THE INFLUENCE OF SUCCINIC ACID 2, 2-DIMETHYLHYDRAZIDE (ALAR) ON NET CO_2 ASSIMILATION RATE, CHLOROPHYLL CONTENT, ANATOMY, AND MORPHOLOGY OF VEGETATIVE GROWTH OF THE APPLE, *MALUS SYLVESTRIS* MILL.

by

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TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	2
III.	MATERIALS AND METHODS	7
	Field Experiment, 1966	7
	Laboratory Studies, 1966	8
	Leaf size and chlorophyll determinations	8
	Net assimilation rate	9
	Light microscopy	13
	Electron microscopy	15
	Greenhouse Experiment, 1967	17
	Laboratory Studies, 1967	19
	Leaf size, weight, and chlorophyll determinations	19
	Net assimilation rate	20
	Light microscopy	20
IV.	RESULTS	22
	Field Experiment, 1966	22
	Laboratory Studies, 1966	27
	Leaf size and chlorophyll determinations	27
	Net assimilation rate	27
	Light microscopy	27
	Electron microscopy	30
	Greenhouse Experiment, 1967	30
	Laboratory Studies, 1967	33

Page

Leaf size, weight, and chlorophyll determinations. . . 33

Net assimilation rate. 44

Light microscopy 44

V. DISCUSSION 63

VI. SUMMARY. 70

VII. LITERATURE CITED 73

VIII. VITA 77

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LIST OF TABLES

Table | Page

I. Alar influence on the number of leaves on the post-treatment portion of the shoot of 3 apple cultivars, 1966. 26

II. Influence of Alar on length and width of Golden Delicious apple leaves from the middle of the post-treatment portion of the shoot, 1966. 28

III. Effect of Alar on chlorophyll content per unit area of fresh tissue of Golden Delicious apple leaves from the middle of the post-treatment portion of the shoot, 1966 . 29

IV. Alar effect on radial diameter and length in microns of parenchyma cells in the pith and cortex of the post-treatment portion of the Golden Delicious shoot, 1966 . . 31

V. The influence of Alar on growth of the pre-treatment portion of the shoot of 2 apple cultivars, 1967 32

VI. Alar influence on growth of the post-treatment portion of the shoot of 2 apple cultivars, 1967 34

VII. The influence of Alar on shoot diameters of 2 apple cultivars, 1967 37

VIII. Influence of Alar on the average length and width in centimeters of leaves of 2 apple cultivars, 1967. 38

IX. Influence of Alar on average area per leaf of 2 apple cultivars, 1967 40

X. Effect of Alar on fresh and dry weight in grams per unit area of leaf tissue of 2 apple cultivars, 1967 . . . 41

XI. Influence of Alar on average dry weight of leaves of 2 apple cultivars, 1967 42

XII. Effect of Alar on total chlorophyll per unit area and per unit weight of fresh leaf tissue of 2 apple cultivars, 1967. 43

XIII. Alar effect on NAR of leaves from the pre-treatment portion of the shoot of 2 apple cultivars, 1967 45

Table		Page
XIV.	Alar effect on NAR of leaves of the post-treatment portion of the shoot of 2 apple cultivars, 1967	46
XV.	Thickness in microns of tissues from the 9th leaf of the post-treatment portion of the shoot of 2 apple cultivars as influenced by Alar, 1967	47
XVI.	The influence of Alar on the number of palisade cells per unit area and per layer per leaf of 2 apple cultivars, 1967	49
XVII.	Alar effect on the diameter of petioles of 2 apple cultivars, 1967	51
XVIII.	Effect of Alar on radial thickness in microns of stem tissues of 2 apple cultivars, 1967	52
XIX.	Estimated number of cells in cross section of the pith and cortex and xylem vessels of 2 apple cultivars as affected by Alar, 1967	54
XX.	Alar effect on radial diameter in microns of parenchyma cells in the pith and cortex and xylem vessels of 2 apple cultivars, 1967	55
XXI.	Influence of Alar on the length of parenchyma cells in the pith and cortex of 2 apple cultivars, 1967	57
XXII.	Estimated number of parenchyma cells per longitudinal column per internode in the pith and cortex of 2 apple cultivars as influenced by Alar, 1967	58

LIST OF FIGURES

Figure		Page
1.	The apparatus used for NAR determinations.	10
2.	The assimilation chambers and lighting system used for NAR determinations	11
3.	Length of the post-treatment portion of Golden Delicious apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966.	23
4.	Length of the post-treatment portion of York Imperial apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966.	24
5.	Length of the post-treatment portion of Delicious apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966.	25
6.	The post-treatment terminal growth of Golden Delicious shoots as influenced by Alar, 1967	35
7.	Effect of Alar concentrations (left to right, 0, 1000, 2000, and 4000 ppm) on the vegetative growth of York Imperial plants, 1967.	36
8.	Cross sections through the palisade layers of Golden Delicious leaf lamellae showing longer and larger palisade cells in the tissue treated with 2000 ppm of Alar (right) than in the untreated tissue (left), 1967. . . .	48
9.	Transverse sections through secondary xylem of untreated (left) and treated with 2000 ppm of Alar (right) of Golden Delicious apple stems. Note smaller vessels in treated xylem than in untreated, 1967	56
10.	Longitudinal sections through central pith parenchyma cells of York Imperial stem tissue showing thicker cell walls in the tissue treated with 2000 ppm of Alar (right) than in the untreated (left), 1967	60
11.	Transverse sections through the pith of untreated (left) and Alar-treated at 2000 ppm (right) York Imperial stems showing parenchyma cells misshaped in the treated tissue, 1967	61

Figure Page

12. Longitudinal sections through the cortical parenchyma cells of untreated (left) and Alar-treated at 2000 ppm (right) York Imperial stem tissue. Note the less organized arrangement of cells and intercellular spaces in the treated tissue than in the untreated, 1967. . . . 62

INTRODUCTION

The potential use of chemical regulators to influence growth and fruiting characteristics of apple trees recently has been receiving considerable attention (3, 5, 13, 15). One of the most promising of these chemical regulators is succinic acid 2, 2-dimethylhydrazide, hereafter referred to as "Alar". The first published account of the use of this compound as a growth retardant on plants was made by Riddell, et al. (34) in 1962.

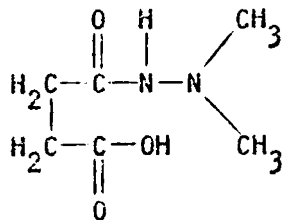
Some of the effects of Alar on apple trees are suppression of terminal growth (2, 3, 13, 14), greener and thicker leaves (13), increase of return bloom (3), less pre-harvest drop (12, 15), increase of fruit color (37), smaller fruit (3, 15, 16), firmer fruit (15, 39), and less storage scald (37, 39). While the effects of Alar on some of the growth and fruiting characteristics of apple trees have been studied, information is limited as to the influence of Alar upon fundamental physiological processes and internal structure.

The experiments reported herein were designed to study the effects of Alar on certain physiological, morphological, and anatomical characteristics of the vegetative growth of apple trees. The following factors were studied (a) net assimilation rate (NAR), (b) chlorophyll content, (c) node number, (d) fresh and dry weights of leaf and stem tissues, (e) stem length, diameter, and anatomy, and (f) leaf size and anatomy.

LITERATURE REVIEW

In recent years many compounds have been reported to have a retarding effect on the growth of various plants. Several of these compounds are unique in that they effectively regulate growth without causing formative effects in the treated plants. The physiology and history of the development of growth retarding chemicals have been reviewed extensively by Cathey (8). Because of the voluminous amount of literature that exists on this subject, this review will be confined largely to the references specifically related to phenomena being investigated.

Succinic acid 2, 2-dimethylhydrazide is a chemical compound developed by the Naugatuck Chemical Division of the United States Rubber Company. At the time of its release as an experimental compound it was designated by the code number B-995 and the chemical name N-dimethylaminosuccinamic acid. Through general usage these became shortened to B_9 and DMAS, respectively. First, the product was marketed for ornamentals under the name B-NINE. Later, a formulation for edible crops was developed under the label ALA -85, a registered trademark of the United States Rubber Company. At that time the chemical name was changed to succinic acid 2, 2-dimethylhydrazide (Alar). The chemical structure of the compound is as follows.



In 1962 Riddell, et al. (34) reported suppression of terminal growth of 14 plant species including the apple with the compound N, N-dimethylaminomaleamic acid, the maleamic analog of Alar. Following this work many investigators reported retarded shoot growth resulting from applications of Alar on numerous horticultural crops including the grape (7), the pear and sweet cherry (3), the bean (6), the cucumber (25), the apple (3, 16), and the rhododendron (9). Shorter internodes accounted for the suppression of shoot length in most crops. The number of nodes produced per shoot was not altered in the bean (6) and grape (7). However, with the cucumber there were fewer nodes due to treatment (25). The effect on node number was not reported for the other crops.

Several reports indicated that growth retardants affected the stem diameter. With 'Delicious' apple trees, Ferree and Stembridge (14) reported smaller shoot diameters from Alar treatment. Conversely, Bukovac (6) found that bean plants treated with N, N-dimethylaminomaleamic acid had larger stem diameters than the controls. According to Scherff (36) there was an increase in stem diameters of bean plants treated with Amo-1618 due to the stimulation of cell production in the cambium. This was accompanied by a delay in cell differentiation and an increase in cell volume of the parenchymatous cortical cells. Sachs and Kofranek (35) found that transverse cell division and expansion in the subapical tissues were stimulated by the retardants Amo-1618, Phosfon D, and Cycocel in chrysanthemum, resulting in stems that were thicker than normal. Brittain (4) reported that pith parenchyma cells in Alar-treated peanut plants were greater in diameter than cells in

untreated plants, thus causing the stem diameter to be larger in treated plants.

Several workers have noted effects of Alar on flowering and fruiting. Batjer, et al. (3) applied Alar to apple, pear, and sweet cherry trees beginning 15 to 17 days after full bloom. A marked increase in the amount of bloom the following spring was observed. Blossoming was delayed in apple trees and fruit size was less in apples and pears but not in sweet cherries. Greenhalgh (16) studied the effect of Alar on flowering and fruiting of several apple cultivars. He found that fruit set, fruit size, and the extent of flower bud initiation were less in Alar-treated plants than untreated.

Various effects of Alar on characteristics of leaves have been reported. Batjer, et al. (3) noted larger leaves on apple trees. Edgerton and Hoffman (13) reported that application of Alar as a foliar spray on 3-year-old 'Delicious' trees in mid-June produced leaves normal in shape but often larger in size. Humphries (24) theorized that the increased leaf area of mustard and tobacco plants was associated with the shorter shoot length. He stated that less internode growth induced by growth retardant application increased meristematic activity in the leaf primordia, producing more cells per leaf, or larger cells, by diverting nutrients from internodes to leaves. However, no evidence was presented to support these theories.

Some researchers have reported less leaf area from growth retardant applications. Bukovac (6) treated bean plants with N, N-dimethylaminomaleamic acid and at high concentrations found smaller leaflets. Crittendon (10) reported that Alar-treated chrysanthemum

and poinsettia plants had smaller leaves. He attributed this to more compact cells in the palisade layer with fewer intercellular spaces. With apple seedlings and 1-year-old cherry trees, Bukovac (5) found that Cycocel treatment resulted in narrower and shorter leaves. Apparently, the effect of growth retardants on leaf size varies with the growth retardant used and the experimental plant.

Many reports indicate that plants treated with Alar have darker green leaves (6, 8, 10, 13). There is evidence that this may be associated with delayed breakdown of chlorophyll since Halevy and Wittwer (17) found that chlorophyll was preserved in detached bean leaves and senescence was delayed in Alar-treated lettuce leaves (18). Crittendon (10) studied the effect of Alar on chlorophyll level in chrysanthemum and poinsettia plants. He found an increase in chlorophyll per unit area of fresh leaf tissue in Alar-treated leaves. He attributed this to less total leaf area, since no increase in chlorophyll was observed on a fresh weight basis.

Along with the reports of darker green leaves on Alar-treated apple trees, Edgerton and Hoffman (13) observed that the leaves were thicker in texture. With Amo-1618 treated bean plants, Scherff (36) also observed that the treated leaves were thicker than leaves of the untreated plants. The increased thickness of the leaves was accounted for by 1 to 3 additional layers of spongy parenchyma and concomitant intercellular spaces. Crittendon (10) found that with chrysanthemum, Alar-treated leaves were thicker than the checks due to longer palisade cells.

The effect of growth retardants on dry matter accumulation in

plant parts appears to be inconsistent. Bukovac ([illegible]) found that apple seedlings and 1-year-old cherry trees produced less dry matter in the tops with treatments of Cycocel and related chemicals. Greenhalgh (16) reported that Alar-treated apple leaves weighed less than untreated leaves. However, with grapes, Bukovac, et al. (7) found that dry matter accumulation was not altered.

Very little information is found in the literature concerning the effect of Alar on apparent photosynthesis. Brittain (4) found that net CO_2 assimilation rates were increased by Alar treatment where peanut plants were densely spaced. He suggested that "Alar may directly increase the photosynthetic efficiency of a unit area of canopy." However, Humphries (24) found that Cycocel treatment resulted in a decline in the NAR of mustard and tobacco leaves.

It is evident from the literature that Alar and related growth regulators can influence growth and fruiting characteristics of plants. While the effects of Alar on certain facets of growth and the external appearance of plants have been studied, information is limited as to the influence of Alar upon fundamental physiological processes and internal structure.

MATERIALS AND METHODS

Field Experiment, 1966

The study was conducted outside at Blacksburg, Virginia. 'York Imperial' and 'Delicious' on Malling Merton 104 rootstocks and 'Golden Delicious' on seedling rootstocks were used as test plants. The 'York Imperial' and 'Delicious' trees were grown in 1 gallon containers containing a soil mix of equal parts by volume of clay loam topsoil, coarse river sand, and German peat. The growing medium for the 'Golden Delicious' trees was an equal mixture by volume of clay loam topsoil and German peat. The soil mix for all plants was previously steam sterilized and the pH was adjusted to 7.1 by adding dolomitic limestone.

The plants were sprayed when necessary to control insects and diseases. Routine watering and fertilizing were carried out during the experiment.

On June 7, 1966, uniform plants, all of which had previously been pruned to 2 shoots, were arranged in 6 replicates. Treatments of 0, 500, 1000, 2000, 4000, and 8000 ppm of technical grade Alar in distilled water were assigned at random within these blocks and applied with a 1 qt compressed air sprayer. Tween 20 at 1000 ppm was used in all sprays. Twenty-four hours prior to spray applications the soil in each container was brought to field capacity. At the time of spraying the temperature was 95° F and the relative humidity was 45%.

The plants were arranged in a randomized complete block design. Plants of each block were mulched with sawdust.

Shoot length and leaf number records were taken at the time of spraying and 1, 2, 4, 6, and 10 weeks after treatment. After observing, sampling, and evaluating the shoots during the growing season, the plants were removed in mid-September.

Laboratory Studies, 1966

Leaf size and chlorophyll determinations

Three 'Golden Delicious' apple leaves from the middle of the post-treatment portion of the shoot (the portion of the shoot that developed after treatment) were removed from 4 replicates and placed in paper bags for transporting to the laboratory. After measuring the length and width of each leaf lamella, 4 disks were cut with a No. 6 cork borer from each of the 3 leaves for chlorophyll determinations.

Chlorophyll concentrations were determined by using a modification of the methods described by AOAC (31) and Comar and Zscheile (11). Preliminary trials indicated that mechanical blending under solvent was more efficient than hand grinding with quartz under solvent in mortar and pestle. The 12 disks for chlorophyll determinations were placed in a Waring blender container with 40 ml of acetone and blended for a total of 3 minutes. The sides of the container were washed down 3 times during the blending process. The extracts were filtered with suction, the filtrate transferred into a 100 ml volumetric flask, and placed in a water bath at 78° F for 10 minutes. Then the filtrate was brought to volume with acetone. A direct spectrophotometric determination of the transmittance was made on a sample of this solution in a Beckman DU-2 Spectrophotometer at 660 and 642.5 mμ. Cal-

culations of the chlorophyll level were made on a unit area basis.

Net assimilation rate

Net assimilation rates (NAR) were determined in the laboratory with a Beckman infrared CO_2 analyzer (Fig. 1). The analyzer used in this study contained a center zero meter and a flowing reference cell instead of the standard reference cell. The instrument was calibrated to read zero by flowing 275 ppm of CO_2 through the reference and sample cells and to read full scale by flowing 275 ppm of CO_2 in 1 cell and 325 ppm of CO_2 in the other.

There are 2 possible ways of using an infrared analyzer to measure CO_2 exchange in a plant (29). One involves the use of an open system and the other involves the use of a closed system. An open circuit apparatus was used in this experiment. In this type of system the incoming air is divided into 2 streams. One of these goes directly into the analyzer and the other stream first enters the leaf chamber and then goes through the analyzer. Both streams, having passed through the analyzer, are discharged to the outside. Knowing the rates of the air flow, and the amount of CO_2 absorbed by a leaf per unit time, the net rate of photosynthesis can be calculated (22).

The layout of the open-circuit apparatus with the assimilation chambers and light system used in this experiment are shown in Fig. 2. The dimensions of each leaf chamber were 2.5 x 8.5 x 18 cm. A lid with weather stripping was bolted on the chamber to seal it. The chambers were constructed of 1/4 inch plexiglas and were surrounded by a continuous flowing water system to control the temperature in

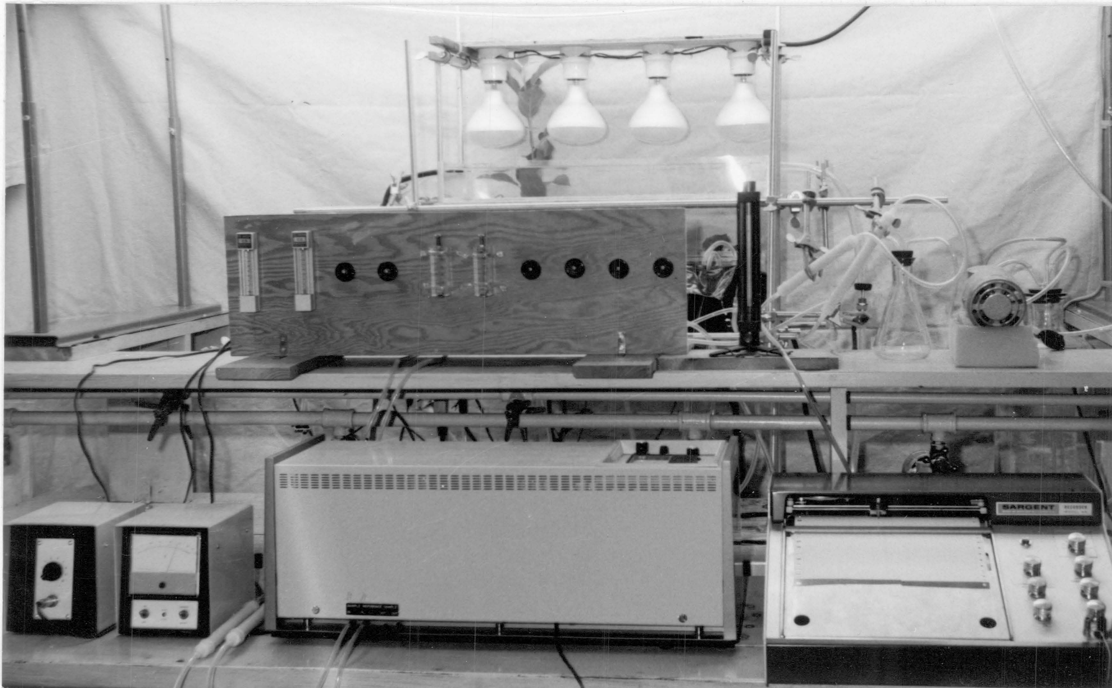


Fig. 1. The apparatus used for NAR determinations.

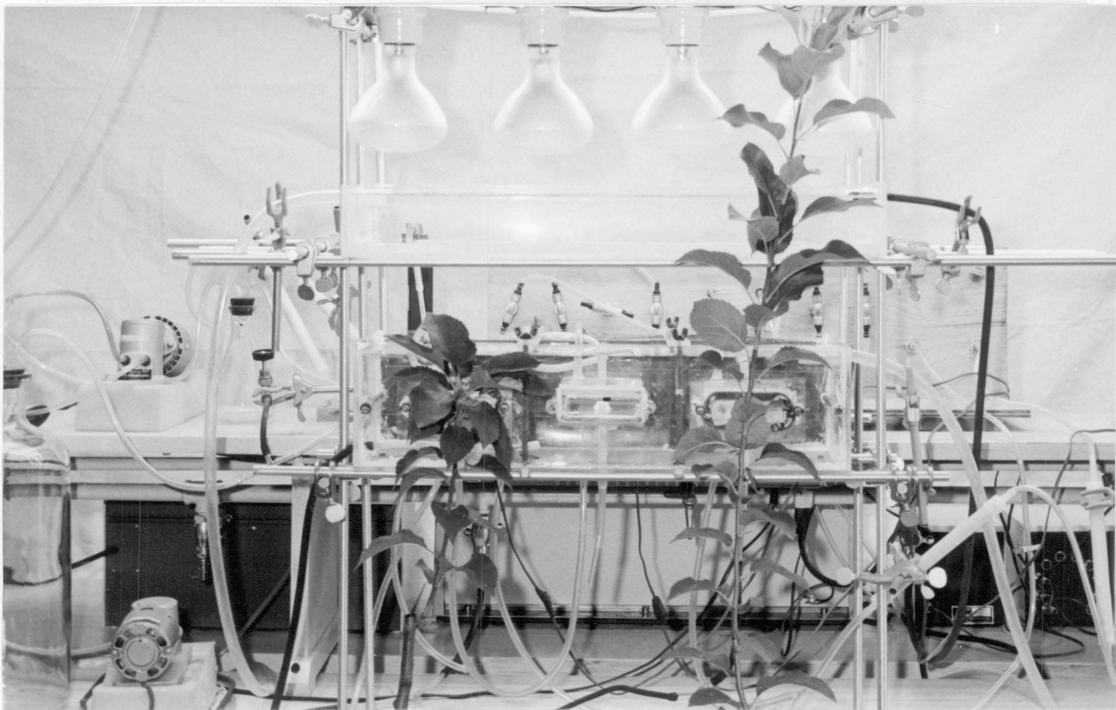


Fig. 2. The assimilation chambers and lighting system used for NAR determinations.

the chamber. A thermistor was mounted in each chamber for temperature readings with an Atkins Temperature Indicator. The temperature was maintained between 73 $\pm$ 1.5° F by controlling the water circulation speed.

Light for the chambers was provided by four 500 w reflector flood lamps 2 cm above a plexiglas trough filled with flowing water 7 cm deep for absorption of infra-red energy. Since Heinicke and Childers (20) found that with apple leaves there was no increase in CO_2 uptake as light intensities increased above 2500 ft-c., a light intensity of 3000 ft-c. was used. Light was measured with a Weston Model 756 Illumination Meter.

The flow rate of air through the leaf chambers and analyzer was maintained at 3 liters/minute. Prior to entering the analyzer the air stream was passed through tubes of drierite. Tygon tubing was used to connect the system since it is impervious to gas exchange. Circulation through the system was accomplished by using Neptune Dyna-Pumps.

A determination for a given leaf usually lasted between 2 and 3 minutes. A leaf in one chamber was allowed to equilibrate while the other was being measured. Then the tubing was switched to that chamber for a reading while a new leaf was equilibrating.

The NAR was computed from the area of the leaf, the rate of air flow (3 liters/minute), and the change in the CO_2 content of the air stream after passing over the leaf. It is this difference between the CO_2 content of the air entering and leaving the leaf chamber which is used to compute the NAR. This net change was a measure of apparent

photosynthesis as it expressed the net difference in the simultaneous processes of CO_2 uptake by photosynthesis and CO_2 evolution by respiration. A more detailed description of systems for measuring NAR is described by Heinicke (21) and Lister, et al. (29).

In the experiment reported herein a leaf from the middle of the post-treatment portion of the shoot from 4 replicates was detached for NAR measurements. Twenty-four hours prior to the removal of the leaves the soil in each container was brought to field capacity. Immediately after removal of the leaf from the plant, the petiole was immersed in an orchid vial containing distilled water. In the laboratory, NAR determinations were made with the apparatus described above.

Light microscopy

A leaf and internode from the middle of the pre-treatment portion of the shoot (the portion of the shoot present at treatment) and the middle of the post-treatment portion of the shoot were harvested 10 weeks after treatment. The leaf was removed with a razor blade and placed between a moistened paper towel for transporting to the laboratory. After measuring the length and width of each leaf, sections were taken from the center of the leaf along the midrib for microscopic study. Also, the portion of the petiole at the base of the leaf lamella was removed for cross sections.

The leaf and stem tissues were placed immediately in a formalin-acetic acid-alcohol (70% ethanol--90 ml, glacial acetic acid--5 ml, and formalin--5 ml) solution for killing and fixing. The samples

were evacuated with a lab-faucet aspirator to accelerate penetration of the killing solution.

All samples were dehydrated and infiltrated using a schedule developed for an Autotechnicon Ultra automatic tissue processing device which utilizes heat, vacuum, and vertical oscillation to hasten and improve processing. This instrument had previously been used successfully with animal tissue but had not been adapted for use with plant material. Experimentation with various alcohol series, clearing agents, and time periods proved the following 8 hour schedule to be best for leaves:

1. Distilled water--20 minutes.

2. Absolute alcohol, 3 changes--20 minutes each, 2 changes--40 minutes each, and 1 change--60 minutes.

3. Technicon clearing agent C-650, 3 changes--20 minutes, 30 minutes, and 70 minutes, respectively.

4. Paraffin, (mp 56.5° C) 2 changes--40 minutes, and 100 minutes, respectively.

Contrary to general belief, the abrupt change between steps 1 and 2 from water to absolute alcohol did not cause shrinkage or distortion. In fact, this immediate change to absolute alcohol was used since it was found to be faster in providing completely dehydrated tissue.

With stem tissue, it was necessary to lengthen the procedure. A 16 hour time disk was used and the specimens were left in the last paraffin for 1 hour after cut-off time before embedding. The following schedule gave the best results:

1. Distilled water--60 minutes.

2. Absolute alcohol, 2 changes--30 minutes each and 4 changes--60 minutes each.

3. Technicon clearing agent C-650, 3 changes--120 minutes each.

4. Paraffin, 2 changes--120 minutes each.

After embedding in plastic molds, serial cross and longitudinal sections were cut with a rotary microtome at 9 microns. In order to cut better stem sections, the blocks with the tissue exposed were immersed in a 1% solution of a mild liquid detergent for 18 hours to soften the material. After cutting, the sections were affixed to the slides with Haupt's adhesive (19) and stained with safranin and fast green according to the schedule described by Johansen (27). The slides were then examined microscopically. An ocular micrometer was used for measuring the radial diameter and length of parenchyma cells in the pith and cortex of 'Golden Delicious' stem tissues. Five cells were measured per tissue and treatment.

Electron microscopy

Stem sections from each treatment were removed from the plants and prepared for study according to the method described by Pease (3[illegible]) with several modifications. Longitudinal and transversal sections were fixed and stained in buffered glutaraldehyde for 20 hours.

Next, the specimens were washed in 3 rapid changes of distilled water and left in the water for 30 minutes. Osmium tetroxide was used as the secondary fixative and stain. The same washing procedure as described above was followed again.

After the tissue blocks had been fixed they were dehydrated by 10 minute exposures to 30, 50, 70, 80, 90 and 95% alcohol, followed by two 20 minute changes of 100% alcohol, and two 20 minute changes of propylene oxide.

Next the tissue was infiltrated with the following resin mixture.

Epon 812	36 ml
DDSA	24 ml
NMA	16 ml
DMP30	1 ml

Propylene oxide was used as the solvent.

The 1st step in infiltration was a 30 minute exposure to 1:1 propylene oxide and the resin mixture. Next the tissue was placed in the undiluted resin mixture for 1 hour with occasional stirring.

Then the tissue was embedded in gelatin capsules containing fresh resin mix. The transfer of tissue was done with a toothpick. The material was polymerized by placing the capsules in an oven at 60° C for 24 hours.

The preparation for the cutting of sections consisted essentially of trimming the embedded block and mounting it in the chuck of the microtome, fitting a knife with a trough for collecting the sections, mounting the knife in the microtome, filling the trough with 10% acetone and adjusting the fluid level and illumination, facing the block with the knife, and adjusting the microtome advance to give sections of the desired thickness (1). Knives for cutting were made from sheets of clean glass.

While studying the fine structure of isolated specialized cells contained in relatively large blocks of tissues, it was necessary to

examine many thick sections under a light microscope before selecting suitable areas for thin-sectioning using a method outlined by Jeon (26). After examination of sections under the light microscope with the above technique, a ribbon was selected and placed on the specimen grid for viewing and photographing under the electron microscope.

Greenhouse Experiment, 1967

One-year-old trees of the 'Golden Delicious' and 'York Imperial' cultivars on seedling rootstocks were grown in 1 gallon contractable plastic-liner containers in the greenhouse at the natural photoperiod during spring and summer. A minimum night temperature of 65° F and maximum day temperature of 95° F was maintained. The growing medium, previously steam sterilized for 30 minutes at 180° F, consisted of equal parts by volume of clay loam topsoil, coarse river sand, and German peat. The pH was adjusted from 5.5 to 7.0 by adding dolomitic limestone. At the time of planting the trees were cut off 38 cm above the soil line. All side branches were removed and 1 shoot was permitted to develop on each tree. Routine watering and fertilizing were carried out during the experiment.

To control insects and diseases the plants were fumigated with Tedion-Dithio gas every 2 weeks. Also, the foliage of all plants was washed twice a week with mist from a fog nozzle.

On May 24, 1967, uniform 'Golden Delicious' plants were selected and arranged in 12 replicates on the basis of their stage of development. The shoots averaged 27 cm long. Alar treatments were assigned at random within each replicate and applied with a 1 qt compressed

air sprayer to the point of runoff. The treatments were aqueous solutions of 0, 1000, 2000, and 4000 ppm of technical grade Alar. Tween 20 at 1000 ppm was used in all sprays to insure adequate wetting. Twenty-four hours prior to spray application, the soil in each container was brought to field capacity. At the time of spraying, the temperature was 92° F and the relative humidity was 30%. The plants were placed in a randomized complete block design.

On June 1, 1967, the 'York Imperial' plants were grouped and treated identically to the 'Golden Delicious' plants. At the time of spraying, the temperature was 94° F and the relative humidity was 35%.

At treatment, the basal leaves on the 1st cm of the shoot were removed from all plants of both cultivars due to the variation in leaf number and size. A magic marker was used to designate this 1 cm measurement. Hereafter this mark was used as the reference point for all determinations on the pre-treatment portion of the shoot.

Shoot length and leaf number records were taken at the time of spraying. All completely unfolded leaves were counted.

The experiment was terminated for each cultivar 10 weeks after treatment. At this time shoot length, leaf number, shoot diameter, node number, and dry weight of the leaf lamella, petiole, and stem were determined. Separate determinations were made on the pre-treatment portion of the shoot and the post-treatment portion. Diameter measurements at the middle of the 7th internode from the base of the pre-treatment portion of the shoot and at the middle of the 2nd and 10th internodes of the post-treatment portion of the shoot were made with a direct reading caliper gage graduated in .1 mm.

Laboratory Studies, 1967

Leaf size, weight, and chlorophyll determinations

Determinations were made on 4 replicates of each cultivar from 2 positions on a shoot from each treatment level. Position I included the 1st, 2nd, and 4th leaves and position II encompassed the 7th, 8th, and 10th leaves of the post-treatment portion of the shoot. Leaves from each position were harvested separately, but they were handled identically in making the determinations.

The 3 leaves were detached at 9 AM and placed in paper bags for transporting to the laboratory. After measuring the length and width of each leaf lamella, the area of each leaf blade was determined by tracing the leaf on a card, weighing the card, cutting the traced portion from the card and weighing this portion, and making the appropriate calculations. For dry weight determinations 4 disks were cut from a given side of each of the 3 leaves with a No. 6 cork borer. Next, 4 disks were cut from the other side of each leaf for fresh weight and chlorophyll determinations. The remaining portion of the 3 leaf lamallae was dried for dry weight determinations.

Chlorophyll levels were determined by the method used in 1966 with a few modifications. Although in the 1st experiment pigment extraction was carried out in the micro-cup of a Waring blender, this assembly was not entirely satisfactory because a bag of ice had to be held around the base of the cup during blending to prevent the temperature of the extract from rising. In 1967 a Servall high speed Omni-Mixer with overhead drive was used. The container could be immersed in an

ice bath during extraction to lower temperature during blending. The maximum temperature of the acetone during the extraction was 59° F. Calculations of the chlorophyll level were made on a unit area and fresh weight basis.

Net assimilation rate

Since in 1966 the excised leaves wilted shortly after transporting them to the laboratory, a slit was cut in the ends of the leaf chambers so that attached leaves could be measured. Modeling clay was used to seal around the petioles (Fig. 2). Otherwise the same apparatus and techniques were used in 1967 as in 1966.

The NAR of the 4th, 5th, and 6th leaves of the pre-treatment portion of the shoot was measured 1, 14, and 35 days after treatment, respectively. Also, determinations were made 40 days after treatment on the 3rd and 9th leaves of the post-treatment portion of the shoot.

Light microscopy

Ten weeks after treatment application, the 9th leaf and internode of the post-treatment portion of the shoot were harvested from 4 replicates of each cultivar for anatomical study. Immediately, leaf lamella sections were taken from the center of the leaf blade along the midrib for cross and paradermal sections. A portion of the petiole at the base of the leaf lamella was removed for cross sections. The middle section of the sampled internode was split into halves for both longitudinal and transverse sectioning of the same portion of the internode.

The same preparation techniques were used in 1967 as in 1966

except the stem tissues were not soaked in a detergent to soften the material. It was found that this increased cell size; therefore, the softening procedure was not used so that more accurate cell size could be determined.

An ocular micrometer was used for measuring the thickness of the various tissues and for measuring the longitudinal and radial diameters of various cells. From 3 to 5 measurements of tissues and cells from 4 plants in each treatment were made.

The number of cells in various tissues was determined by making cell counts within a grid of a known area. Then, calculations were made of the area of the particular tissue being studied. This and other information at hand permitted calculation of an estimate of the number of cells per tissue being studied.

The photomicrographs, which are presented under results, were taken of sections representative of the effects on an entire treatment of 4 replicates.

RESULTS

Field Experiment, 1966

The amount and duration of terminal growth suppression by Alar varied among cultivars (Figs. 3, 4, and 5). However, the data show a consistent pattern in that the amount of growth retardation was related directly to the concentration of Alar applied.

The 'Golden Delicious' shoot length was apparently not affected during the 1st week after treatment with Alar (Fig. 3). Then the rate of growth was suppressed by Alar until 4 weeks after treatment. At this time the plants treated with the lower concentrations began to "grow out" of the retardation effect whereas at 8000 ppm the effect was present at the termination of the experiment. With the 'York Imperial' plants, which were the most responsive to Alar, the treatment effect was obvious during the 1st week's growth and was still present at the 6 to 10 week interval of growth with all Alar treatments (Fig. 4). The 'Delicious' plants, the least responsive to treatment, were influenced by Alar during the 1st week's growth but began to "grow out" of the treatment effect after only 2 weeks except at 8000 ppm (Fig. 5). Furthermore, total terminal growth of the 'Delicious' shoots was not affected at the lower concentrations; whereas with the 'Golden Delicious' and 'York Imperial' cultivars, 500 ppm of Alar suppressed shoot growth.

The influence of Alar on leaf number on the post-treatment portion of the shoot of 3 apple cultivars is presented in Table 1. With the 'Golden Delicious' and 'York Imperial' plants there were fewer

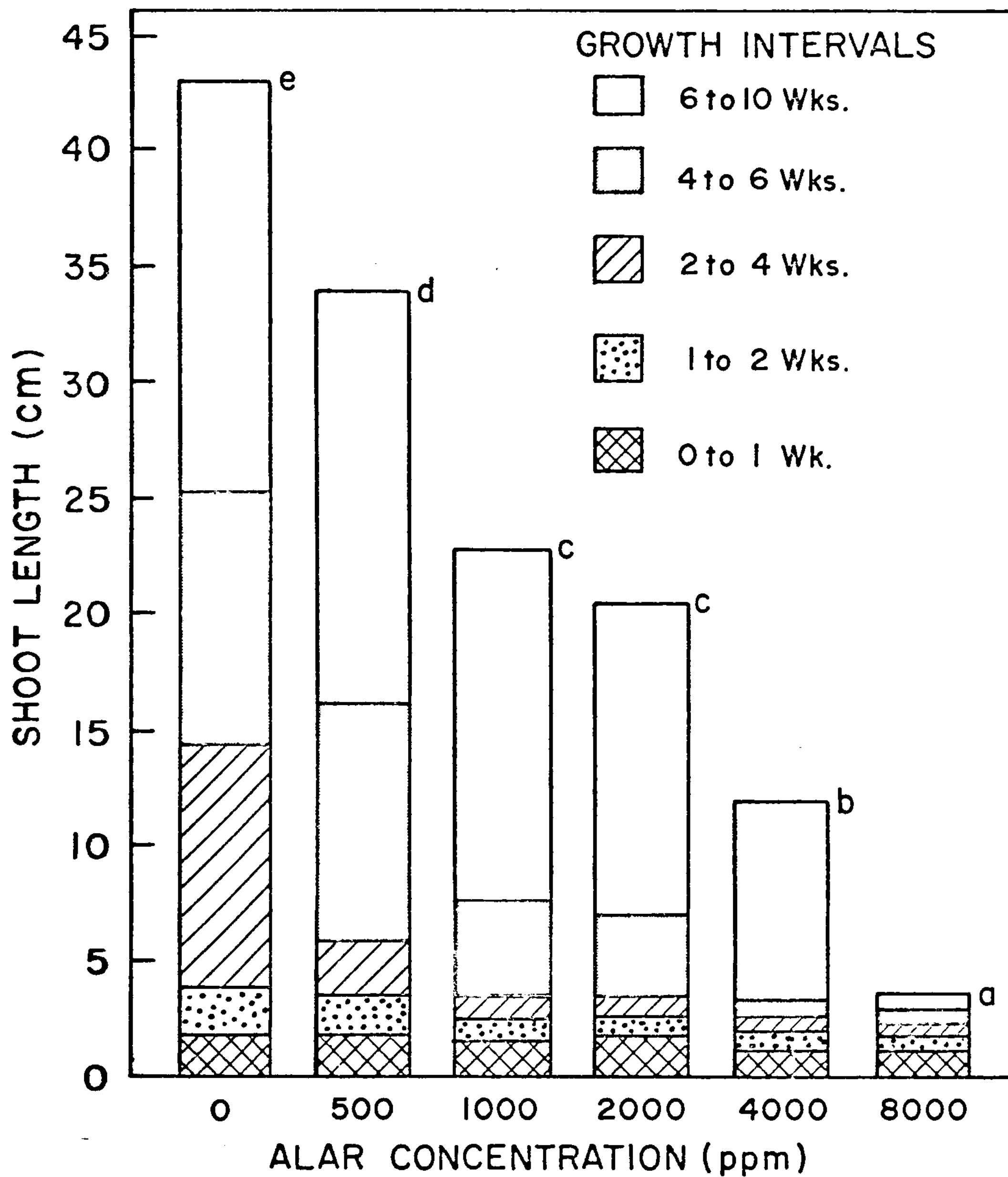


Fig. 3. Length of the post-treatment portion of Golden Delicious apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966. Bars with a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

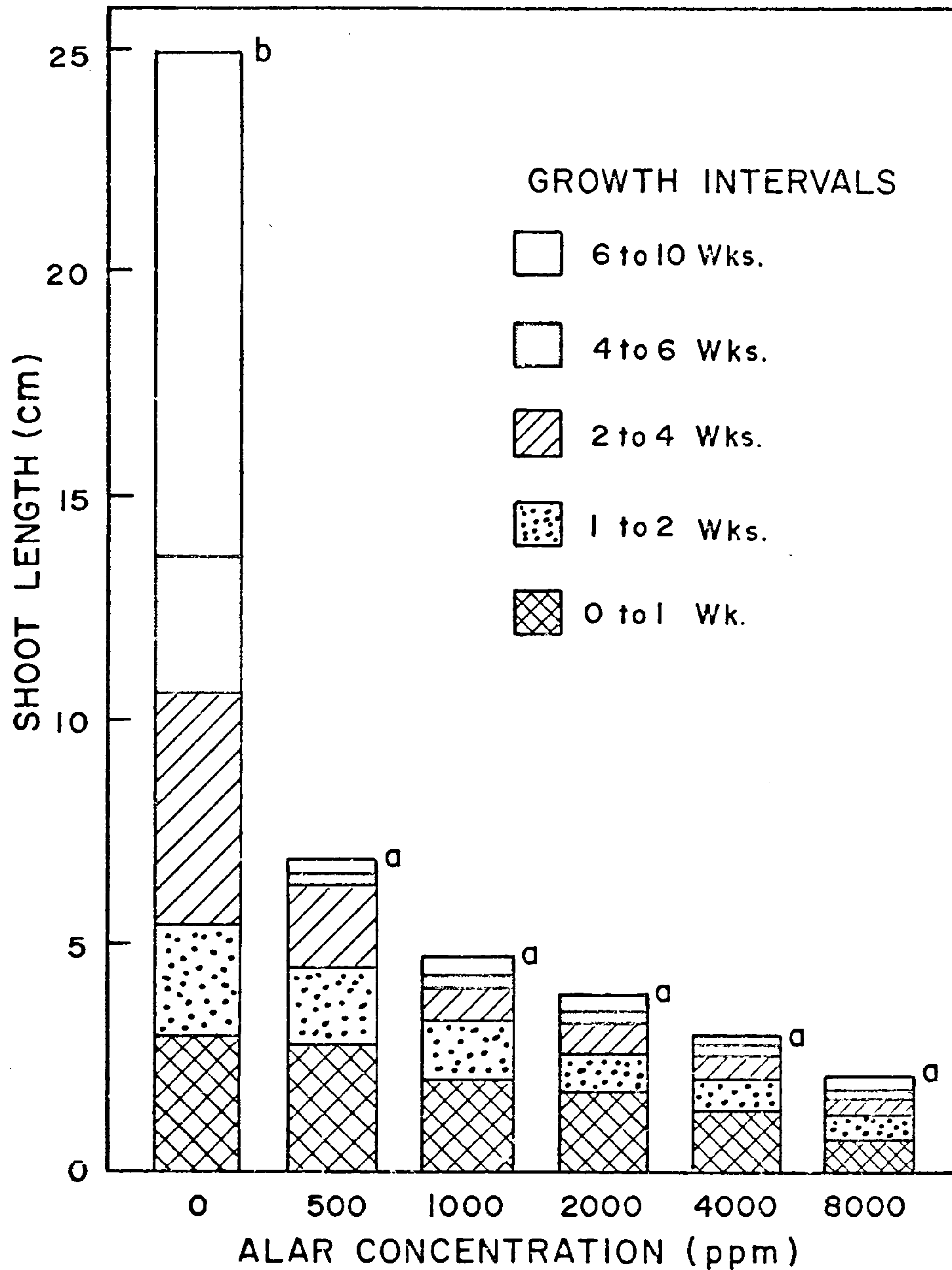


Fig. 4. Length of the post-treatment portion of York Imperial apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966. Bars with a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

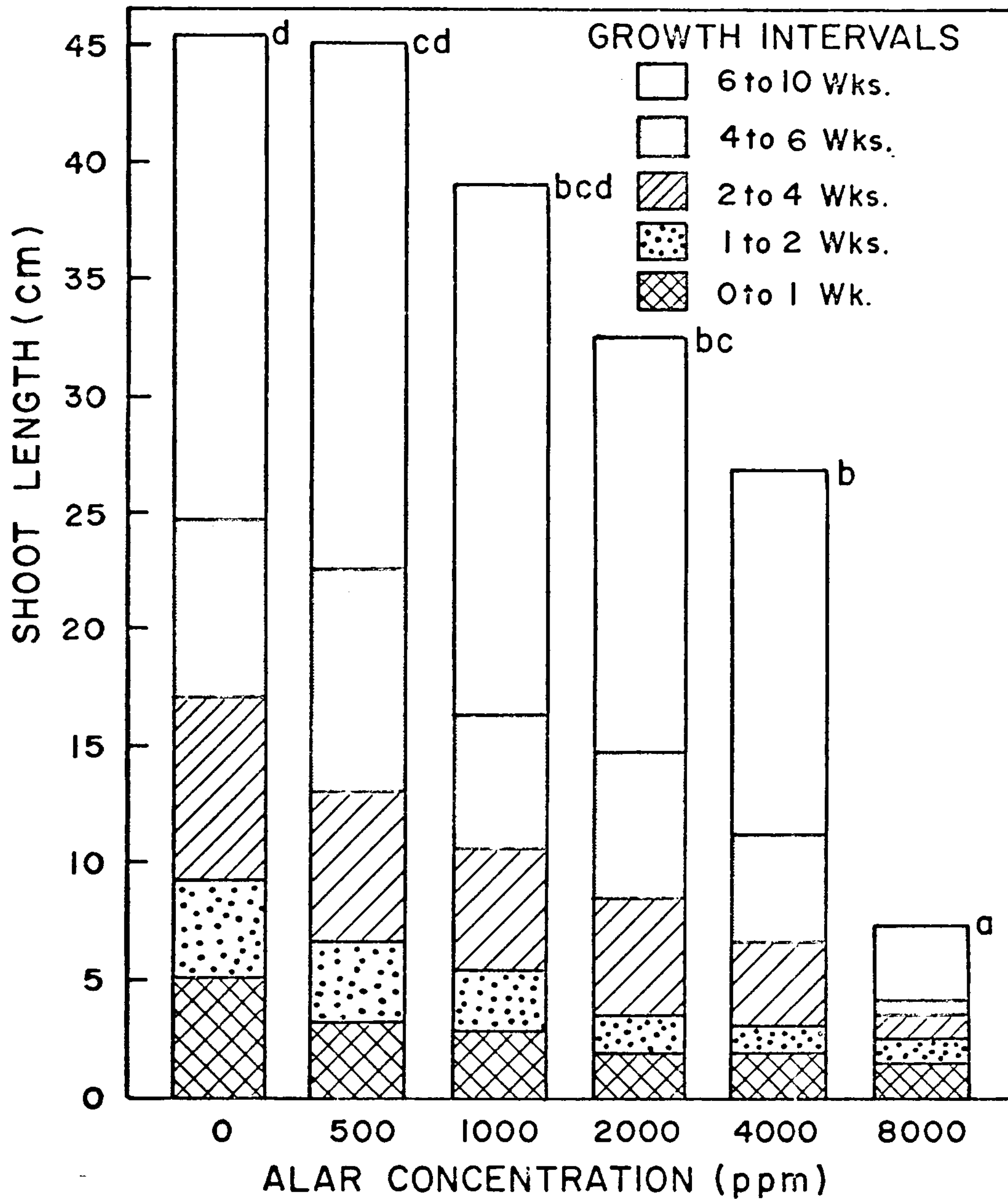


Fig. 5. Length of the post-treatment portion of Delicious apple shoots 1, 2, 4, 6, and 10 weeks after treatment with various concentrations of Alar, 1966. Bars with a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

Table I. Alar influence on the number of leaves on the post-treatment portion of the shoot of 3 apple cultivars, 1966.

Alar conc. (ppm)	Number of leaves at harvest Golden Delicious	York Imperial	Delicious
0	18d[x]	11c	18b
500	17d	6b	17b
1000	13c	5ab	14ab
2000	12c	4ab	13ab
4000	9b	3ab	12ab
8000	5a	2a	9a

[x]Within each column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

leaves at harvest on the treated than on the untreated shoots. However, with the 'Delicious' shoots, 8000 ppm was the only treatment that limited leaf number.

Laboratory Studies, 1966

Leaf size and chlorophyll determinations

The 'Golden Delicious' leaves from the middle of the post-treatment portion of the shoot were longer and wider when treated with rates of 500, 1000, and 2000 ppm of Alar (Table II). However, leaves from plants treated with a concentration of 4000 ppm of Alar were smaller than leaves from the untreated plants. At rates of 2000 and 4000 ppm of Alar chlorophyll concentration was increased per unit area of fresh tissue of 'Golden Delicious' leaves from the middle of the post-treatment portion of the shoot (Table III).

Net assimilation rate

Attempts to determine the effect of Alar on the NAR were unsuccessful using the procedure outlined. The excised leaves wilted shortly after being put in the assimilation chambers.

Light microscopy

A schedule was developed which reduced the time necessary for preparation of plant material for paraffin embedding from several days to 8 hours for leaf tissue and 17 hours for stem tissue. Within these time periods up to 100 samples were prepared using this procedure and instrument.

Microscopic examination of leaf lamella sections from 'Golden

Table II. Influence of Alar on length and width of Golden Delicious apple leaves from the middle of the post-treatment portion of the shoot, 1966.

Alar conc. (ppm)	Length (cm)	Width (cm)
0	7.8b[x]	6.0b
500	8.3c	6.3c
1000	8.8d	6.6cd
2000	9.1d	6.7d
4000	7.4a	5.5a

[x]Within each column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table III. Effect of Alar on chlorophyll content per unit area of fresh tissue of Golden Delicious apple leaves from the middle of the post-treatment portion of the shoot, 1966.

Alar conc. (ppm)	Total chlorophyll ($\mu g/cm^2$)
0	257a[x]
500	296ab
1000	302abc
2000	320bc
4000	354c

[x]Any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Delicious' plants indicated that treated leaves were thicker than the untreated leaves. The stem diameter of the post-treatment portion of the shoot was observed to be greater as a result of Alar treatment. However, the pre-treatment portion of the shoot had a smaller diameter in treated plants after it matured than in untreated plants.

To determine a cause for the thicker stems and shorter internodes of the post-treatment portion of the shoot in Alar-treated plants, measurements were made of cell size in the pith and cortex. Data presented in Table IV indicate that transverse cell expansion was stimulated by Alar while longitudinal cell expansion was inhibited.

Electron microscopy

Since replica specimens were not suitable for obtaining the information needed in this study an attempt was made to work with thin sections. It was found that the tissue was too hard to obtain intact sections. A softening process will have to be developed before thin section study of apple stem tissue can be continued with the electron microscope.

Greenhouse Experiment, 1967

The effect of Alar on growth of the pre-treatment portion of the shoot for the 2 cultivars studied is presented in Table V. There was no treatment effect except in dry weight of the 'Golden Delicious' leaf lamellae and 'York Imperial' stem tissues. Total weight of leaf blades per 'Golden Delicious' plant was increased due to Alar treatment at 2000 ppm. The stem tissues of treated 'York Imperial' plants

Table IV. Alar effect on radial diameter and length in microns of parenchyma cells in the pith and cortex of the post-treatment portion of the Golden Delicious shoot, 1966.

Alar conc. (ppm)	Pith		Cortex	
	Radial diameter	Length	Radial diameter	Length
0	43[x]	54	34	39
500	46	49	36	38
1000	50	45	39	35
2000	55	42	44	33
4000	57	39	45	32

[x]Mean measurement of 5 cells.

Table V. The influence of Alar on growth of the pre-treatment portion of the shoot of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	No.of nodes	Leaf no. at harvest	Dry wt. (g) / plant Stem	Leaf blade	Petiole
Golden Delicious	0	11[x]	7	6.23	1.97a	.15
	1000	11	8	5.24	2.08a	.14
	2000	11	8	5.89	2.56b	.18
	4000	11	9	4.73	2.41ab	.16
York Imperial	0	14	11	6.40c	2.98	.26
	1000	14	12	4.74b	3.06	.25
	2000	14	11	3.74ab	2.99	.24
	4000	14	12	3.38a	3.01	.24

[x]Within each cultivar in a column, unlettered means or means having a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

weighed less than stems from untreated plants. The trend was the same in the 'Golden Delicious' plants but was not significant at the .05 level.

The post-treatment shoot of both cultivars was drastically changed by Alar treatment (Table VI). However, the 'York Imperial' plants were more responsive than the 'Golden Delicious' plants. Terminal growth (Fig. 6) and stem weight were less in treated 'Golden Delicious' plants than untreated. At Alar concentrations of 2000 and 4000 ppm on 'Golden Delicious' plants there were fewer nodes and leaves than on the untreated plants. Also, the 'Golden Delicious' leaf blades and petioles weighed less on the plants treated at 4000 ppm than on the check plants. The 'York Imperial' shoots were shorter (Fig. 7) with fewer nodes and leaves, and all tissues weighed less due to Alar treatment.

Shoot diameters of both cultivars were less in the treated than untreated plants at positions I and II (Table VII). However, at position III Alar resulted in an increase in the stem diameter.

Laboratory Studies, 1967

Leaf size, weight, and chlorophyll determinations

The influence of Alar on length and width of leaves is presented in Table VIII. At position I the 'Golden Delicious' leaves were shorter and narrower as a result of Alar treatment at 4000 ppm. At position II the treated leaves were longer at 1000 and 2000 ppm but shorter at 4000 ppm than the check leaves. Width of 'Golden Delicious' leaves was not affected by Alar at position II. 'York Imperial' plants

Table VI. Alar influence on growth of the post-treatment portion of the shoot of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Term.gr. (cm)	No.of nodes	Leaf no. at harvest	Dry wt. (g) / plant Stem	Leaf blade	Petiole
Golden Delicious	0	75.2c[x]	33b	27b	7.87c	9.83b	1.01b
	1000	38.3b	32b	26b	3.90b	8.24b	.92b
	2000	23.8a	26a	21a	3.04ab	7.39ab	.86b
	4000	14.8a	23a	18a	1.79a	5.67a	.64a
York Imperial	0	70.8c	30d	24d	8.11c	10.12d	1.03d
	1000	26.0b	24c	18c	2.63b	6.14c	.77c
	2000	7.7a	17b	11b	.92a	2.43b	.34b
	4000	6.3a	11a	5a	.64a	1.37a	.16a

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

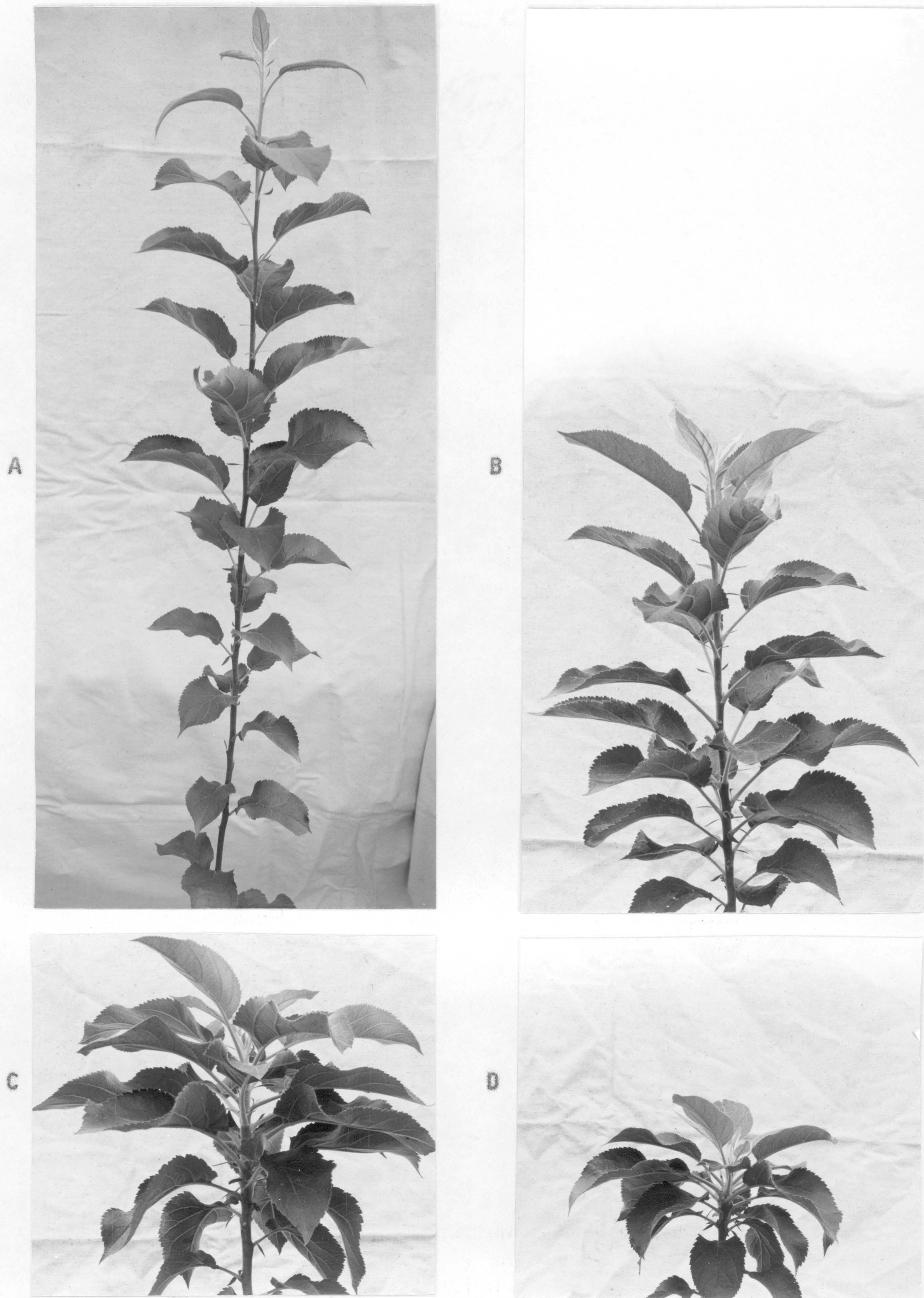


Fig. 6. The post-treatment terminal growth of Golden Delicious shoots as influenced by Alar, 1967. A=0, B=1000, C=2000 and D=4000 ppm of Alar. Photographed 8 weeks after treatment.

Fig. 7. Effect of Alar concentrations (left to right, 0, 1000, 2000 and 4000 ppm) on the vegetative growth of York Imperial plants, 1967. The horizontal line separates the pre-treatment and the post-treatment portions of the shoot. Photographed 8 weeks after treatment.

Table VII. The influence of Alar on shoot diameters of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Mean diameters, cm Position I[w]	Position II[x]	Position III[y]
Golden Delicious	0	.75b[z]	.69b	.60a
	1000	.73b	.64a	.69b
	2000	.69a	.65a	.76c
	4000	.66a	.61a	.81d
York Imperial	0	.79c	.70c	.64a
	1000	.72b	.63b	.66a
	2000	.61a	.58a	.78b
	4000	.60a	.57a	.76b

[w]The middle of the 7th internode of the pre-treatment portion of the shoot.

[x]The middle of the 2nd internode of the post-treatment portion of the shoot.

[y]The middle of the 10th internode of the post-treatment portion of the shoot.

[z]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table VIII. Influence of Alar on the average length and width in centimeters of leaves of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Position I[x] Length	Position I[x] Width	Position II[y] Length	Position II[y] Width
Golden Delicious	0	9.1b[z]	5.6b	11.0b	6.9
	1000	9.0b	5.5b	12.0c	6.7
	2000	9.0b	5.3ab	12.1c	6.6
	4000	8.2a	5.0a	10.2a	6.2
York Imperial	0	10.8c	7.1b	11.3b	7.1b
	1000	10.1b	6.4a	10.1ab	5.4a
	2000	9.6ab	6.3a	9.5a	5.4a
	4000	9.3a	6.2a	9.2a	5.3a

[x]The 1st, 2nd, and 4th leaves of the post-treatment portion of the shoot.

[y]The 7th, 8th, and 10th leaves of the post-treatment portion of the shoot.

[z]Within each cultivar in a column, unlettered means or means having a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

treated with Alar had shorter and narrower leaves at both positions.

At position I Alar-treated 'Golden Delicious' leaves had less leaf area (Table IX). At position II treatments of 1000 and 2000 ppm increased leaf area but there was less leaf area with the 4000 ppm treatment. With the 'York Imperial' cultivar, treated plants had less leaf area at both positions than untreated plants.

The influence of Alar on fresh and dry weight per unit area of leaf tissue is presented in Table X. There was an increase in fresh and dry weight per unit area of leaf tissue at both positions and cultivars with the exception of dry weight of tissue at position I of the 'Golden Delicious' leaves. In this case only the 2000 ppm Alar concentration increased dry weight.

Dry weight was measured on the portion that remained after disks were removed for chlorophyll and weight determinations of the 7th, 8th, and 10th leaves from the post-treatment part of the shoot. With the 'Golden Delicious' cultivar, the 1000 and 2000 ppm Alar treatments increased dry weight per leaf whereas at 4000 ppm there was no effect (Table XI). However, the dry weight per 'York Imperial' leaf was less due to Alar treatment.

Chlorophyll per unit area of fresh tissue of both cultivars and at both positions was increased as a result of treatment (Table XII). There was no effect of Alar on chlorophyll concentration on a weight basis except at position I with the 'York Imperial' leaves where there was an increase.

Table IX. Influence of Alar on average area per leaf of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Leaf area, cm^2 Position I^x	Position II^y
Golden Delicious	0	$40.0c^z$	54.9b
	1000	36.5b	61.6c
	2000	33.8b	62.4c
	4000	30.4a	49.1a
York Imperial	0	61.0c	64.9c
	1000	53.0b	47.7b
	2000	50.0b	42.2ab
	4000	43.9a	37.1a

[x]The 1st, 2nd, and 4th leaves of the post-treatment portion of the shoot.

[y]The 7th, 8th, and 10th leaves of the post-treatment portion of the shoot.

[z]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table X. Effect of Alar on fresh and dry weight in grams per unit area[w] of leaf tissue of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Position I[x] Fresh wt.	Position I[x] Dry wt.	Position II[y] Fresh wt.	Position II[y] Dry wt.
Golden Delicious	0	.201a[z]	.091a	.228a	.095a
	1000	.218bc	.092a	.273b	.108b
	2000	.226c	.102b	.289c	.121c
	4000	.214b	.093a	.275b	.112bc
York Imperial	0	.214a	.090a	.231a	.097a
	1000	.252b	.103b	.272b	.109b
	2000	.250b	.111b	.284c	.127c
	4000	.254b	.109b	.283c	.122c

[w]Equal to 13 cm^2 of tissue removed by a cork borer from 3 leaves.

[x]The 1st, 2nd, and 4th leaves of the post-treatment portion of the shoot.

[y]The 7th, 8th, and 10th leaves of the post-treatment portion of the shoot.

[z]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XI. Influence of Alar on average dry weight of leaves[x] of 2 apple cultivars, 1967.

Alar conc. (ppm)	Dry weight (g)	
	Golden Delicious	York Imperial
0	1.10a[y]	1.24b
1000	1.32b	1.01a
2000	1.44b	.98a
4000	1.09a	.89a

[x]The 7th, 8th, and 10th leaves from the post-treatment portion of the shoot minus the 26 cm^2 of tissue used for fresh and dry weight determinations.

[y]Within each column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XII. Effect of Alar on total chlorophyll per unit area and per unit weight of fresh leaf tissue of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Position I[x] ($\mu g/cm^2$)	Position I[x] ($\mu g/g$)	Position II[y] ($\mu g/cm^2$)	Position II[y] ($\mu g/g$)
Golden Delicious	0	308a[z]	1987	310a	1685
	1000	338ab	2039	350b	1772
	2000	356b	2065	384bc	1764
	4000	360b	2160	395c	1813
York Imperial	0	314a	1769a	334a	1887
	1000	392b	2009b	411b	1966
	2000	410bc	2151c	440c	2040
	4000	432c	2231c	471d	2173

[x]The 1st, 2nd, and 4th leaves of the post-treatment portion of the shoot.

[y]The 7th, 8th, and 10th leaves of the post-treatment portion of the shoot.

[z]Within each cultivar in a column, unlettered means or means having a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

Net assimilation rate

The influence of Alar on the NAR 1, 14, and 35 days after treatment of leaves from the pre-treatment portion of the shoot is presented in Table XIII. One and 14 days after treatment, Alar at rates of 2000 and 4000 ppm resulted in a lower NAR of 'Golden Delicious' leaves. With the 'York Imperial' cultivar, all Alar treatments caused a reduction in the CO_2 assimilation rate. However, 35 days after treatment leaves from both cultivars that had been treated with Alar had a higher NAR than the untreated leaves.

The data for the influence of Alar on the NAR 40 days after treatment of the 3rd and 9th leaves of the post-treatment portion of the shoot of 2 apple cultivars are presented in Table XIV. All Alar treatments caused a reduction in the NAR of leaves of both cultivars except the 1000 ppm treatment in the 'York Imperial' leaves.

Light microscopy

The thickness of leaf tissues as influenced by Alar is presented in Table XV. There was no effect on the thickness of the epidermal layers. However, the palisade and spongy mesophyll were thicker in the treated than in the untreated plants. Therefore, the total leaf thickness was increased as a result of Alar treatment. The increased thickness of the palisade region of the treated leaves was due to longer palisade cells rather than there being more palisade cell layers (Fig. 8).

Data presented in Table XVI show the effect of Alar on the number of palisade cells per unit area and per layer per leaf as determined

Table XIII. Alar effect on NAR of leaves from the pre-treatment portion of the shoot of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	CO_2 assimilation, mg dm^{-2} hr^{-1} Tr. + 1 day	Tr. + 14 days	Tr. + 35 days
Golden Delicious	0	18.8b[x]	24.7b	15.4a
	1000	16.8b	22.6ab	17.2b
	2000	14.6a	21.1a	18.2bc
	4000	13.6a	20.8a	18.8c
York Imperial	0	21.3b	20.5c	14.3a
	1000	17.0a	17.9b	16.6b
	2000	16.4a	16.0a	19.4c
	4000	13.7a	15.7a	19.9c

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XIV. Alar effect on NAR[x] of leaves of the post-treatment portion of the shoot of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	CO_2 assimilation, mg $dm^{-2}hr^{-1}$	
		Third leaf after tr.	Ninth leaf after tr.
Golden Delicious	0	19.1c[y]	18.7d
	1000	17.7b	17.4c
	2000	16.4a	16.7b
	4000	16.0a	15.7a
York Imperial	0	21.6b	19.7b
	1000	19.6a	18.4b
	2000	18.8a	16.1a
	4000	17.9a	16.0a

[x]The measurements were made 40 days after treatment.

[y]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XV. Thickness in microns of tissues from the 9th leaf of the post-treatment portion of the shoot of 2 apple cultivars as influenced by Alar, 1967.

Cultivar	Alar conc. (ppm)	Upper epidermis	Palisade	Spongy mesophyll	Lower epidermis	Total
Golden Delicious	0	16[x]	89a	91a	12	208a
	1000	17	102b	100b	14	233b
	2000	19	110c	105b	14	248c
	4000	18	108c	102b	13	241bc
York Imperial	0	16	87a	89a	12	204a
	1000	16	99b	100b	14	229b
	2000	18	111c	106b	15	250c
	4000	18	111c	107b	16	252c

[x]Within each cultivar in a column, unlettered means or means having a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

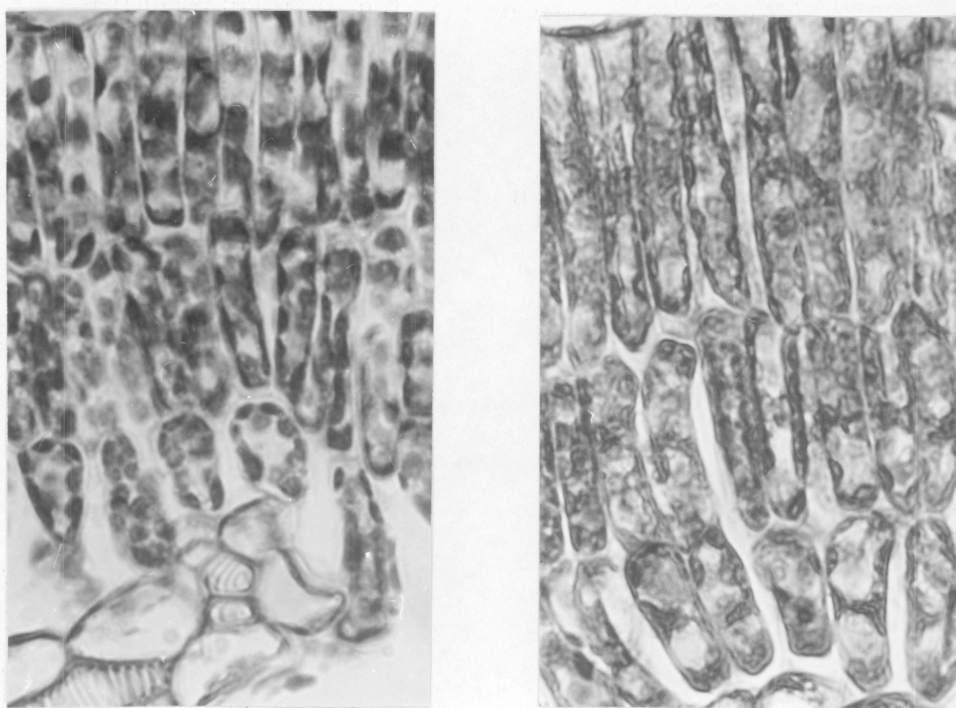


Fig. 8. Cross sections through the palisade layers of Golden Delicious leaf lamellae showing longer and larger palisade cells in the tissue treated with 2000 ppm of Alar (right) than in the untreated tissue (left), 1967. (X1000).

Table XVI. The influence of Alar on the number of palisade cells per unit area and per layer per leaf of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Cells/.01 square mm	Est. no. of cells/layer/leaf (x 10^{-5})
Golden Delicious	0	104c[x]	527
	1000	92b	575
	2000	85a	574
	4000	109d	531
York Imperial	0	107a	645
	1000	116b	522
	2000	123c	457
	4000	123c	340

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

from paradermal sections. At 1000 and 2000 ppm of Alar, 'Golden Delicious' leaves contained fewer palisade cells per .01 square mm indicating more intercellular spaces and larger cells whereas at 4000 ppm the contrary occurred. The estimated number of cells per layer per 'Golden Delicious' leaf was increased at 1000 and 2000 ppm but unaffected at 4000 ppm. With the 'York Imperial' cultivar, the treated leaves had more cells per unit area but fewer cells per layer per leaf.

Petioles of treated 'Golden Delicious' leaves were thicker in both the lateral and vertical planes than petioles of untreated plants (Table XVII). Also, the Alar-treated 'Golden Delicious' petioles were circular whereas the untreated were oval. There was no consistent effect of treatment on the diameter and shape of 'York Imperial' petioles.

The stem radius was increased by Alar. In 'Golden Delicious' plants this was due to an increase in the radial thickness of the pith, phloem, and cortex tissues (Table XVIII). There was no consistent effect on the transverse thickness of the 'Golden Delicious' xylem. The 'York Imperial' stem radius was increased as a result of the pith and cortex tissue being thicker even though the xylem was thinner in the treated plants. The radial thickness of 'York Imperial' phloem was not affected by Alar.

From the radial thickness data presented in Table XVIII, the area of each of the tissues was calculated by assuming that each tissue was circular. The same trends for area determinations were present as described above. However, since the radius of the pith was increased by Alar, the differences between treatments of other tissues

Table XVII. Alar effect on the diameter of petioles of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Mean diameter, microns Lateral	Vertical
Golden Delicious	0	2130a[x]	1874a
	1000	2440b	2459bc
	2000	2599b	2637c
	4000	2355ab	2356b
York Imperial	0	2589ab	2778b
	1000	2784c	2874b
	2000	2658bc	2832b
	4000	2455a	2629a

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XVIII. Effect of Alar on radial thickness in microns of stem tissues of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Pith radius	Xylem	Phloem	Cortex	Stem radius
Golden Delicious	0	766a[x]	1478ab	257a	485a	3012a
	1000	1029b	1659b	324b	660b	3698b
	2000	1309c	1513b	377c	817c	4044c
	4000	1635d	1315a	407c	941c	4326c
York Imperial	0	838a	1414c	237	609a	3124a
	1000	1086b	1033b	256	854b	3257a
	2000	1541c	676a	292	1253c	3790b
	4000	1729d	582a	257	1267c	3854b

[x]Within each cultivar in a column, unlettered means or means having a letter in common are not significantly different at the .05 level by Duncan's Multiple Range Test.

showing an increasing trend due to Alar treatment were magnified and the ones showing a decreasing trend were less significantly different.

The estimated number of cells in cross section of the pith and cortex and xylem vessels as affected by Alar is presented in Table XIX. Treatment increased the number of parenchyma cells in cross section in the pith and cortex. However, there were fewer xylem vessels in the treated than untreated 'York Imperial' plants.

The radial diameter of parenchyma cells in the pith and cortex was increased by Alar (Table XX). However, the xylem vessels of the treated plants were smaller than vessels of the untreated plants (Fig. 9). It is apparent from comparisons of Tables XIX and XX that Alar affects radial cell number more than radial cell diameter.

The influence of Alar on the length and number of parenchyma cells per longitudinal column per internode in the pith and cortex is presented in Tables XXI and XXII. The treated plants had shorter and fewer cells per longitudinal column per internode. By comparing the values in these 2 tables it is obvious that Alar influences longitudinal cell division more than it affects cell length.

By multiplying the values in Table XIX by values in Table XXII of the respective tissues, an estimate of the total number of cells for each of the tissues studied per half of an internode was obtained. It was found that Alar treatment resulted in fewer total cells per internode in all tissues studied except in the pith of the 'Golden Delicious' cultivar. In the latter case, there was enough increase in cell number in cross section to more than offset the decrease in longitudinal cell number.

Table XIX. Estimated number of cells in cross section of the pith and cortex and xylem vessels of 2 apple cultivars as affected by Alar, 1967.

Cultivar	Alar conc. (ppm)	Number of cells x 10^{-2}		
		Pith	Xylem	Cortex
Golden Delicious	0	14	61	74
	1000	23	62	114
	2000	33	63	136
	4000	47	63	145
York Imperial	0	13	43	68
	1000	19	37	86
	2000	35	30	131
	4000	39	30	123

Table XX. Alar effect on radial diameter in microns of parenchyma cells in the pith and cortex and xylem vessels of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Pith	Xylem	Cortex
Golden Delicious	0	39a[x]	45b	30a
	1000	45b	45b	34b
	2000	48c	38a	38c
	4000	51c	34a	40c
York Imperial	0	46a	49d	37a
	1000	51ab	42c	40b
	2000	53b	37b	42c
	4000	55b	32a	44c

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

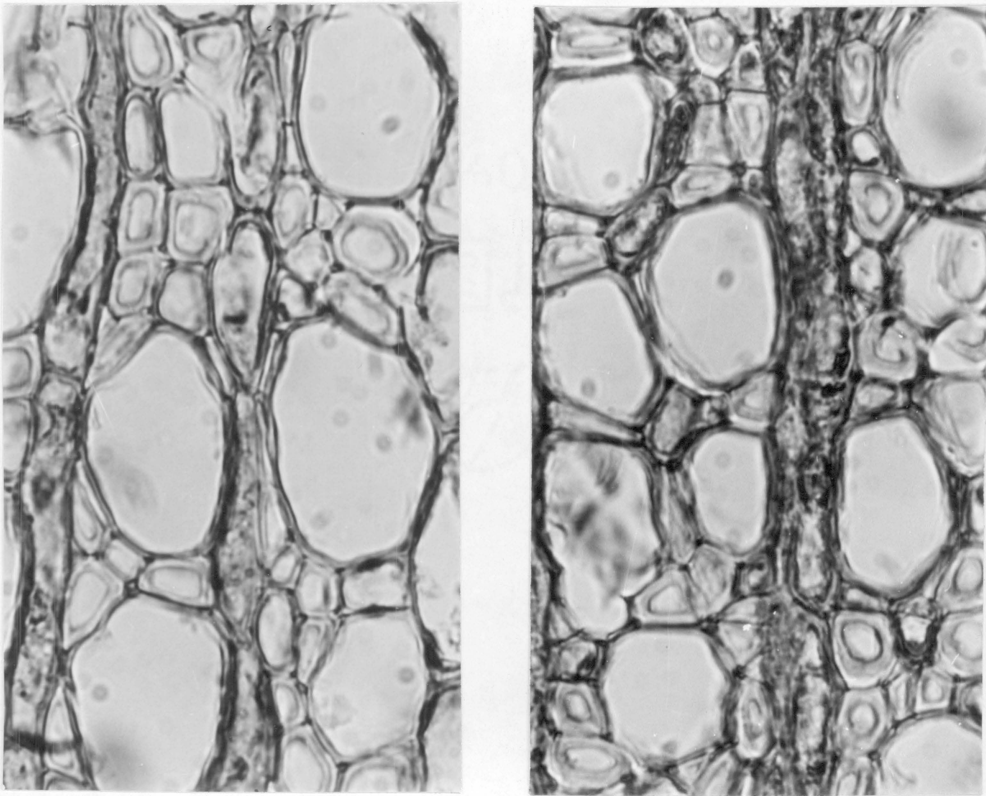


Fig. 9. Transverse sections through secondary xylem of untreated (left) and treated with 2000 ppm of Alar (right) of Golden Delicious apple stems. Note smaller vessels in treated xylem than in untreated, 1967. (X1000).

Table XXI. Influence of Alar on the length of parenchyma cells in the pith and cortex of 2 apple cultivars, 1967.

Cultivar	Alar conc. (ppm)	Mean length, microns	
		Pith	Cortex
Golden Delicious	0	51c[x]	34b
	1000	38b	30ab
	2000	33a	28a
	4000	32a	27a
York Imperial	0	40c	35d
	1000	34b	31c
	2000	31a	29b
	4000	30a	27a

[x]Within each cultivar in a column, any 2 means not followed by a letter in common are significantly different at the .05 level by Duncan's Multiple Range Test.

Table XXII. Estimated number of parenchyma cells per longitudinal column per internode in the pith and cortex of 2 apple cultivars as influenced by Alar, 1967.

Cultivar	Alar conc. (ppm)	Number of cells Pith	Cortex
Golden Delicious	0	453	683
	1000	312	401
	2000	269	326
	4000	201	237
York Imperial	0	582	677
	1000	312	342
	2000	167	208
	4000	143	152

Cells in treated plants had thicker walls and were abnormally oriented in most tissues (Figs. 10, 11, and 12). The effect of Alar on cell wall thickness is revealed in Fig. 10. Note the thicker cell walls in the treated pith parenchyma cells than in the untreated. A comparison of the cross sections through the pith of untreated and treated 'York Imperial' stems shows misshaped parenchyma cells in the treated tissue (Fig. 11). The longitudinal section through the cortical parenchyma cells of untreated and treated 'York Imperial' stem tissue shows the less organized arrangement of cells and intercellular spaces as a result of Alar treatment (Fig. 12).

The sieve and companion cells of the petiole and stem tissues were closely examined microscopically for treatment effects. It was observed that no collapse or obliteration had occurred due to Alar treatment.

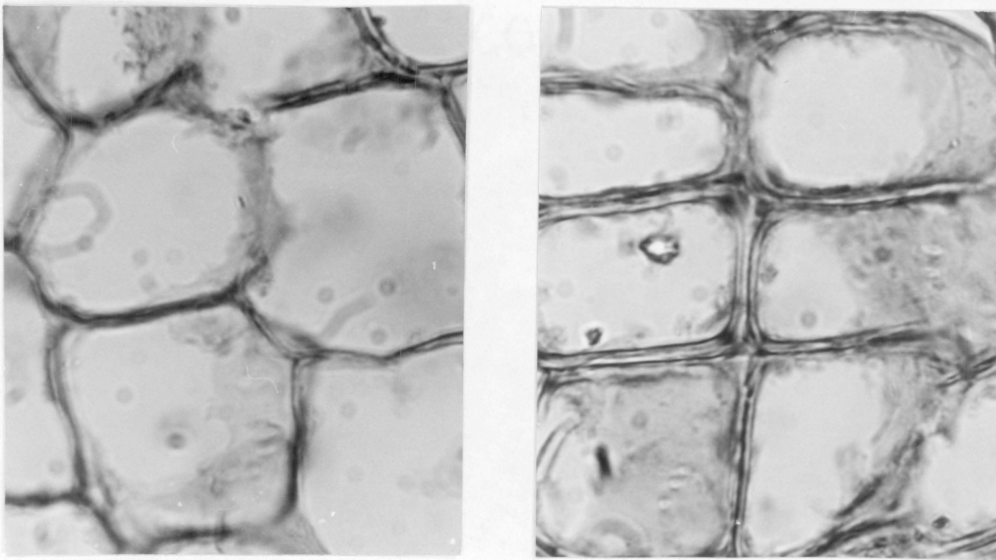


Fig. 10. Longitudinal sections through central pith parenchyma cells of York Imperial stem tissue showing thicker cell walls in the tissue treated with 2000 ppm of Alar (right) than in the untreated (left), 1967. (X1000).

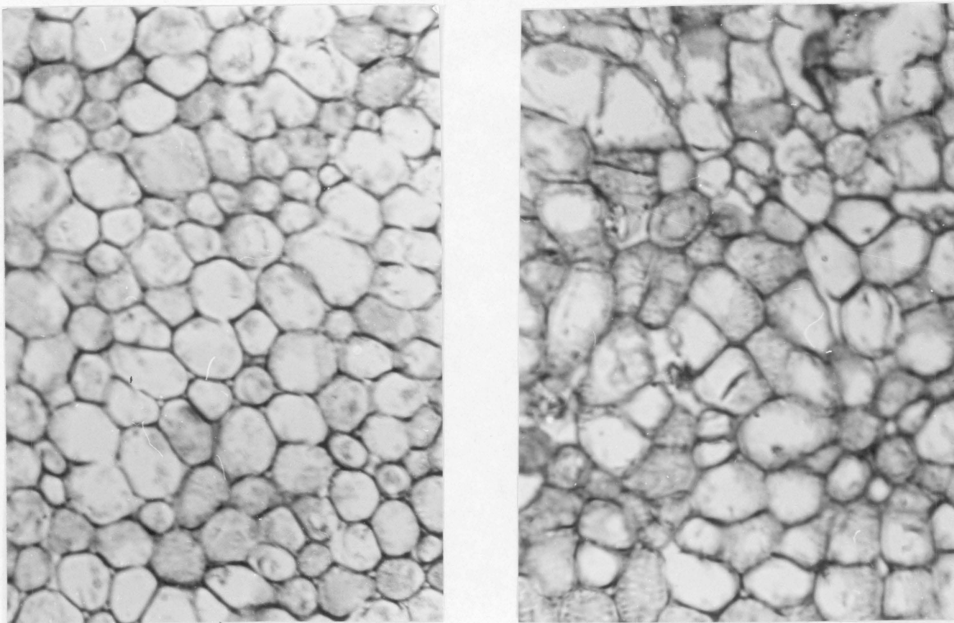


Fig. 11. Transverse sections through the pith of untreated (left) and Alar-treated at 2000 ppm (right) York Imperial stems showing parenchyma cells misshaped in the treated tissue, 1967. (X250).

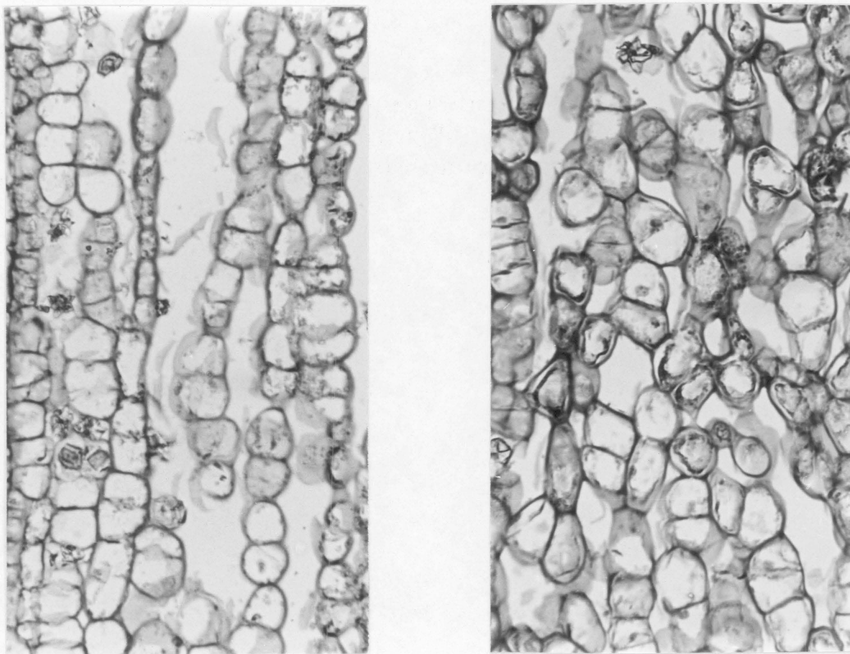


Fig. 12. Longitudinal sections through the cortical parenchyma cells of untreated (left) and Alar-treated at 2000 ppm (right) York Imperial stem tissue. Note the less organized arrangement of cells and intercellular spaces in the treated tissue than in the untreated, 1967. (X250).

DISCUSSION

The suppression of terminal growth by Alar and its effect on morphological characteristics varied among cultivars. This agrees with the findings of Fisher and Looney (15) that a diversity of response to Alar occurs among apple cultivars. By comparing Figs. 3, 4, and 5, it is evident that of the 3 cultivars studied in these experiments, the 'Delicious' trees were the least affected by Alar and the 'York Imperial' plants were the most responsive. For instance, total terminal growth of the treated 'Delicious' shoots was not affected by Alar at the lower concentrations; whereas 500 ppm of Alar limited shoot growth of the 'Golden Delicious' and 'York Imperial' cultivars. Furthermore, with the exception of the 8000 ppm treatment, the 'Delicious' trees began to "grow out" of the treatment effect after only 2 weeks; whereas with the 'York Imperial' plants, the treatment effect was obvious at the 6 to 10 week growth interval with all Alar treatments.

The fewer nodes and leaves on 'Golden Delicious' and 'York Imperial' shoots due to treatment with Alar concurs with the findings of Jaffe and Isenberg (25) with cucumbers. With the 'Delicious' trees leaf number was not affected as a result of treatment. With the bean (6) and grape (7), the leaf number also was reported to be unaffected by Alar treatment.

Although total dry weight accumulation in treated plants was less than in untreated plants, a redistribution of the dry weight in Alar-

treated plants was found. In these experiments an attempt was made to determine the specific growth response of the various regions by making separate determinations at several positions. The lack of such determinations could be responsible in part for the contradictory results reported with measurements of stem diameter and leaf area. For example, in the data presented in this report stem diameter was less in the treated than untreated plants at positions I and II but increased at position III due to Alar treatment (Table VII). Alar treatment of the 'Golden Delicious' trees resulted in less leaf area at position I but increased leaf area at position II (position III of the stem diameter measurement) at 1000 and 2000 ppm (Table IX).

The increase in leaf area on apple trees due to Alar treatment reported herein is in agreement with findings of Batjer (2) and Edgerton and Hoffman (13). Less leaf area as a result of Alar treatment to apples has not been reported. However, Crittendon (10) and Bukovac (6) found that Alar-treated leaves of other crops were smaller than untreated leaves.

Alar treatment resulted in increased fresh and dry weights per unit area of leaf tissue (Table X). This substantiates the report of thicker leaves by Edgerton and Hoffman (13).

Many reports have indicated that plants treated with Alar have darker green leaves (6, 8, 10, 13). In this experiment chlorophyll per unit area of fresh tissue was increased as a result of treatment (Table XII). Also, Alar caused an increase in chlorophyll on a weight basis at position I with the 'York Imperial' leaves. There was a trend toward an increase by Alar in chlorophyll on a weight basis at both

positions with both cultivars. Crittendon (10) found an increase in chlorophyll with Alar on a per unit area basis but not on a weight basis. He attributed this to less total leaf area and more compact tissue. However, Halevy and Wittwer (17) found that chlorophyll was preserved in detached bean leaves treated with Alar. Since in the experiment reported herein the increase in chlorophyll can not be related to the effect on leaf area, it appears that the increase in chlorophyll may be a result of accelerated or prolonged synthesis or a delay in breakdown.

The reduction in NAR of all leaves studied except those senescing (Tables XIII and XIV) and the decrease in total dry weight accumulation (Table VI) by treatment suggest that Alar may have a direct adverse effect on the efficiency of the photosynthetic mechanism. Although contradictory to Brittain's (4) work with peanuts, this is supported by Humphries' (24) finding that the NAR of mustard and tobacco leaves was lower due to Cycocel treatment. Secondly, the reduction in NAR may be caused by the influence of Alar on the stem size and development. According to Mason and Maskell (30) the rate at which carbohydrates move away from a leaf depends on the existence of suitable sinks or reservoirs. Therefore, since the stem, a main sink to which carbohydrates are diverted in plants, has a much smaller capacity for utilization and storage of carbohydrates in Alar-treated plants, the accumulation of carbohydrates at the point of manufacturing would be greater. This would explain thicker leaves (Table XV) and, in some cases, larger leaves on treated plants (Tables II and IX). The reduction in NAR as a result of Alar treatment is supported by

Humphries (23) who found that the NAR depends on sink size. In other words, productivity is less in Alar-treated leaves because of a lower rate of stem growth.

Thirty-five days after treatment the leaves from the pre-treatment portion of the shoot had a higher NAR on the treated plants than untreated (Table XIII). One explanation for this would be that the leaves had begun to "grow out" of the treatment effect. However, this seems unlikely since the 3rd and 9th leaves that developed 40 days after treatment on the post-treatment portion of the shoot had a lower NAR on the treated plants. A more logical explanation would be that Alar delayed senescence. Since the NAR declines as individual leaves age after maturity (28), this would explain why higher rates of CO_2 assimilation were obtained in the treated leaves than on the untreated where senescence had begun. This is supported by the finding of Halevy and Wittwer (18) that senescence is delayed in Alar-treated lettuce leaves.

Alar exerts its effect on the structure of 'Golden Delicious' and 'York Imperial' apple leaves and stems by affecting cell division and expansion. Whether Alar inhibits or stimulates these developmental processes is dependent upon the cultivar, tissue, direction of growth, and concentration of treatment.

Edgerton and Hoffman (13) reported that Alar-treated apple leaves were thicker but they did not examine the leaves microscopically. In the study reported herein, leaf lamellae of treated plants were thicker as a result of longer palisade cells and a looser arrangement of the spongy parenchyma cells (Table XV and Fig. 8). Longer palisade

cells due to Alar treatment have been reported previously by Crittendon (10) in chrysanthemum leaves.

It appears that with 'Golden Delicious' leaves Alar stimulates transverse palisade cell production and expansion at 1000 and 2000 ppm and inhibits these processes at 4000 ppm (Table XVI). With the 'York Imperial' cultivar, Alar appears to inhibit cell division and expansion at all concentrations. Although there is no evidence in the literature to substantiate these findings, these results can be correlated with the different effects on leaf area of the 2 cultivars studied in these experiments.

Alar increased the diameter of the 'Golden Delicious' petioles but did not affect appreciably the 'York Imperial' petioles (Table XVII). Microscopic examination of the phloem elements revealed that no collapse or obliteration of the sieve and companion cells occurred due to Alar treatment as had been reported in petioles of leaves treated with another growth regulator, maleic hydrazide (32, 38). McIlrath (32) hypothesized that with maleic hydrazide the impaired translocation due to collapse of the phloem elements resulted in accumulation of carbohydrates in the leaves; however, with Alar there is an accumulation in treated leaves without abnormalities of the phloem occurring.

Several reports indicate that there is an increase in the stem diameter of growth-regulator treated plants. Scherff (36) found in bean stems treated with Amo-1618, that this increase was due to the stimulation of cell production in the cambium accompanied by a delay in cell differentiation and an increase in cell volume of the paren-

chymatous cortical cells. In the study reported herein, the increase in stem radius by Alar treatment (Table XVIII) was due to stimulation of cell division and expansion of parenchyma cells in the pith and cortex (Tables XIX and XX). There was an inhibition of vessel expansion in both cultivars as a result of treatment (Fig. 9) and the 'York Imperial' stems treated with Alar had fewer vessels in the xylem. It thus appears that in the same plant Alar has the capacity to stimulate cell production and expansion in one tissue in the same plane of growth while it inhibits these processes in another tissue. This variability in effect of Alar indicates that there is a rather delicately balanced mechanism in which cell environment plays a major determinative role.

Although limiting terminal growth of apples with Alar by inhibiting internode elongation has been reported numerous times (2, 3, 13, 14), no evidence has been presented to indicate why this phenomenon occurs. On the basis of this study, it seems apparent that Alar-treated plants have shorter and fewer cells per longitudinal column per internode (Tables XXI and XXII).

Microscopic examination of cellular components and structure of treated plants revealed abnormally oriented and misshaped cells (Figs. 11 and 12) with thicker walls (Fig. 10). However, no obliteration or collapsing of the phloem elements were observed in the stem as a result of Alar treatment.

It seems apparent from this study that the most significant cytological effect of Alar is on cell division. The greater part of Alar stimulation and inhibition of growth is due to the effect on

mitosis in the apical meristems and vascular cambium. Although Alar affects cell enlargement, this effect is minor when compared to cell division in transverse stimulation and longitudinal inhibition of growth. Radial diameters of parenchyma cells in the pith and cortex from treated plants were 20% greater than cells from untreated stems. However, there were 153% more parenchyma cells in cross section in the Alar-treated stems than in the controls. Longitudinally, untreated plants contained cells 37% longer than treated plants in comparison to 242% more cells per longitudinal column per internode in the untreated tissue than in the Alar-treated. Consequently, the mechanism of Alar growth stimulation and inhibition is to be looked for in factors which affect cell division rather than cell enlargement.

It is hoped that this study has contributed significantly to our understanding of the fundamental effects of Alar on apple trees. On the basis of this study, as well as the available literature, Alar undoubtably has a great potential for modifying growth of apple trees but it will be necessary to consider the cultivar and concentration when making recommendations for its use.

SUMMARY

A 2 year study was conducted to investigate the influence of Alar on (a) net assimilation rate (NAR), (b) chlorophyll content, (c) node number, (d) dry weight of leaf and stem tissues, (e) stem length, diameter, and anatomy, and (f) leaf size and anatomy of young apple trees. In 1966, concentrations of 0, 500, 1000, 2000, 4000, and 8000 ppm of Alar were applied to 'Golden Delicious', 'York Imperial' and 'Delicious' cultivars. All plants were grown outside in 1 gallon containers. In 1967, Alar was applied at 0, 1000, 2000, and 4000 ppm to 'Golden Delicious' and 'York Imperial' trees grown in the greenhouse.

The amount and duration of terminal growth suppression by Alar varied among cultivars. The response to Alar was greatest with 'York Imperial' and least with 'Delicious' plants. Alar-treated 'Golden Delicious' and 'York Imperial' cultivars contained fewer leaves; but with the 'Delicious' shoots only the 8000 ppm treatment limited leaf number.

The stem diameter was less in the treated than untreated plants of the 7th internode of the pre-treatment portion of the shoot and 2nd internode of the post-treatment portion of the shoot. However, stem diameter of the 10th internode on the post-treatment portion was increased due to Alar treatment.

Although total dry weight accumulation in treated plants was less than in untreated plants, a redistribution occurred in Alar-treated shoots. The pre-treatment portion of the shoot was generally not af-

fected, but the post-treatment part of the shoot was drastically changed.

Dry weight per unit area of leaf lamellae from the post-treatment portion of the shoot was increased by Alar treatment. However, on a per leaf basis only the 'Golden Delicious' leaves from the mid-post-treatment portion of the shoot weighed more as a result of treatment. Leaf blades from other determinations weighed less due to Alar treatment.

Leaf area of treated leaf lamellae was less at all positions and cultivars except with the 'Golden Delicious' leaves from the mid-post-treatment part of the shoot. In this case the treated leaf blades were larger.

Chlorophyll per unit area of fresh tissue was increased by Alar treatment. Also, there was a trend toward an increase in chlorophyll on a fresh weight basis by treatment.

The NAR of treated leaves on the pre-treatment and post-treatment portions of the shoot was less than untreated leaves except where senescence had begun. In this case, applications of Alar increased the NAR.

All anatomical samples were taken from the post-treatment portion of the shoot 10 weeks after treatment. Leaf blades of treated plants were thicker as a result of longer palisade cells and a looser arrangement of the spongy mesophyll cells. In 'Golden Delicious' leaf blades Alar stimulated transverse palisade cell production and expansion at 1000 and 2000 ppm and inhibited these processes at 4000 ppm. With the 'York Imperial' cultivar, Alar inhibited cell division and expansion at all concentrations.

Petioles of treated 'Golden Delicious' plants were thicker and more circular in shape. However, Alar did not appreciably affect petioles of the 'York Imperial' leaves.

The stem radius of the 'Golden Delicious' plants was increased by Alar treatment due to an increase in the radial thickness of the pith, phloem, and cortex tissues. The 'York Imperial' stem radius was increased as a result of the pith and cortex tissue being thicker even though the xylem was thinner in the treated plants. The radial diameter and number of parenchyma cells in the pith and cortex in cross section were increased by Alar, but xylem vessels were smaller and there were fewer vessels in the 'York Imperial' cultivar as a result of treatment.

In longitudinal section the treated plants had fewer and shorter cells per internode. However, cell division was affected more than cell expansion.

Microscopic examination of cellular components and structure of treated plants revealed abnormally oriented and misshaped cells with thicker walls. However, no obliteration or collapsing of the sieve or companion cells of the phloem was observed in the stem or petiole as a result of Alar treatment.

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THE INFLUENCE OF SUCCINIC ACID 2, 2-DIMETHYLHYDRAZIDE (ALAR) ON NET CO_2 ASSIMILATION RATE, CHLOROPHYLL CONTENT, ANATOMY, AND MORPHOLOGY OF VEGETATIVE GROWTH OF THE APPLE, MALUS SYLVESTRIS MILL.

Robert Gordon Halfacre

ABSTRACT

A 2 year study was conducted to investigate the influence of Alar on young apple trees. In 1966 concentrations of 0, 500, 1000, 2000, 4000 and 8000 ppm of Alar were applied to 'Golden Delicious', 'York Imperial' and 'Delicious' cultivars growing outside in 1 gallon containers. In 1967 Alar was applied at 0, 1000, 2000, and 4000 ppm to 'Golden Delicious' and 'York Imperial' trees in the greenhouse.

The amount and duration of terminal growth suppression by Alar varied among cultivars. The response was greatest with 'York Imperial' and least with the 'Delicious' plants. Treated 'Golden Delicious' and 'York Imperial' cultivars contained fewer leaves; but with the 'Delicious' shoots, only the 8000 ppm treatment limited leaf number.

The stem diameter was less in the treated than untreated plants of the pre-treatment portion of the shoot (shoot present at treatment) and the base of the post-treatment portion of the shoot (shoot portion that developed after treatment). However, the diameter of the mid-post-treatment portion was increased due to Alar treatment.

Although dry weight accumulation in treated plants was less than in untreated plants, a redistribution occurred in Alar-treated shoots. The pre-treatment shoot was generally not affected, but the post-treatment part of the shoot was drastically changed.

Treated leaves were smaller at both positions and cultivars except

the 'Golden Delicious' leaves from the mid-post-treatment part of the shoot. In this case the treated leaves were larger.

Chlorophyll per unit area of fresh tissue was increased by Alar treatment. Also, there was a trend toward an increase in chlorophyll on a weight basis in the treated plants.

The net assimilation rate (NAR) of treated leaves on the pre-treatment and post-treatment portions of the shoot was less than untreated leaves except where senescence had begun. In this case, applications of Alar increased the NAR.

Anatomical samples were taken from the mid-post-treatment portion of the shoot 10 weeks after treatment. Leaves of treated plants were thicker as a result of longer palisade cells and a looser arrangement of the spongy mesophyll cells. In 'Golden Delicious' leaves Alar stimulated transverse palisade cell production and expansion at low concentrations and inhibited these processes at high concentrations. With the 'York Imperial' cultivar, Alar inhibited cell division and expansion at all concentrations. Petioles of treated 'Golden Delicious' plants were thicker and more circular in shape.

The stem radius of the 'Golden Delicious' shoot was increased by Alar treatment due to an increase in the radial thickness of the pith, phloem, and cortex tissues. The 'York Imperial' stem radius was increased as a result of the pith and cortex tissue being thicker even though the xylem was thinner in the treated plants. The radial diameter and number of parenchyma cells in the pith and cortex in cross section were increased by Alar, but xylem vessels were smaller and there were fewer vessels in the 'York Imperial' cultivar as a result

of treatment. In longitudinal section the treated plants had fewer and shorter cells per internode. Cell division was affected more than cell expansion transversally and longitudinally.

Microscopic examination of cellular components and structure of treated plants revealed abnormally oriented and misshaped cells with thicker walls. However, no obliteration or collapsing of the phloem elements was observed in the stem or petiole as a result of Alar treatment.