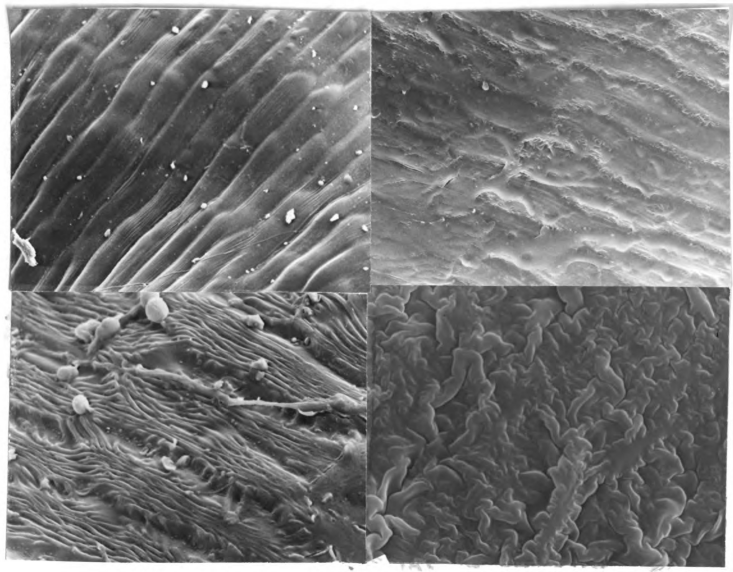
Figure 9 A-D Morphology of fruit surface. Scanning electron micrographs of Ilex verticillata (L.) Gray

A--fruit surface near calyx end (control)

B--fruit surface near calyx end (Vapor Gard treatment)

C--fruit surface near stylar end (control)

D--fruit surface near stylar end (Vapor Gard treatment)



9A

1.6 µm

9B

1.6 µm

9C

0.8 µm

9D

1.6 µm

DISCUSSION

When fruiting branches of *Ilex verticillata* were harvested and their stem ends placed in water, the glossy red colored fruit lost their sheen and shrivelled up within a period of 10 to 14 days. This keeping quality was extended during preliminary work by top dipping fruiting branches in wax and plastic based antitranspirants. A further experiment was conducted using 4 of these compounds, the most effective of which was Vapor Gard. When applied at 2.5% the keeping quality of common winterberry fruit was extended to 26 days. This considerable increase over the control (10 to 14 days) was a result of reduced transpiration. These results agree with work on other plant species using antitranspirants such as Vapor Gard and silicone (10, 31, 48). Transpiration for Vapor Gard 2.5 and 5% treatments after a period of 23 days was 13.2 and 10.1 ml respectively (Figure 2A).

One would expect the effects of a 5% treatment to be greater than or equivalent to a 2.5% treatment. This was the case with loss in fresh weight, water uptake and transpiration. However, observations of fruit revealed a loss in keeping quality with 5% treatments 3 days earlier than with 2.5% treatments. This difference was due to inhibition of water uptake by twigs and fruit treated with 5% Vapor Gard (Figure 1C). Under similar conditions, antitranspirants have been reported to reduce water uptake by sweet cherry fruit (30).

In addition to the above advantages, fruit treated with Vapor Gard exhibit a greater gloss than untreated fruit. The antitranspirant material providing this gloss was easily observed by means of the SEM

(Figures 9B and 9D). Positive identification of such material was not established when the fruit cuticle was viewed in cross section under the light microscope.

Differences in gas exchange between control, Vapor Gard and Concord Alcoat SE-12 treatments, as measured by oxygen uptake, were slight (Figures 3A, 3B and 3C). Respiration rates for fruit treated with Concord Alcoat SE-12 (Figure 3C) displayed a different pattern from the control or those treated with Vapor Gard (Figures 3A and 3B). Inhibitory effects of Vapor Gard on photosynthesis have been reported (31). Results of this experiment, on respiration rate, do not show an inhibition of gas exchange with I. verticillata stems and fruit.

In previous experiments when the stems of untreated and antitranspirant treated fruiting twigs were inserted in water (without being cut under water) a small quantity of fruit later abscised. This occurred in greater frequency when the fruit were shrivelled and the twigs handled roughly. In this experiment all stems were cut under water to prevent entry of air, which would interfere with water uptake, into the vascular tissue. A faster rate of water loss may increase fruit abscission because keeping quality of fruit was maintained 3 to 4 days longer than in previous experiments and abscission was limited to the fruit infected with the holly midge larvae. Since all treatments were uniformly infected with this insect, its contribution to treatment variances was ignored. This pest is more prevalent in evergreen hollies where the larvae pupate and the adult emerges in spring (45).

SECTION III

PHYSIOLOGY OF FRUIT ABSCISSION IN *ILEX VERTICILLATA* (L.) GRAY

The process of organ separation from plants is termed abscission. Early studies on fruit abscission were with immature fruit but with the advent of mechanical harvesters and the high cost of labor, research on abscission of mature fruit was initiated.

Avocado, mango (11), cherry (100) and citrus fruit (110) abscise at the upper zone between the pedicel and the twig when immature, and at the lower zone between the pedicel and the fruit when mature. Small cells are typical of such zones (106) and the process may involve cell division (41), middle lamella dissolution and part of the primary wall or entire cell disintegration (20).

The characteristics of abscission layer formation in mature sour cherry fruit were outlined by Stosser et al 1969 (99). The abscission layer (first detected by less affinity for haematoxylin stain) develops by middle lamella dissolution and cell collapse and does not include the vascular tissue and peripheral cells. In sweet cherry, histochemical differences were not observed (111). The abscission process in apples has been described by MacDaniels 1936 (59) and McCown in 1938 (64) and 1943 (65).

Changes in pectin (32, 71, 83), calcium (81, 99), polysaccharides (84, 100, 111) and cellulose (84) during abscission layer formation have been documented. Various growth regulators are reported to affect abscission. Auxin treatments distal to the abscission zone: or applied early or in high concentrations (10^{-3}M) delayed abscission. Whereas proximal, late applications or low concentrations (10^{-6}M) increased abscission (6, 16, 40, 88). Leaf fall in cut holly was reduced by application of α-naphthaleneacetic acid (NAA) during storage (68). Ethylene increased abscission (14, 15, 47) and auxin applications

enhanced abscission by stimulation of ethylene production (3, 19, 74). This gas was also produced by such compounds as (2-chloroethyl) phosphonic acid (ethephon) which decomposed to release ethylene, and cycloheximide (CH) which caused ethylene production by tissue injury.

CH therefore has been associated with an increase in abscission in citrus fruit (25). But it has also delayed abscission in explants of cotton, bean and Coleus (2) and sour and sweet cherry (112) probably by inhibition of de novo protein synthesis. Gibberellic acid A_3 (GA) delayed (13, 20) and enhanced abscission (20, 112). Ga also caused ethylene production (1, 26) and recent evidence indicates that it promotes ethylene induced abscission (70). Response from cytokinins varies with concentration and position of application. Abscission was accelerated with distal applications in beans (75) and proximal applications in cotton (13), whereas placement on the abscission zone in bean delayed abscission (75). High concentrations (10^{-2}M) delayed and low concentrations (10^{-5}M) increased abscission of the primary leaf in bean if the opposite leaf was intact (21, 83). Alar (Succinic acid -2,2-dimethylhydrazide, SADH, B-9), an inhibitor of gibberellin biosynthesis is associated with increasing firmness and delaying senescence in apples (57). Senescence of peaches and cherries (104) was advanced but only peach abscission (39) was increased with alar treatment.

These experiments were designed to investigate the abscission process in fruit of Ilex verticillata (L.) Gray using growth regulators and studying anatomical changes in the pedicel. The objective was to maintain keeping quality of detached fruiting branches by application of growth regulator and antitranspirant treatment combinations.

MATERIALS AND METHODS

Two plants of Ilex verticillata were selected for a growth regulator study. Individual branches on all sides of each plant were randomly selected and sprayed with: GA, NAA, CH and sodium azide (NaN_3) at 10^{-5}, 10^{-4} and 10^{-3}M concentrations; ethephon at 10, 100 and 1000 ppm, plus water as a control. The surfactant X77 at a final concentration of 0.1% was added to all temperatures. Some branches were sprayed on September 1; others on September 15 and an additional number on both dates. The treatments were replicated 5 times, in a completely randomized design.

Fruit removal force (FRF) was determined with a Hunter Mechanical Force Gauge (Hunter Springs, Hatfield, Pa) for 5 fruit in each replication on September 1 and at weekly intervals thereafter until November 17. The position of fruit separation from the twig was recorded. Abscission between the fruit and the pedicel was termed distal; between the pedicel and the stem proximal; and breakage through the pedicel was described as intermedial.

Anatomical studies were conducted on pedicel tissue selected from the control and 1000 ppm ethephon treatments at each recording date. Detached specimens were immediately killed and fixed in formalin-acetic caid-alcohol (54, 91). Three embedding media: paraffin, Spurr's firm epoxy resin mixture and glycol methacrylate plastic resin were used. The paraffin technique involved dehydration in a gradient tertiary butyl alcohol series and embedding in Tissuemat (melting range 56-58°C) (54). Ten to 12 μm sections were cut on a Leitz Wetzlar rotary microtome and affixed to glass slides with Haupt's adhesive.

The slides were then placed on a warming plate and flooded with 4% formalin to expand and flatten the sections. After removal of paraffin the sections were stained with safranin/fast green combinations and iron haematoxylin.

Infiltration with Spurr's firm epoxy resin mixture (51) was achieved in graded steps after ethanol dehydration. Resin (100%) infiltration was facilitated by subjecting the samples to a high pressure for 12 hours in an Emerson Bomb Fuel Calorimeter. The pressure was increased to 250 kg/6.25 cm^2 with nitrogen gas. Sections at 6 µm were cut on a Leitz Wetzlar and at 2 µm on a Porter Blum MT-2 ultra microtome and stained with toluidine blue.

The third resin, a monomer of glycol methacrylate is described by Feder and O' Brien (33). Fixed specimens were dehydrated at 0°C by transfer through solvents, each of which was changed twice in a 48 hour period, methyl cellosolve (synonyms: ethylene glycol monomethyl ether, 2-methoxyethanol), ethanol, n-propanol and n-butanol in that order. The monomer was hydroxyethyl methacrylate -96% (Rohm and Haas Company, Philadelphia, Pa.) in which final concentrations of 0.5% (w/v) of 2,2-azobis (2-methylpropionitrile) (Fisher Scientific Company) (the catalyst) and 5% (v/v) polyethylene glycol 400 (Fisher Scientific Company) (the plasticizer) were dissolved. Dehydrated specimens were transferred at room temperature from n-butanol to the monomer mixture. Following two 24 hour changes infiltration was aided by high pressure as stated above. Polmerization was obtained in gelatin capsules, size #00, at 38°C for 2 days and the temperature increased to 55°C for a further day. Sections, 2 µm in thickness, were cut on the ultramicrotome and stained with toluidine blue (51); Periodic acid-Schiff (PAS) stain for total polysaccharides (33);

hydroxylamine-ferric chloride for pectins; phlorglucinol for lignin (54). All determinations were performed at least 3 times.

In 1974, a plant was selected and FRF was periodically determined from fruit set to maturity (July 8 through October 18). The position of separation was identified and recorded as above. Samples of pedicels at each recording date beginning on September 14 were carried through the procedure of glycol methacrylate resin infiltration, sectioning and staining.

Laboratory studies 1. Fruiting branches with leaves obtained from a single plant, on September 27, 1973 were stored in 10 µm opaque polyethylene bags at 5°C for 8 days. After removal from storage uniformly fruiting twigs approximately 15 cm in length were selected. Treatments consisted of dipping approximately 14 cm of each twig in an antitranspirant material and inserting the untreated proximal ends in water containing alar at 0, 10^{-3}, 10^{-4} and 10^{-5}M concentrations. This was contained in bottles (2.3 cm diameter, 8 cm high). Antitranspirant materials included: Vapor Gard (polyterpene) (Miller Chemical and Fertiliser Corp., Hanover, Pa.) 5% and 2.5%, Geon Latex (B.F. Goodrich Chemical Co., 6100 Oak Tree Blvd, Cleveland, Ohio) 5% and water dips for control.

The environment was 24°C and 40% relative humidity and treatment combinations were replicated 4 times in a completely randomised design. Fresh weight of the fruiting twigs (after drying the stem end with tissue paper) was recorded at intervals of 2 to 3 days from October 6 until October 26 when shrivelled fruit and twigs were discarded. Other treatments were continued until October 31. Data on loss in fresh weight was transformed to a per cent of original fresh weight

and analyses of variance carried out for days 7 and 17. In addition, qualitative observations of fruit condition were made at each recording date.

Fruiting branches from a single plant obtained on October 9, 1973 were prepared as above and the experiment initiated on October 12. Factorial treatment combinations consisted of top dipping the fruiting twigs in antitranspirants and transferral to various media combinations after drying. The antitranspirant materials included: Vapor Gard and Geon Latex at 2.5%, controls were top dipped in water. Media treatments were: CH, NaN_3 and NAA at 10^{-3} and 10^{-4}M concentrations; calcium chloride 4%, sucrose 2%, 8 hydroxyquinoline sulfate (8 HQS) 200 ppm, water as a control. Half of the material was further treated by addition of polyvinylpyrrolidone (PVP) (a phenol inhibitor) to the media. Proximal ends of fruiting twigs were inserted in media combinations contained in bottles. Treatment combinations were replicated 5 times and randomly distributed in environmental conditions similar to those of the previous experiment. Fresh weight determinations were made on October 14 and at intervals of 2 to 3 days until November 3 (20 days). On October 17, leaves present at collection of plant material abscised and further data on weight loss was transformed as a percentage of October 17 readings. Analyses of variance was conducted on per cent loss in fresh weight for days 8 and 10, and qualitative information on keeping quality of treatments was collected at each recording date.

In another experiment, leafless plant material was collected and prepared as in the previous experiment. On November 2, 1973, before stem ends of fruiting twigs were inserted in bottles of water their

distal ends were dipped in solutions of NaN_3 40, 70 and 100 ppm; NAA 10, 20 and 30 ppm; 8 HQS 100, 200 and 300 ppm; CH 15, 30 and 45 ppm; kinetin (KIN) and benzyladenine (BA) at 10^{-4}, 10^{-5} and 10^{-6}M; calcium chloride 4 and 8% and controls were dipped in water. Upon drying, one half of the total number received a further dip in Vapor Gard 2.5% and the remainder were dipped in water. All were transferred to the bottles. Treatment combinations were replicated 5 times in a completely randomized design with the same environmental conditions as before. Loss in fresh weight determinations were transformed to per cent of original fresh weight for statistical analyses on days 10 and 17 and qualitative information was collected on each recording date.

RESULTS

Field experiment. Within 1 week of spraying, leaf and fruit abscission occurred on branches treated with ethephon at 1000 ppm and CH at 10^{-3}M concentrations. This effect was most pronounced on the September 1 treatments. FRF for these and all other chemical treatments were similar to the control of 272 g after 1 week and 342 g after 2 weeks (Table 1). FRF for all applications on September 1 and 15 were similar. Average frequencies of abscission types were 65% distal, 30% proximal and 5% intermedial.

The paraffin technique was satisfactory for soft summer material but was unsuccessful with more lignified pedicels taken in the fall when excessive tearing was experienced during sectioning. The pedicel, in longitudinal section approximately 2 weeks after corolla abscission

Table 1. Fruit removal force (FRF) (g) of Ilex verticillata (L.) Gray as affected by September 1 application of 10^{-5} M, 10^{-4}M, 10^{-3}M concentrations of gibberellic acid A3 (GA), α naphthaleneacetic acid (NAA), cycloheximide (CH), sodium azide (NaN3); 10, 100 and 1000 ppm (2-chloroethyl) phosphonic acid (ethephon) and control for intervals of 1 to 4 weeks from September 9 to November 17.

	Fruit removal force (g)					
Treatment	Sept. 8	Sept. 15	Sept. 22	Oct. 6	Oct. 20	Nov. 17
GA 10^{-5}M	263	337	305	316	248	335
GA 10^{-4}M	288	370	318	306	308	354
GA 10^{-3}M	283	337	318	306	356	358
NAA 10^{-5}M	294	352	294	297	358	289
NAA 10^{-4}M	250	274	290	314	315	---
NAA 10^{-3}M	306	356	301	313	356	323
CH 10^{-5}M	284	336	320	301	347	299
CH 10^{-4}M	267	319	265	311	332	---
CH 10^{-3}M	242	330	271	278	314	---
NaN3 10^{-5}M	284	362	335	342	341	---
NaN3 10^{-4}M	301	366	346	333	333	297
NaN3 10^{-3}M	283	342	302	332	309	304
Ethephon 10 ppm	264	383	341	324	335	355
Ethephon 100 ppm	297	353	291	309	341	318
Ethephon 1000ppm	272	374	307	317	359	---
Control	272	342	343	353	323	352
Total mean	278	346	309	316	336	325

Initial FRF on September 1 = 244 g

--- = missing data

and stained with haematoxylin, is depicted for the distal or fruit end in Figure 1A and the twig or proximal end in Figure 1B.

The method of Spurr's firm epoxy resin mixture infiltration was an improvement over the paraffin technique. However, tearing of sections was also encountered due to poor resin infiltration. Resin penetration was facilitated by pressure but some component of the pedicel inhibited its polmerization. Sections exhibited little evidence of structural changes at any point along the pedicel (Figure 1C).

The most successful embedding media was glycol methacrylate. Polmerization was a lengthy procedure but it sectioned quite easily on the ultramicrotome. Structural changes indicative of abscission layer formation, were not evident in untreated pedicels or those treated with ethephon. There were no changes in total polysaccharides or pectins. Sclereids in the cortex were associated with the vascular tissue and identified by means of toluidine blue (Figure 1D), and phlorglucinol (Figure 2A) stains. This layer of cells, continuous from the calyx tip to the stem end of the pedicel and discontinuous in cross section, was occasionally ruptured during sectioning. Separation of cells in the cortex was observed from the proximal end of the pedicel, continued along its entire length through the distal end (Figure 2B) to the peripheral cells in the calyx tip (Figures 2C and 2D).

From fruit set to maturity the frequency of abscission in the distal zone (between fruit and pedicel) increased from approximately 40% in July to 80% in October (Figure 3A). Breakage through the pedicel (intermedial type) was relatively infrequent in the early and late stages of fruit development but occurred with approximately

Figure 1 A-D Longitudinal section of Ilex verticillata (L.) Gray pedicel.

A--distal or fruit end B--proximal or twig end 2 weeks after corolla abscission, stained with haematoxylin

C--distal end on September 8, 1974 stained in toluidine blue and viewed with a phase microscope

D--center of pedicel on September 15, 1974 stained in toluidine blue

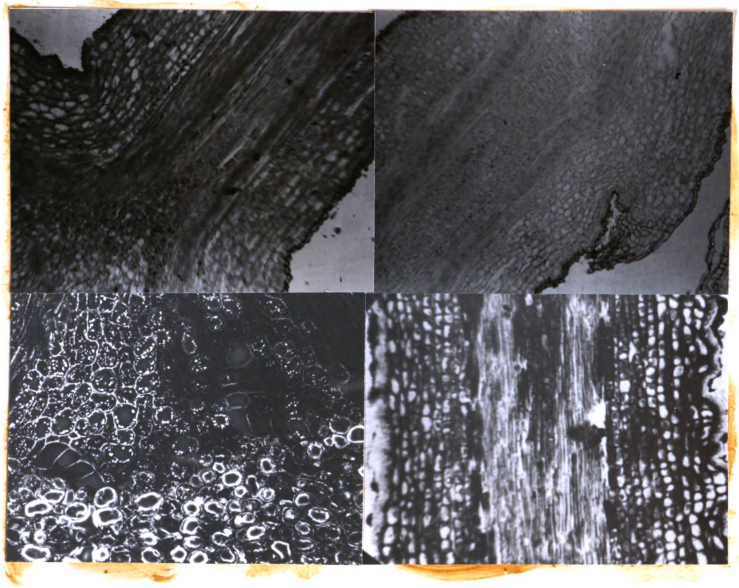

1A
5 µm
1B
5 µm
1C
4 µm
1D
2 µm

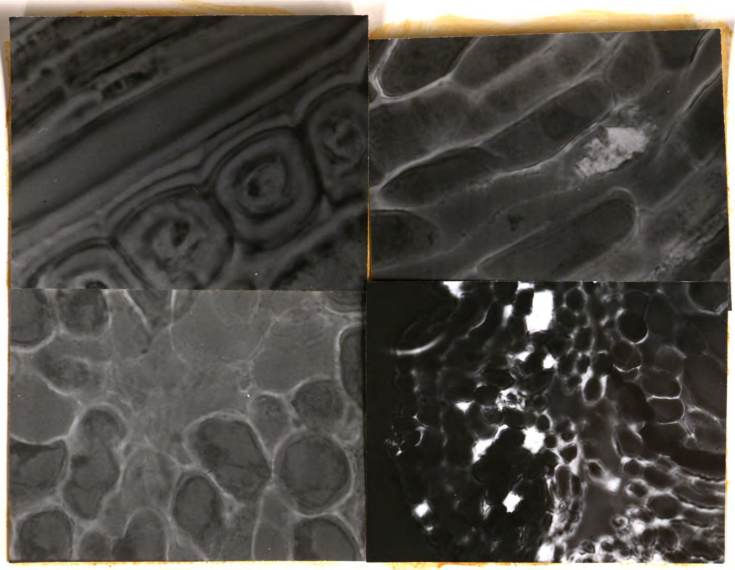
Figure 2 A-D Longitudinal section of Ilex verticillata (L.) Gray pedicels. Glycol methacrylate resin, bright field microscope.

A--sclereids in center of pedicel, phlorglucinol stain

B--cortex juncture of pedicel and calyx stained with toluidine

C--cell lysis at calyx tip (high magnification)

D--cell lysis at calyx tip (low magnification)



2A

0.8 µm

2B

1.4 µm

2C

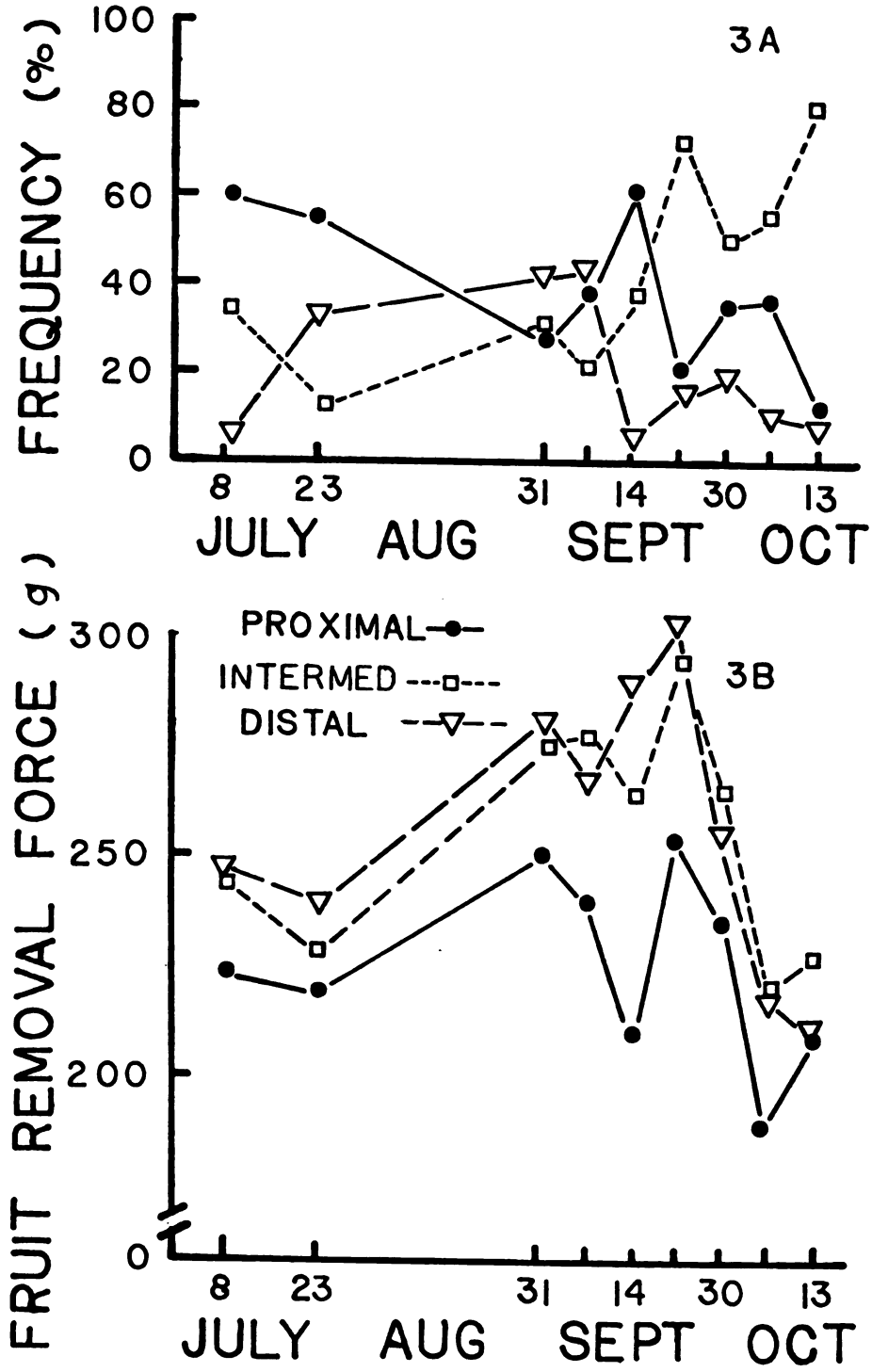
1.4 µm

2D

4 µm

Figure 3 A-B A-- frequency (%) of proximal, intermedial and distal abscission

B-- fruit removal force (g) of Ilex verticillata (L.) Gray fruit from July 8 through October 13, 1974.


3A
FREQUENCY (%)
100
80
60
40
20
0
8
23
31
14
30
13
JULY
AUG
SEPT
OCT
3B
PROXIMAL
INTERMED
DISTAL
FRUIT REMOVAL FORCE (g)
300
250
200
0
8
23
31
14
30
13
JULY
AUG
SEPT
OCT

40% frequency from July 23 to September 8. Proximal abscission (between twig and pedicel) declined from 60% frequency in July to 28% in late August. By September 14 frequency was again 60% and then declined to 12% on October 13.

FRF for intermedial and distal abscission increased from a mean value of 235 g in July to 295 g on September 22 and then decreased to 218 g on October 13 (Figure 3B). FRF for proximal abscission, which were in the region of 20 g less than intermedial or distal, followed a similar pattern except on September 14 when they declined to 210 g expanding this difference to 65 g. In anatomical studies of the pedicel in longitudinal section, structural or staining differences were not detected.

Laboratory studies. Water loss indexed by % loss in fresh weight of fruiting branches as affected by 10^{-3}, 10^{-4} and 10^{-5}M concentrations of alar and all antitranspirant treatments were similar to the control. Keeping quality of the fruit was maintained for approximately 1 week with controls; 9 to 10 days with alar and 14 to 21 days with antitranspirants.

Loss in fresh weight with NaN3 10^{-4}M treatments was 18.4% on day 8 and insignificant from the control (24.6%). On day 8, means of CH 10^{-3}M (32.5%) and NaN3 10^{-3}M (29.2%) solutions were larger than and significantly different from the control, and on day 20 the same was true for CH 10^{-3}M (41.2%) and 8 HQS (46.5%) solutions. All other treatments were similar to the control on day 20 (Table 2).

By day 8 the addition of PVP caused greater loss of weight compared with a control but by day 20 values for both treatments reached similar levels (Table 3). The interaction between media treatment and PVP was significant on day 8 but not on day 20. The control

Table 2. Loss in fresh weight (%) of *Ilex verticillata* (L.) Gray fruiting branches when proximal ends were inserted in 10^{-3}M and 10^{-4}M concentrations of α-naphthaleneacetic acid (NAA), cycloheximide (CH) and sodium azide (NaN_3); calcium chloride 4%; sucrose 2% and 8 hydroxyquinoline sulfate (8 HQS) 200 ppm and water (control) at days 8 and 20.

Treatment	Loss in fresh weight (%)	
	Day 8	Day 20
NAA 10^{-3}M	23.0a	36.1a
NAA 10^{-4}M	26.4a	39.6a
CH 10^{-3}M	32.5b	41.2b
CH 10^{-4}M	27.2a	38.1a
NaN_3 10^{-3}M	29.2b	37.4a
NaN_3 10^{-4}M	18.4a	37.0a
Calcium chloride 4%	23.4a	30.2a
Sucrose 2%	28.5a	40.4a
8 HQS 200 ppm	27.8a	46.5b
Control	24.6a	37.9a

Means within a column followed by a different letter are significantly different at the 0.05 level with Tukey's HSD test (98).

Table 3. Loss in fresh weight (%) of Ilex verticillata (L.) Gray fruiting branches when the proximal ends were inserted in various media containing polyvinylpyrrolidone (PVP) 1% and control on days 8 and 20.

	Loss in fresh weight (%)	
Treatment	Day 8	Day 20
PVP 1%	30.7a	39.1a
Control	21.5b	37.9a

Means within a column followed by a different letter are significantly different at the 0.05 level with Tukey's HSD test (98).

Table 4. Loss in fresh weight (%) of Ilex verticillata (L.) Gray fruiting branches when the proximal ends were inserted in various media and the distal ends dipped in water (control) and 5% concentrations of Vapor Gard and Geon Latex.

	Loss in fresh weight (%)	
Treatment	Day 8	Day 20
Vapor Gard 5%	20.8b	33.3c
Geon Latex 5%	27.3a	39.5b
Control	30.3a	42.6a

Means within a column followed by a different letter are significantly different at the 0.05 level with Tukey's HSD test (98).

plus PVP combination on day 8 lost 12.2% of its weight whereas without PVP, weight loss was 38.8% (Table 5). Some treatments showed the opposite effect for example CH 10^{-3}M and NaN_3 10^{-4}M (Figure 4A).

Antitranspirants significantly reduced weight loss. Vapor Gard 5% treatments resulted in 20.8% weight loss on day 8 compared with 30.3% for the control and 33.3% versus 42.6% on day 20. Geon Latex treatments were similar to the control on day 8 but were statistically separated from it on day 20 (Table 4).

Fruit in NaN_3 10^{-4}M and 8 HQS treatments were in good condition for 12 days; all other treatments for 6 to 9 days. Treatment combinations, with and without PVP maintained keeping quality for 7 and 12 days respectively. Antitranspirant treatments prevented loss of fruit turgidity for 14 to 21 days and the control treatments shrivelled in 7 days.

In the experiment on top dipping of fruiting twigs, the effects of various chemicals were similar to the control. On days 10, 17 and 22 significant differences were recorded for antitranspirant treatments and the interaction between chemical dips and the antitranspirants. Water loss indexed by loss in fresh weight of fruiting branches was reduced by application of Vapor Gard (Figure 4B). Loss in fresh weight of fruiting twigs when dipped in water or most chemicals and later dipped in Vapor Gard was usually less than those not treated with Vapor Gard. NaN_3 100ppm and the control in Figures 5A and 5B are an example. CH 45 ppm and BA 10^{-4}M treatments on days 10 and 17 respectively lost more weight when treated with Vapor Gard than when untreated (Figures 5A and 5B). Keeping quality, evidenced by fruit condition, lasted approximately 10 days for controls and 23

Table 5. Loss in fresh weight (%) of Ilex verticillata (L.) Gray fruiting branches for the interaction between proximal insertion in 10^{-3}M and 10^{-4}M concentrations of α naphthaleneacetic acid (NAA), cyclohexiimide (CH) and sodium azide (NaN3); calcium Chloride 4%; sucrose 2% and 8 hydroxyquinoline sulfate (8 HQS) 200 ppm and water (control) with and without the addition of polyvinylpyrrolidone (PVP) 1% on day 8.

	Loss in fresh weight (%)	
Media	Without PVP	With PVP
NAA 10^{-3}M	19.7abcd	26.1abcde
NAA 10^{-4}M	16.0ab	39.4e
CH 10^{-3}M	29.0bcde	39.2e
CH 10^{-4}M	22.7abcde	33.6de
NaN3 10^{-3}M	31.1cde	36.3de
NaN3 10^{-4}M	12.0a	32.7cde
Calcium chloride 4%	20.3abcd	26.9abcde
Sucrose 2%	22.8abcde	33.5cde
8 HQS 200 ppm	12.3ab	46.9f
Control	38.8e	12.2ab

Means within a column followed by a different letter are significantly different at the 0.05 level with Tukey's HSD test (98).

Figure 4 A-B Loss in fresh weight (%) of Ilex verticillata (L.) Gray fruiting branches.

A--8 days after proximal ends were inserted in cycloheximide (CH) 10^{-3}M, sodium azide (NaN_3) 10^{-3}M and control with and without the addition of polyvinylpyrrolidone (PVP). Vertical lines indicate standard deviations above the mean.

B--when the tops were treated with Vapor Gard 2.5% and compared with a control dipped in water. Vertical lines indicate standard deviations.

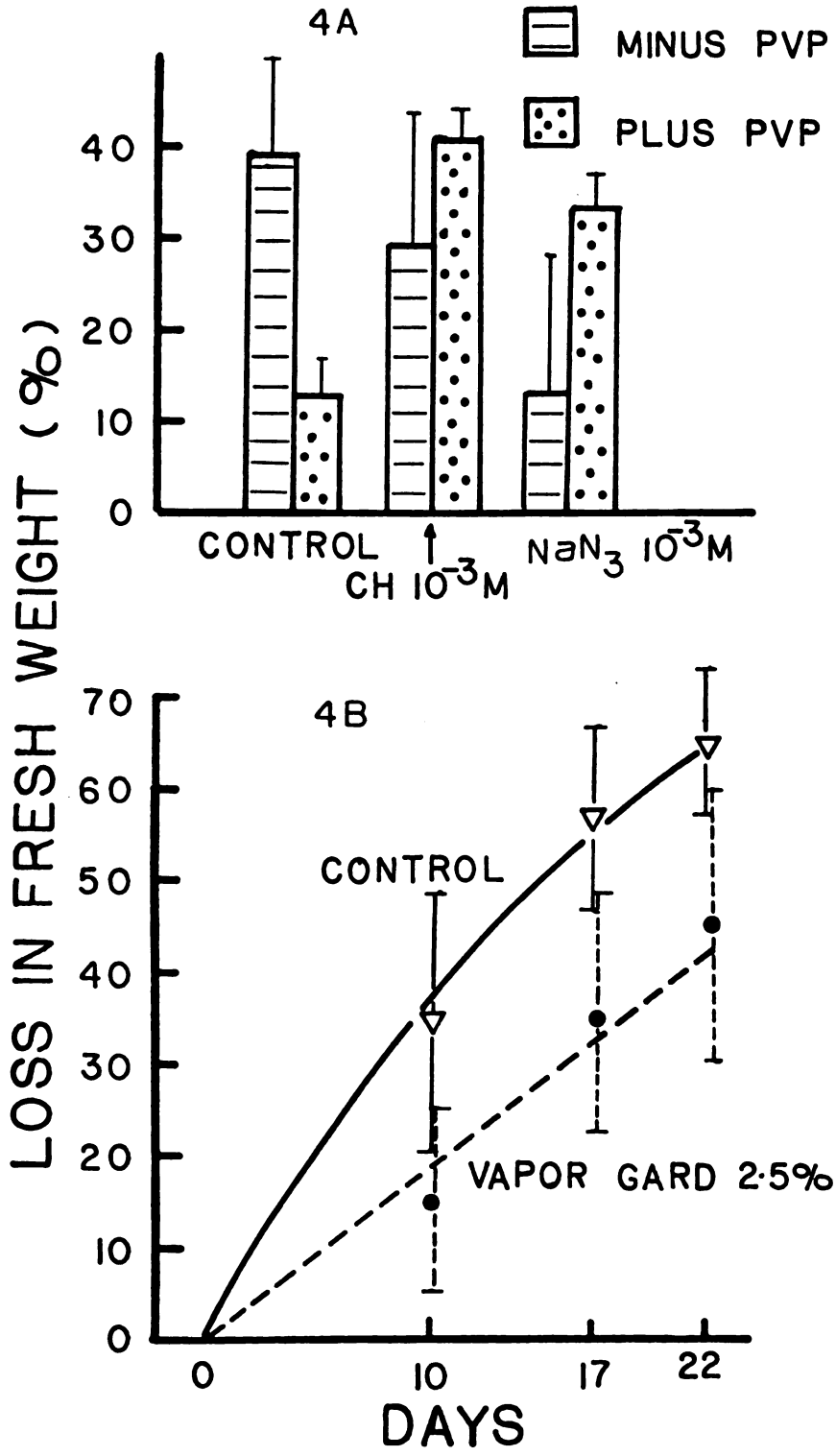
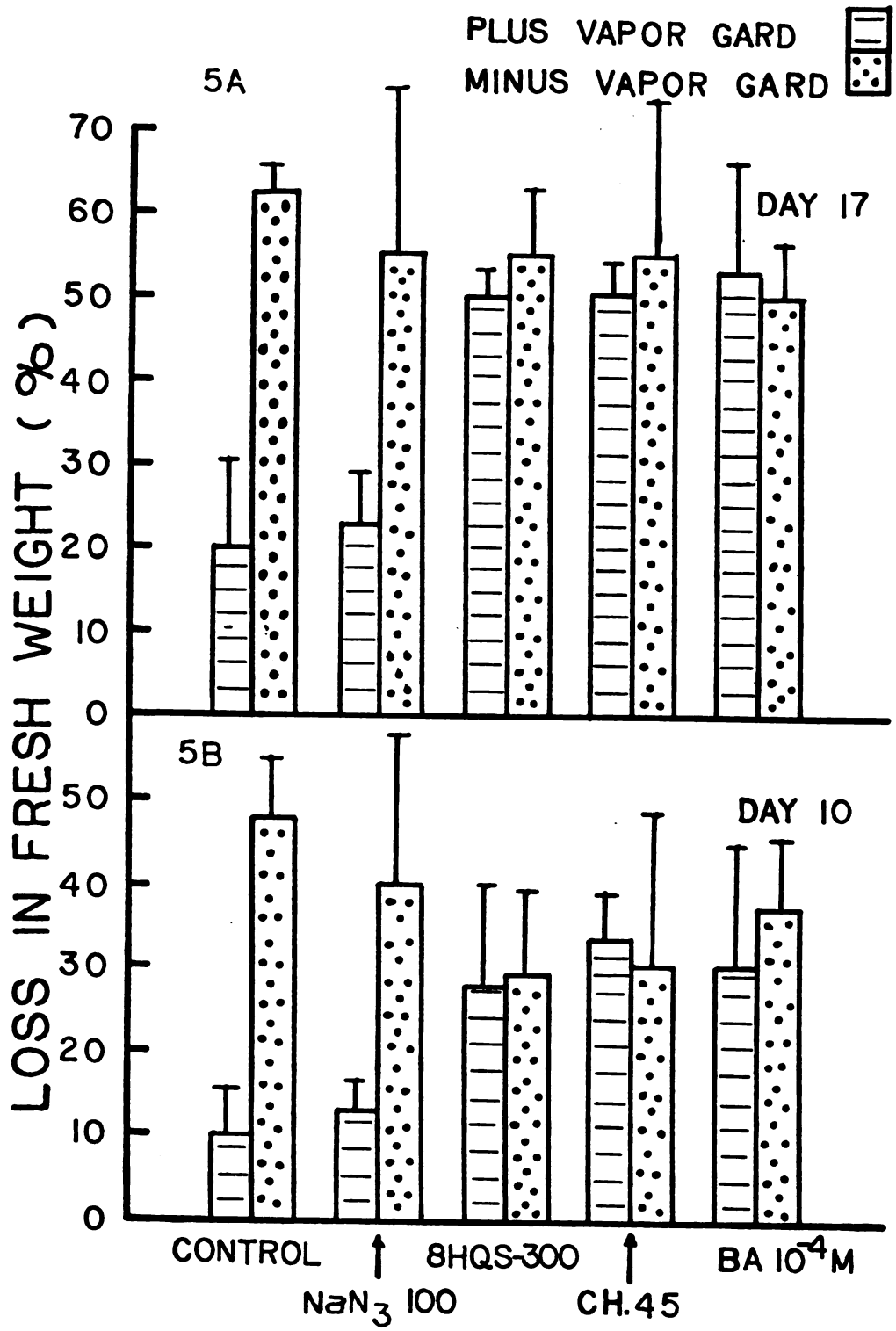

4A
MINUS PVP
PLUS PVP
LOSS IN FRESH WEIGHT (%)
0
10
20
30
40
CONTROL
CH 10^{-3}M
NaN_3 10^{-3}M
4B
CONTROL
VAPOR GARD 2·5%
0
10
20
30
40
50
60
70
0
10
17
22
DAYS

Figure 5 A-B Loss in fresh weight (%) of Ilex verticillata (L.) Gray fruiting branches

A-- 10 days and

B-- 17 days after proximal ends were inserted in water (control), sodium azide (NaN_3) 100 ppm, 8 hydroxyquinoline sulfate (8 HQS) 300 ppm, cycloheximide (CH) 45 ppm and benzyladenine (BA) 10^{-4}M and the tops dipped in water and Vapor Gard 2.5%. Vertical lines indicate standard deviations above the mean.


PLUS VAPOR GARD
MINUS VAPOR GARD
5A
DAY 17
5B
DAY 10
LOSS IN FRESH WEIGHT (%)
70
60
50
40
30
20
10
0
CONTROL
NaN3 100
8HQS-300
CH.45
BA 10-4M

days for treatments receiving Vapor Gard.

DISCUSSION

Evergreen holly, when harvested for Christmas displays, is immersed in a bath of 40 ppm NAA (68) to prevent leaf fall. When fruiting branches of Ilex verticillata are harvested for the same purpose some loss of fruit occurs during the handling process. A preharvesting treatment which would reduce this loss was envisioned. But studies in the field show that FRF could not be increased by application of GA, CH, NAA or NaN_3 (Table 1). FRF for untreated branches increased slightly over time. Ethephon increased fruit fall but did not change FRF when recorded one week later (Table 1). A response from ethephon occurred within 1 week thus FRF measurements after 1 week were obtained from the remaining unresponsive fruit on these branches. CH also caused fruit abscission, probably by increasing ethylene production (1, 26).

Some plants of Ilex verticillata are noted to lose their fruit just before leaf fall. For cropping purposes one would not propagate such a plant. Instead, selection of plants, which retain their fruit until December or January, was desirable. Such were the type of plants selected for this study. It is understandable therefore that staining or structural differences indicative of abscission layer formation were not detected in histological and histochemical studies. The fruit was firmly held with no tendency towards reduction in FRF, as winter approached. The ability of the fruit to remain attached to the plant must relate to a discontinuous ring of sclereid tissue in the cortex

outside the vascular tissue. In apple (65) and cherry (112) there was a reduction in sclerenchyma in the abscission zone.

Surrounding the layer of lignified cells the cortex loosened and cell separation was visible along the entire length of the pedicel. With the paraffin technique this was thought to be part of the torn section artifact, but glycol methacrylate resin (which was also stained) proved otherwise (Figure 2B).

The following year FRF data (for a different plant) was measured over time beginning approximately 2 weeks after fruit set. FRF increased from July to September but decreased in October (Figure 3A). On September 14, a large quantity of proximal type abscission occurred naturally and when abscised samples were studies only cell loosening (previously described) was observed. All histological and histochemical studies recorded similar findings to the previous experiment. There are 2 possibilities for the reduced FRF for proximal abscission on September 14 (Figure 3A). The first is low temperature effect. On September 4, 2°C was recorded in the Lansing area. The temperature could have been much less in the low area where the plant was growing. The other possibility is a natural abscission process. If this is true, then abscission occurred with little change in pedicel structure.

Former abscission studies on cherry, cotton, bean etc. were conducted with varieties of known performance (13, 83, 112). Ilex verticillata plants are very variable in fruit size, color and pedicel length etc. from one habitat to another. This diversification limits conclusions of the fruit abscission process. Further studies should be conducted with individual plants all propagated from 1 selected specimen. In spite of the present limitations, a generalization can

be formulated from the present study. There are at least 2 classes of fruit abscission within the native I. verticillata plants. Some lose most of their fruit before leaf fall and the process may be similar to that of other fruits for example sweet cherry (111). Other plants retain their fruit until December or January; no abscission occurs and eventually fruit fall is a result of the fruit and pedicel being killed by sub zero temperatures (17).

Proximal application (insertion of stem ends) of NAA, CH, alar or NaN_3 did not alter weight loss of fruiting branches. The same results occurred with sucrose (an energy source); 8 HQS (a fungicide) and calcium chloride (for cell firmness). Distal applications of similar compounds and cytokinins had no effect on fruit fall.

Use of inhibitors such as NaN_3 were expected to cause browning of the fruit and a phenol inhibitor (PVP) was included to prevent this occurrence. Fruit color was not affected but PVP increased weight loss and negatively affected all treatments (Table 3). Fruit abscission and weight loss were reduced when moisture loss was impeded with antitranspirants and Vapor Gard at 5% and 2.5% was better than Geon Latex (Table 4).

SECTION IV

THE EFFECT OF TEMPERATURE, RELATIVE HUMIDITY AND WIND ON FRUIT ABSCISSION OF *ILEX VERTICILLATA* (L.) GRAY

The effects of ecological factors on abscission of plant parts were reviewed by Addicott in 1968 (5) and by Addicott and Lyon in 1973 (8). Changes in temperature affected abscission. Abscission was increased in sour cherry fruit when the temperature was increased from 15 to 35°C (112) and in olive fruit when the temperature was increased from 4.5 to 29.5°C (46). In Citrus, subfreezing temperatures of -5°C increased abscission (115). Petioles exposed to low temperatures become brittle and break easily in strong winds (52).

Abscission of buds, flowers, fruit or leaves (8) can be initiated by moisture stress. And the process is accelerated during warm weather (7). Abscission of cotton leaves induced by ethylene was enhanced (55) and abscission of olive fruit induced by cycloheximide was inhibited by water stress (46). Moisture stress can also result from drying winds (8); and strong winds remove leaves and branches from plants. Shaking of 10 day old Cucurbita melopepo plants inhibited the growth of stems and petioles (102) and seedlings of Robinia pseudoacacia were smaller, had less dry weight and fewer leaves after being subjected to a wind velocity of 3.7 m/sec for 4 weeks (92).

The following experiments were designed to test the effects of temperature and relative humidity (RH) on fruit abscission of Ilex verticillata (L.) Gray (common winterberry). Fruit fall during the winter months was hypothesised to be due to a combination of low temperature killing of the pedicel and fruit tissue followed by periods of high temperature and low RH which cause dessication and the shrunken browned fruit and pedicels fall during heavy windstorms.

MATERIALS AND METHODS

Temperature and relative humidity. Two experiments were conducted to determine the effect of temperature and RH on abscission. Plant material for both experiments was harvested on September 18, 1974 and stored in sealed 10 µm opaque polyethylene bags. On December 15, fruiting twigs approximately 10 cm in length, were selected and the number of fruit on each twig recorded for the first experiment. The twigs were exposed to -9°C and -18°C temperatures, followed by storage at 0°C or 20°C at low or high RH. Four twigs packed in aluminum foil, were inserted in a vacuum cylinder flask fitted with thermocouple wire and placed in a Revco freezer. The temperature was monitored with a potentiometer; the sensor was a thermocouple inserted into a separate fruit and enclosed with the twigs. This comprised 1 of 2 replications for low temperature treatments.

The temperature was decreased at 10°C/hour until the desired level of -9°C or -18°C was reached and immediately the fruiting twigs were removed from the flasks and transferred to the storage environment chambers. These were cardboard boxes approximately 0.04 m^3, encased in 10 µm opaque polyethylene bags. Low RH at 0°C (35%) and at 20°C (25%) were obtained by placement of dry calcium chloride within the chamber. High RH at 0°C (98%) and at 20°C (94%) were obtained by placement of distilled water in the chambers.

Fruit removal force (FRF) (g) was determined for 4 fruit on each twig with a Hunter Mechanical Force Gauge (Hunter Springs, Hatfield, Pa.) before, at time zero and at 2, 4, 6 and 16 days after low temperature treatment. Abscission between the pedicel and the fruit

was termed distal; between the pedicel and the stem proximal; breakage through the pedicel intermedial. Analyses of variance for a split plot design were conducted with data on FRF. Data on fruit fall in a wind tunnel and FRF were correlated. The proximal ends of randomly selected fruiting twigs were placed through holes (0.5 cm in diameter) in a plexiglass sheet and inverted in the ceiling of a wind tunnel (0.75 m in cross section). Quantitative measurements of fruit fall from each twig were made at wind velocities (supplied by a turbine engine) of 6.1, 12.2, 16.3 and 24.4 m/sec and qualitative observations of the condition of the fruit were recorded.

The second experiment was begun on January 2 and designed to confirm the findings of the former experiment. The fruiting twigs were gradually exposed to -9°C or -18°C for 24 hours within sealed flasks which were later removed and the foil covered twigs left in the freezer for an additional 5 hours. Storage temperatures were 0°C and 20°C with high RH combinations as in the first experiment. Data on FRF was determined for 10 fruit on each twig before, at time zero and at 2, 4 and 8 days after treatment.

Determination of killing point. A thermocouple was inserted in a fruit; wrapped in aluminum foil and enclosed in a vacuum cylinder flask. The flask was placed in a freezer and the temperature decrease (10°C/hour) monitored with a potentiometer. The first and third exotherms (66, 107) were determined for 5 fruit.

Ten excised pedicels were attached to masking tape. A micro-thermocouple (76 um) was inserted into an eleventh pedicel. The tape, covered with foil, was inserted in a flask and placed in a freezer. Temperature decrease (10°C/hour) was monitored with a potentiometer.

Two flasks were used for each treatment of -9°C, -12°C, -15°C, -18°C. When the desired temperature was reached and the foil removed, the pedicels and masking tape were placed in high humidity chambers at 22°C. Four days later sectioned pedicels were examined for browning under the dissecting microscope. This process was repeated with temperatures of -11.1°C, -12.2°C, -13.3°C, -14.4°C and -15°C.

RESULTS

FRF for -18°C temperature treatments were lower than those recorded for -9°C treatments (Figures 1A and 1B). Treated branches stored at 20°C had lower FRF than those stored at 0°C (Figures 1C and 1D), Both low temperature and storage temperature treatment differences were significant at 2, 4 and 6 days in the first experiment. The interaction between storage temperature and RH was highly significant on day 16 (Figure 2). Differences for RH and all other interactions were insignificant. In both experiments, abscission at the distal end fluctuated between 50 to 80%; at the proximal end 15 to 45% and the intermedial type was always less than 10%.

Fruit fall for all treatments at wind speed velocities of 6.1, 12.2, 16.3 and 24.4 m/sec was zero. Most fruit exposed to -18°C and some exposed to -9°C turned brown. Browning had occurred by day 2 and reached a maximum by day 6 or 8 (Figure 3C).

Determination of killing points. The fruit when super-cooled, produced 2 significant exotherms (Table 1). The mean freezing point was -3.7°C and the fruit were killed at a mean of -10.8°C. Samples of pedicel tissue were alive at -9°C and -12°C (Figure 3B) but were

Table 1. Freezing and killing temperatures °C (exotherms) of Ilex verticillata (L.) Gray fruit when super-cooled at 10°C/hour.

#	Freezing temperature	Killing temperature
1	-3.3	-10.8
2	-3.5	-10.6
3	-4.2	-10.7
4	-3.9	-11.0
5	-3.6	-10.8
Mean	-3.7	-10.8

Table 2. Number of Ilex verticillata (L.) Gray pedicels alive after subfreezing temperature treatments of -11.1°C, -12.2°C, -13.3°C, -14.4°C and -15.5°C.

	Temperature °C				
#	-11.1	-12.2	-13.3	-14.4	-15.5
1	10	8	0	0	0
2	10	7	0	0	0

Figure 1 A-D Fruit removal force (g) of Ilex verticillata (L.) Gray

A-- before, at time zero, 2, 4, 6, and 16 days

B-- before, at time zero, 2, 4 and 8 days after exposure of fruiting branches to subfreezing temperatures of -9°C and -20°C.

C-- before, at time zero, 2, 4, 6 and 16 days

D-- before, at time zero, 2, 4 and 8 days of storage at 0°C and 20°C after exposure of fruiting branches to subfreezing temperatures. Subfreezing and storage temperature differences were significantly different at days 2, 4 and 6.

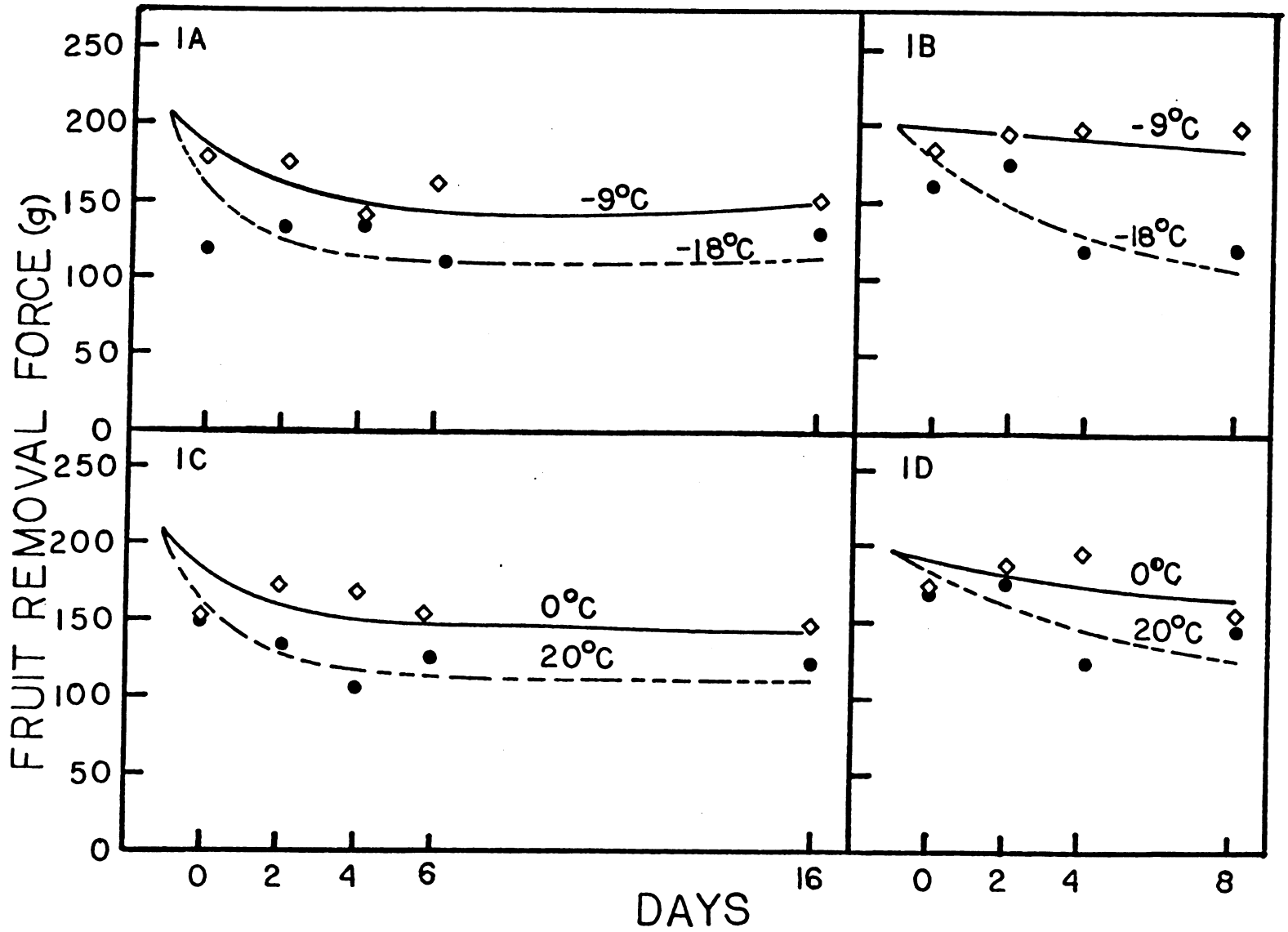

1A
1B
1C
1D
-9°C
-18°C
0°C
20°C
FRUIT REMOVAL FORCE (g)
DAYS
250
200
150
100
50
0
0
2
4
6
16
8

Figure 2 Fruit removal force (g) of Ilex verticillata (L.) Gray 16 days after exposure to subfreezing temperatures (temp) followed by storage at 0°C and 20°C in low and high relative humidity (RH) environments.

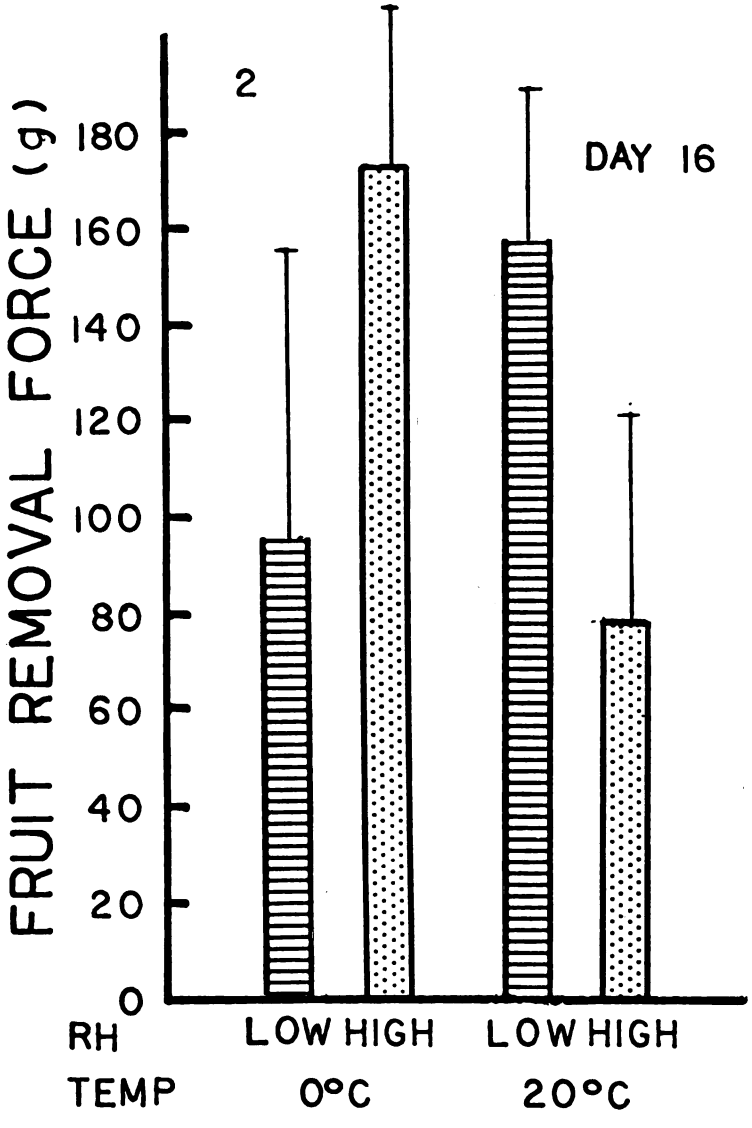
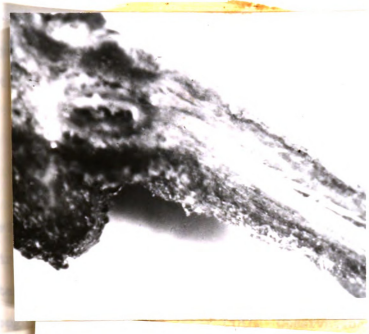

2
DAY 16
FRUIT REMOVAL FORCE (g)
180
160
140
120
100
80
60
40
20
0
RH
LOW HIGH
LOW HIGH
TEMP
0°C
20°C

Figure 3 A-C Pedicels of Ilex verticillata (L.) Gray after exposure to

A-- -18°C B-- -12°C

C-- browning of fruit after exposure to -18°C compared with red fruit exposed to -9°C.



3A

3B

3C

killed at -15°C and -18°C (Figure 3A). In the second experiment (Table 2) pedicel tissue was killed at -13.3°C, -14.4°C and -15.5°C. Some dead tissue was observed at -12.2°C but none at -11.1°C.

DISCUSSION

In their natural habitat the majority of Ilex verticillata plants retain their fruit until January. Physiological and anatomical studies determined that fruit from these plants were firmly attached with no recognisable abscission later (18). Observations of weather conditions preceding fruit fall led to the conclusion that weather conditions and fruit fall were related and a hypothesis was formulated.

Results show that fruits were killed at subfreezing temperatures between -10°C and -11°C (Table 1) and pedicels between -12.2°C and -13.3°C (Table 2). Temperatures of -18°C therefore killed both the fruit (Figure 3C) and the pedicel (Figure 3A). FRF was less for these treatments than for the -9°C treatments. When fruiting branches were subsequently stored at 20°C FRF was less than treatment at 0°C. Differences in FRF between RH levels were not significant, but the interaction between storage temperature and RH became significant on day 16. When fruiting twigs were subjected to wind velocities of 6.1, 12.2, 16.3 and 24.4 m/sec fruit fall was not observed.

The hypothesis as stated was rejected because only 2 factors, low temperature kill and storage temperature were proven significant. Dehydration for this limited period did not influence fruit fall. Wind forces were insignificant for 2 reasons. In nature, they can be gusty

and multidirectional, and fruiting branches brush against one another as they respond to such forces. These conditions were not simulated in the laboratory. Secondly, fluid flowing past a body produces pressure (normal to the area) and viscous shear (parallel or tangent to the area) forces; these components taken in the direction of motion are collectively called the profile drag (76). The equation for total drag (assuming the fruit is a sphere and held by a stationary twig in the laboratory experiment) calculated, showed that the wind force at 24.4 m/sec was equivalent to 0.25 grams. Obviously this was not enough force to remove the fruit when most values of FRF were greater than 100 g. But profile drag also varies with temperature and barometric pressure. High pressures and low temperatures increase its values.

It is suggested that the primary growth of I. verticillata pedicels are killed in winter. The dead fruit on these pedicels remain red when temperatures are below 0°C. Periodic bursts of sunshine increase the temperature causing browning and shrivelling. Heavy winter storms disturb the shrubs causing many fruit to fall off because of friction between branches. Those that remain are eventually pushed off with growth increases the following spring. Fruiting plants transferred to a greenhouse shortly after leaf fall retained their fruit in excellent condition for 2 years, when they turned yellow and the mesocarp was dehydrated. Evergreen holly (for example I. opaca American holly) retain some fruit for 2 years. This could be due to protection from the elements by the evergreen leaves.

SUMMARY AND CONCLUSIONS

Fruiting branches of Ilex verticillata (L.) Gray were stored at 9 environment combinations of low, intermediate and high RH in 0°, 8.8°, or 10° and 20°C chambers for a period of 8 weeks in 1973 and repeated in 1974. Water loss from fruit and twigs and shrivelling of fruit was minimized with 0°C/98% RH. Abscission indexed by reduction in FRF was correlated with VPD during storage. Minimum levels of O_2 were 20% and maximum levels of CO_2 were 0.5% in all chambers. Ethylene concentration in chambers at 20°C/94% RH (in which fruit were contaminated with fungi) reached 315 ppb at the end of week 4. A study on ethylene production rate showed that green, red-green and red fruit produced peaks of 5.8, 4.6 and 1.1 µl/kg/hr at 5½, 6½ and 9½ days after harvest. It is concluded that fruiting branches of this species can be maintained for at least 2 months at 0°C/98% RH.

The keeping quality of fruiting branches, as affected by 4 antitranspirants,was studied. One of these, Vapor Gard at 2.5%, significantly reduced loss in fresh weight, water uptake and transpiration and fruit remained in excellent condition until day 23 when shrivelling occurred. The presence of Vapor Gard on fruit surfaces was detected by use of a SEM but not with the light microscope. Fruit respiration rate differences between treatments were slight. It is concluded that Vapor Gard is an effective antitranspirant for maintaining fruit quality 2 to 3 times longer than untreated fruit.

Field and laboratory experiments were conducted to determine the process of fruit abscission. FRF from branches treated with NAA, CH, GA, NaN3 and ethephon and non treated branches were similar. Fruit and leaf abscission occurred on branches treated with ethephon and CH. An abscission layer was not detected by histological or histochemical studies but sclereids were associated with the vascular tissue along the entire length of the pedicel. In another experiment FRF increased from approximate values of 240 g at fruit set to 280 g at maturity and then declined to 220 g. Approximately 50% of fruit with pedicels attached abscised just before leaf fall, proximal application of CH, NAA, NaN_3, calcium chloride, sucrose, 8 HQS and alar; and distal applications of CH, NAA, NaN3, calcium chloride, kinetin and benzyladenine caused loss in weight for detached fruiting branches which was similar to or greater than the control. Loss in fresh weight was increased when PVP was included with proximal treatments. And antitranspirant applications reduced loss in fresh weight of fruiting branches. It is concluded that the plants of this species studied do not have a fruit abscission layer; instead fruit fall is prevented by the presence of the sclereids in the pedicel.

Fruit and pedicels were killed between -10°C and -11°C and -12°C and -13°C respectively. FRF of fruiting branches exposed to -18°C was less than those exposed to -9°C. FRF for branches subsequently stored at 20°C was lower than at 0°C. Fruiting branches subjected to a maximum wind force of 24.4 m/sec retained 100% of their fruit. It is concluded that fruit and pedicels of this species are killed by subfreezing temperatures, browning and shrivelling occurs when temperatures fluctuate above 0°C and abscission occurs by rubbing of

branches during heavy winter storms or with pressure from enlarging twigs during spring growth.

LIST OF REFERENCES

LIST OF REFERENCES

1. Abeles, F.B. 1967. Mechanism of action of abscission accelerators. Physiol. Plantarum 20: 442-454.

2. Abeles, F.B. and R.E. Holm. 1967. Abscission: Role of protein synthesis. Ann. N.Y. Acad. Sci. 144: 367-373.

3. Abeles, F.B. and B. Rubenstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963-969.

4. Addicott, F.T. 1965. Physiology of abscission. Ency. Plant Physiol. 15/2: 1094-1126.

5. Addicott, F.T. 1968. Environmental factors in the physiology of abscission. Plant Physiol. 43: 1471-1479.

6. Addicott, F.T. and R.S. Lynch. 1951. Acceleration and retardation of abscission by IAA. Science 114: 688-689.

7. Addicott, F.T. and R.S. Lynch. 1957. Defoliation and dessication: harvest-aid practices. Advan. Agron. 9: 67-93.

8. Addicott, F.T. and J.L. Lyon. 1973. Physiological ecology of abscission. In: T.T. Kozlowski ed. Shedding of Plant Parts. Academic Press, New York and London.

9. Allen, F.W. and W.T. Pentzer. 1935. Studies on the effect of humidity in the cold storage of fruits. Proc. Amer. Soc. Hort. Sci. 33: 215-223.

10. Angus, D.E. and H. Bielorai. 1965. Transpiration reduction by surface films. Aust. Jour. Agr. Res. 16: 107-112.

11. Barnell, E. 1939. Studies in tropical fruits V Some anatomical aspects of fruit fall in two tropical arboreal plants. Ann. Bot. 3: 77-89.

12. Barnwell, M.G. 1973. The role of the cold store in the hardy plant nursery. Gardeners Chronicle 174(11): 26-27, 29.

13. Berman, T. 1969. The effect of amino acids and growth regulators on abscission in cotton explants. Israel Jour. of Agr. Res. 19: 129-130.

14. Beyer, Elmo M. Jr. 1973. Abscission: Support for a role of ethylene modifications of auxin transport. Plant Physiol. 52: 1-5.

15. Beyer, Elmo M. Jr. and P.W. Morgan. 1971. The role of ethylene modification of auxin transport. Plant Physiol. 48: 208-212.

16. Biggs, R.H. and A.C. Leopold. 1958. The two-phase action of auxin on abscission. Amer. Jour. Bot. 45: 547-551.

17. Boylan, H.C. 1975. The effect of temperature, relative humidity and wind on fruit abscission of Ilex verticillata (L.) Gray. Ph. D. thesis Section IV, Michigan State University.

18. Boylan, H.C. 1975. Physiology of fruit abscission in Ilex verticillata (L.) Gray. Ph. D. thesis, Section III, Michigan State University.

19. Burg, S.P. and E.A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. 55: 262-266.

20. Carns, H.R., F.T. Addicott, K.C. Baker and R.K. Wilson. 1961. Acceleration and retardation of abscission by gibberellic acid. In: R.M. Klein ed. Plant Growth Regulation. Iowa State University Press, Ames, Iowa.

21. Chatterjee, S.K. and A.C. Leopold. 1964. Kinetin and gibberellin actions on abscission processes. Plant Physiol. 39: 334-337.

22. Christopher, E.P., S.A. Pieniazek, V. Shutak and L. McElroy. 1948. Transpiration of apples in cold storage. Proc. Amer. Soc. Hort. Sci. 51: 114-118.

23. Clark, W.L. and R.J. Shirk. 1965. A hot melt transparent peelable coating for food. Food Technology 19 (10): 105-111.

24. Comin, Donald and W. Junnila. 1946. Water loss from vegetables in storage. Ohio Agr. Expt. Sta. Bull. 31(243): 159-166.

25. Cooper, W.C., G.K. Rasmussen and D.J. Hutchinson. 1969. Promotion of abscission of orange fruits by cycloheximide as related to site of treatment. BioScience 19: 443-444.

26. Cooper, W.C., G.K. Rasmussen, B.J. Rogers, P.C. Reece and W.H. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43: 1560-1576.

27. Crossley, J.H. 1954. Instructions for cutting, dipping, packing and shipping holly. (Mimeo publ.) Saanichton Exp. Sta., B.C.

28. Davenport, D.C., M.A. Fisher and R.M. Hagan. 1972. Some counteractive effects of antitranspirants. Plant Physiol. 49: 722-724.

29. Davenport, D.C., P.E. Martin and R.M. Hagan. 1972. Antitranspirants for conservation of leaf water potential of transplanted Citrus trees. HortScience 7 (5): 511-512.

30. Davenport, D.C., K. Uriu and R.M. Hagan. 1972. Antitranspirant film: Curtailing intake of external water by cherry fruit to reduce cracking. HortScience 7 (5): 507-508.

31. Davies, W.J. and T.T. Kozlowski. 1974. Short- and long-term effects of antitranspirants on water relations and photosynthesis of woody plants. Jour. Amer. Soc. Hort. Sci. 99 (4): 297-304.

32. Facey, V. 1950. Abscission of leaves in Fraxinus americana L. New Phytol. 49: 103-116.

33. Feder, N. and T.P. O' Brien. 1968. Plant microtechnique: Some principles and new methods. Amer. Jour. Bot. 55 (1): 123-142.

34. Fee, Harry A. 1931. Transplanting the winterberry. Horticulture. 9: 50.

35. Fenton, Daniel G. 1970. Orcharding in the middle Atlantic area. Handbook of Hollies. Amer. Hort. Mag. 49 (4): 290-293.

36. Forsyth, F.B. and I.V. Hall. 1969. Ethylene production with accompanying respiration rates from the time of blossoming to fruit maturity in three Vacinnium species. Natur. Canad. 96: 257-259.

37. Gale, J. 1961. Studies on plant antitranspirants. Physiol. Plant. 14: 777-786.

38. Gale, J. and R.M. Hagan. 1966. Plant antitranspirants. Ann.Rev. Plant Physiol. 17: 269-282.

39. Gambrell, C.E., E.T. Sims, G.E. Stenbridge and W.H. Rhodes. 1972. Response of peaches to succinic acid-2 2-dimethylhydrazide as an aid in mechanical harvesting. Jour. Amer. Soc. Hort. Sci. 97: 265-268.

40. Gaur, B.K. and A.C. Leopold. 1955. The promotion of abscission by auxin. Plant Physiol. 30: 487-490.

41. Gawadi, A.G. and G.S. Avery. 1950. Leaf abscission and the so-called "abscission layer". Amer. Jour. Bot. 37: 172-180.

42. George, William L. Jr. 1972. Fruit waxing to increase storage life of European forcing cucumbers. Ohio Agr. Res. and Devel. Center. Research Summary 58.

43. Hall, E.G. and S.A. Trout. 1944. Some effects of waxing on weight loss from oranges and certain vegetables. Jour. Aust. Inst. Agr. Sci. 10: 80-82.

44. Hamblin, Stephen F. 1927. Shrubs with winter berries. Nature Magazine. 10: 357-358.

45. Hamilton, Clyde C. 1959. Insect pests of holly. Bulletin #2. Holly Soc. of America.

46. Hartmann, Hudson T., Mostafa El-Hamady and John Whisler. 1972. Abscission induction in the olive by cycloheximide. Jour. Amer. Soc. Hort. Sci. 97 (6): 781-785.

47. Harvey, E.M. 1913. The castor bean plant and laboratory air. Bot. Gaz. (Chicago) 56: 439.

48. Heinlein, J.D. and W.G. Haigh. 1971. Transpiration control with silicone emulsion. Plant Physiol. 47 (suppl.) 38.

49. Heinlein, J.D. and W.G. Haigh. 1972. Are silicone antitranspirants practical? Plant Physiol. 49 (suppl.): 22.

50. Hitz, C.W. and I.C. Haut. 1942. Effects of waxing and pre- storage treatments upon prolonging the edible and storage qualities of apples. Md. Agr. Expt. Stat. Bulletin A14.

51. Hooper, G.R. and J.E. Bath. Pressure resin infiltration of aphids for electron microscopy. Stain Technology 48: 167-171.

52. Hoshaw, R.W. and A.T. Guard. 1949. Abscission of marcescent leaves of Quercus palustris and Quercus coccinea. Bot. Gaz. (Chicago) 110: 587-593.

53. Jard, Leonard. 1972. Some advances in the chemistry of anthocyanin type plant pigments. In: C.O. Chichester ed. The Chemistry of plant pigments. Academic Press, New York and London.

54. Jensen, W.A. 1962. Botanical histochemistry. W.H. Freeman & Co., San Francisco and London.

55. Jordan, Wayne R., Page W. Morgan and T.L. Davenport. 1972. Water stress enhances ethylene mediated leaf abscission in cotton. Plant Physiol. 50: 756-758.

56. Lebovits, Alexander. 1966. Permeability of polymers to gases, vapors and liquids. Modern Plastics 43 (7): 139.

57. Looney, N.E. 1968. Inhibition of apple ripening by succinic acid 2, 2-dimethylhydrazide and its reversal by ethylene. Plant Physiol. 43: 1133-1137.

58. Lutz, J.M. and R.E. Hardenburg. 1968. The commercial storage of fruits, vegetables and florist and nursery crops. U. S. Department of Agriculture Handbook # 66.

59. MacDaniels, L.H. 1936. Some anatomical aspects of apple flower and fruit abscission. Proc. Amer. Soc. Hort. Sci. 34: 122-129.

60. Mahlstede, J.P. and W.E. Fletcher. 1960. Storage of nursery stock. 62 pp. Amer. Assoc. Nurserymen, Washington, D.C.

61. Malcolm, C.V. and L.H. Stolzy. 1968. Effect and mode of action of latex and silicone coatings on shoot growth and water use by Citrus. Agron. Jour. 60: 598-602.

62. Mansfield, W.W. 1953. Effect of surface films on the evaporation of water. Nature 172: 110.

63. Martin, John D. 1974. Antitranspirants and their possible uses in floriculture. The Maryland Florist 190: 1-9.

64. McCown, M. 1938. Abscission of flowers and fruits of the apple. Proc. Amer. Soc. Hort. Sci. 36: 320.

65. McCown, M. 1943. Anatomical and chemical aspects of abscission of fruits of the apple. Bot. Gaz. 105: 212-220.

66. McLeester, R.C., C.J. Weiser and T.C. Hall. 1969. Multiple freezing points as a test for viability of plant stems in the determination of frost hardiness. Plant Physiol. 44: 37-44.

67. Meheriuk, M. and S.W. Porritt. 1972. Effects of waxing on respiration, ethylene production, and other physical and chemical changes in selected apple cultivars. Can. Jour. Plant Sci. 52: 257-259.

68. Milbrath, J.A. and H. Hartman. 1940. Holly defoliation prevented by α naphthaleneacetic acid. Science 92: 401.

69. Milbrath, J.A. and H. Hartman. 1942. The cause and control of defoliation in cut holly. Oregon Agr. Expt. Sta. Bull. 413.

70. Morgan, P.W. and J.I. Durham. 1975. Ethylene-induced leaf abscission is promoted by gibberellic acid. Plant Physiol. 55 (2): 308-311.

71. Morre, D.J. 1968. Cell wall dissolution and enzyme secretion during leaf abscission. Plant Physiol. 43: 1545-1559.

72. Neal, O.M. 1954. Deciduous holly, a new ornamental crop. W. Va. Agr. Expt. Stat. Bull. 363, pt. 2: 5-11.

73. Neumann, P.M. 1974. Senescence of attached bean leaves accelerated by sprays of silicone oil antitranspirants. Plant Physiol. 53: 638-640.

74. Osborne, D.J. 1968. Hormonal mechanisms regulating senescence and abscission. In: F. Wightman and G. Setterfield eds. Biochemistry and Physiology of Plant Growth Substances. The Runge Press Ltd., Ottawa, Canada. pp 815-840.

75. Osborne, D.J. and S.E. Moss. 1963. Effects of kinetin on senescence and abscission in explants of Phaseolus vulgaris. Nature 200: 1299-1301.

76. Olson, Reuben M. 1966. Essentials of engineering fluid mechanics (Second edition) International Textbook Company, Scanton, Pa.

77. Pallas, J.E. Jr., G.D. Harris, C.B. Elkins and A.R. Bertrand. 1961. Research in plant transpiration. U. S. Department of Agriculture Prod. Res. Report 70. 37pp.

78. Parkinson, K.J. 1970. The effects of silicone coatings on leaves. Jour. Exp. Bot. 21: 566-579.

79. Platenius, Hans. 1939. Wax emulsions for vegetables. Cornell University Agr. Expt. Sta. Ithaca, N.Y. Bull. 723.

80. Poljakoff-Mayber, A. and J. Gale. 1972. Physiological basis and practical problems of reducing transpiration. In: T.T. Kozlowski ed. Water Deficits and Plant Growth Volume III, Chapter 9. Academic Press, New York.

81. Poovaiah, B.W. and H.P. Rasmussen. 1973. Calcium distribution in the abscission zone of bean leaves. Plant Physiol 52: 683-684. (Short Communication).

82. Potter, C.W. 1962. Holly spells Christmas. Florists Review. 131 (3389): 29-30.

83. Rasmussen, H.P. 1965. Chemical and physiological changes associated with abscission layer formation in the bean (Phaseolus vulgaris L. cv. contender). Ph. D. Thesis, Michigan State University.

84. Rasmussen, H.P. and M.J. Bukovac. 1969. A histochemical study of abscission layer formation in the bean. Amer. Jour. Bot. 56 (1): 69-76.

85. Reinsmith, Winton H. 1948. Some southern shrubby hollies. Amer. Nurseryman 88 (9): 12.

86. Ricker, P.L. 1924. Our Christmas green. Nature Magazine 4: 351-354.

87. Roberts, A.N. and R.L. Ticknor. 1970. Commercial production of English holly in the Pacific northwest. Handbook of Hollies. Amer. Hort. Mag. 49(4): 301-314.

88. Rubinstein, B. and A.C. Leopold. 1963. Analysis of the auxin control on bean leaf abscission. Plant Physiol. 38: 262-267.

89. Ryall, A.L. and W.T. Pentzer. 1974. Handling, Transportation and Storage of Fruits and Vegetables. AVI Publishing Co. Inc., Westport, Conn.

90. Santamour, F.S. Jr. 1973. Anthocyanins of holly fruits. Phytochemistry 12: 611-615.

91. Sass, J.E. 1958. Botanical Microtechnique. The Iowa State University Press, Ames, Iowa.

92. Satoo, T. 1961. Wind, transpiration and tree growth. In: T.T. Kozlowski ed. Tree Growth. 299-310. Ronald Press, N.Y.

93. Schomer, H.A. and C.F. Pierson. 1968. The use of wax on apples and pears. Proc. Wash. Sta. Hort. Assoc. 198-200.

94. Sheehan, T.J. 1962. Market development for horticultural specialty products. A. R. Fla. Agr. Expt. Sta. p 137.

95. Simerl, L.E. 1953. Waxes in protective packaging. Food Technol. 7 (6): 256-257.

96. Singleton, V.L. 1972. Common plant phenols other than anthocyanins, contribution to coloration and discoloration. In: C.O. Chichester ed. The Chemistry of Plant Pigments. Academic Press, N. Y. and London.

97. Smith, W.H. 1933. Evaporation of water from apples in relation to temperature and atmospheric humidity. Ann. Appl. Biol. 20 (2): 220-235.

98. Steel, R.G.D. and J.H. Torrie. 1960. Principles and practices of statistics. McGraw-Hill Book Co. Inc., New York, Toronto and London.

99. Stosser, R., H.P. Rasmussen and M.J. Bukovac. 1969. A histological study of abscission layer formation in cherry fruits during maturation. Jour. Amer. Soc. Hort. Sci. 94: 239-243.

100. Stosser, R., H.P. Rasmussen and M.J. Bukovac. 1969. Histochemical changes in the developing abscission layer in fruits of Prunus cerasus L. Planta. 86: 151-164.

101. Trout, S.A., E.G. Hall and S.M. Sykes. 1953. Effects of skin coatings on the behaviour of apples in storage I. Physiological and general observations. Aust. Jour. of Agr. Res. 4: 57.

102. Turgeon, R. and J.A. Webb. 1971. Growth inhibition and mechanical stress. Science 174: 961-962.

103. Umbreit, W.W., R.H. Burris and J.F. Stauffer. 1959. Manometric techniques. Burgess Publishing Company, Minneapolis, Minn.

104. Umrath, C.R., A.L. Kenworthy and C.L. Bedford. 1969. The effect of alar on fruit maturation, quality and vegetative growth of sour cherries (Prunus cerasus L. cv. Montmorency). Jour. Amer. Soc. Hort. Sci. 94: 387-391.

105. Waggoner, P.E. 1966. Decreasing transpiration and the effect upon growth. In: W.H. Pierre et al ed. Plant environment and efficient water use. p 49. Amer. Soc. Agron., Madison,Wis.

106. Webster, Barbara D. 1973. Ultrastructural studies of abscission in Phaseolus: ethylene effects on cell walls. Amer. Jour. Bot. 60 (5): 436-447.

107. Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science. 169: 1269-1278.

108. Wells, A.W. 1962. Effects of storage temperature and humidity on loss of weight by fruit. U. S. Department of Agriculture Marketing Research Report # 539.

109. Wilkinson, B.G. 1965. Some effects of storage under different conditions of humidity on the physical properties of apples. Jour. Hort. Sci. 40: 58-65.

110. Wilson, W.C. and C.H. Hendershott. 1968. Anatomical and histochemical studies of abscission of oranges. Proc. Amer. Soc. Hort. Sci. 92: 203-210.

111. Wittenbach, V.A. and M.J. Bukovac. 1972. A morphological and histochemical study of (2-chloroethyl) phosphonic acid enhanced abscission of sour and sweet cherry fruit. Jour. Amer. Soc. Hort. Sci. 97 (5): 628-631.

112. Wittenbach, V.A. and M.J. Bukovac. 1973. Cherry fruit abscission. Effect of growth substances, metabolic inhibitors and environmental factors. Jour. Amer. Soc. Hort. Sci. 98 (4): 348-351.

113. Wooley, Joseph T. 1967. Relative permeabilities of plastic films to water and carbon dioxide. Plant Physiol. 42: 641-643.

114. Wright, R.C. and T.M. Whiteman. 1931. Deterioration of Christmas holly in transit and storage. U. S. Department of Agriculture Circular # 207.

115. Young, R. and F. Meredith. 1971. Effect of exposure to subfreezing temperature on ethylene evolution and leaf abscission in Citrus. Plant Physiol. 48: 724-727.