AN INVESTIGATION INTO THE MODE OF ACTION OF ALAR (SUCCINIC ACID 2,2-DIMETHYL HYDRAZIDE) ON APPLE

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Horticultural Science

at

Massey University

MURRAY EDWIN HOPPING

1968

A C K N O W L E D G E M E N T S

The author wishes to thank his supervisor, Professor J.A. Veale for his continued interest and assistance throughout this study. Thanks are also due to my wife for typing this thesis; to the Central Photography Unit; to the Massey Library Staff; to Mr D.B. Thomas for laboratory assistance; and to all others who have given help when needed.

TABLE OF CONTENTS

Chapter		Page
	ACKNOWLEDGEMENTS	
	ABSTRACT	
1	REVIEW OF LITERATURE	
	A. INTRODUCTION	2
	B. THE EFFECTS OF ALAR ON WHOLE PLANT SYSTEMS	3
	I. (a) Stem growth.	3
	(b) Stem girth.	4
	(c) Leaf growth.	4
	(d) Shoot apical dominance.	4
	II. Plant Dry Weight	5
	III. Flowering	5
	(a) Flower bud initiation.	5
	(b) Delayed flowering.	6
	(c) Fruit set.	6
	(d) Fruit size.	6
	C. GIBBERELLINS IN RELATION TO SHOOT GROWTH	7
	I. Anatomical Considerations	7
	II. Physiological Considerations	7

Chapter		Page
	(a) Gibberellins.	7
	(b) Auxins.	9
	(c) Auxin - Gibberellin interaction.	10
	III. Environmental Considerations	11
D.	THE INTERACTION BETWEEN GROWTH RETARDANTS AND GIBBERELLIN	14
	I. The Interaction on Whole Plant Systems	14
	II. The Interaction on Cell Division and Elongation	18
	III. Growth Retardant Influence on Cellular Metabolism	21
E.	GROWTH RETARDANT INDUCED INHIBITION OF GIBBERELLIN BIOSYNTHESIS	24
	I. Studies on *Fusarium moniliforme*	24
	II. Studies on Higher Plants	25
F.	GROWTH RETARDANT INTERACTION WITH GIBBERELLIN SITE OF ACTION	28
G.	INTERACTION BETWEEN GROWTH RETARDANTS AND AUXIN	28
H.	MOVEMENT AND FATE OF ALAR IN TREATED PLANTS	30
	I. (a) Movement and Fate of Alar in Apple.	30
	(b) Movement and fate in cherry.	32

Chapter		Page
	II. Interaction and General Effects of Alar and Gibberellin on Apple Shoot Growth	32
	I. DISCUSSION	33
2	MATERIALS AND METHODS	
	A. INTRODUCTION	37
	B. EXPERIMENTAL PLOTS AND SAMPLING TECHNIQUES	38
	I. Experimental Apple Planting	38
	(a) Block 1.	38
	(b) Block 2.	38
	II. Sampling Technique	39
	(a) Block 1.	39
	(b) Harvest dates.	39
	III. Treatment of Each Harvest	39
	(a) Stem and leaf measurement.	39
	(b) Dry weight measurement.	40
	(c) Fruit harvest.	40
	(d) Photography.	41
	(e) Anthocyanin determinations.	41
	C. GIBBERELLIN	42
	I. Extraction of Gibberellin-like Substances	42

Chapter		Page
	II. Thin Layer Chromatography	43
	III. Barley Endosperm Bioassay	44
	(a) Materials.	44
	(b) Method.	44
	(c) Reducing sugar estimation.	45
	IV. Standard Curves	46
	D. AUXIN	46
	I. Auxin Extraction	46
	II. Chromatography	47
	III. Auxin Bioassay	48
	IV. Standard Curve	49
	E. HORMONE INTERACTION WITH ALAR ON AVENA FIRST INTERNODE TISSUE	49
3	THE EFFECT OF ALAR TREATMENT ON APPLE SHOOT GROWTH	
	A. SHOOT PHOTOGRAPHY	51
	B. THE EFFECT OF ALAR ON SHOOT NODE NUMBER	52
	C. THE EFFECT OF ALAR TREATMENT ON SHOOT INTERNODE LENGTH	53

Chapter		Page
	D. THE EFFECT OF ALAR TREATMENT ON SHOOT LEAF AREA	56
	E. THE EFFECTS OF ALAR TREATMENT ON SHOOT DRY WEIGHT	57
	I. Shoot Dry Weight	57
	II. Leaf Dry Weight	58
	III. Petiole Dry Weight	59
	F. EFFECTS OF ALAR TREATMENT ON FRUIT SIZE, WEIGHT, AND STORAGE	60
	I. Fruit Photography	60
	II. The Effects of Alar Treatment on Apple Fruit	61
	(a) Fruit size.	61
	(b) Fruit weight.	61
	III. Fruit Storage	62
	G. ANTHOCYANIN DETERMINATIONS	63
	H. DISCUSSION	64
4	ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES EXTRACTED FROM ALAR TREATED APPLE SHOOTS	
	A. INTRODUCTION	68
	B. DERIVATION OF THE STANDARD CURVE	68
	C. ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES	71

Chapter		Page
	I. Purification of Ethyl Acetate Extracts	71
	II. Recovery Values	74
	III. Gibberellin-like Activity	76
	D. DISCUSSION	78
5	ENDOGENOUS AUXINS EXTRACTED FROM ALAR TREATED APPLE SHOOTS	
	A. INTRODUCTION	82
	B. DERIVATION OF STANDARD CURVE	82
	C. ENDOGENOUS AUXINS	84
	I. General Considerations	84
	II. Acidic Auxins	85
	III. Neutral Auxins	86
	IV. Basic Auxin	87
	D. DISCUSSION	88
6	INTERACTION BETWEEN ALAR, GIBBERELLIN, AND AUXIN ON Avena 1st INTERNODE SECTIONS	
	A. GROWTH RETARDATION OF Avena SEEDLINGS	90
	B. HORMONE-ALAR INTERACTIONS	91
	C. DISCUSSION	

Chapter		Page
7	FINAL DISCUSSION	97
	APPENDIX I	104
	APPENDIX II	106
	APPENDIX III	119
	REFERENCES	

L I S T O F T A B L E S

Table		Face Page
1	Alar effects on apple shoots selected for photography.	51
2	Comparison between initial and final shoot length.	52
3	Total internode number from fifteen decapitated samples.	53
4	Mean internode length.	55
5	Average leaf area from five shoot samples.	56
6	Dry weight (gm) from fifteen shoot samples.	58
7	Comparison between internode length (average) and internode dry weight (average) from shoots harvested on 2/1/68.	58
8	Mean leaf dry weight (gm) from thirty leaf samples.	59
9	Petiole dry weight from fifteen shoot samples.	60
10	Fruit number and weight (oz) from Alar treated apple trees.	62

Table		Face Page
11	Apple fruit quality after storage - fruit from size distribution 2.50-2.75 inches.	63
12	Anthocyanin levels extracted from photographed fruit.	64
13	Reducing sugar release from barley endosperm incubated in distilled water.	70
14	Recovery values from petroleum ether purification steps.	73
15	Recovery values from petroleum ether and ethyl acetate purification steps.	74
16	Gibberellin (GA_3) equivalents from Alar treated shoots.	76
17	Endogenous auxin levels extracted from Alar treated apple shoots.	85
18	The degree of Alar induced retardation on *Avena* seedlings.	90
19a	Interaction between GA_3, IAA, and buffer on *Avena* 1st internode sections (mean values calculated from two replicates).	91
19b	Interaction between GA_3, IAA, and Alar on *Avena* 1st internode sections (mean values calculated from two replicates).	91

L I S T O F F I G U R E S

Figures		Face Page
1	Experimental Gravenstein planting - Massey University Orchard.	38
2	Summary of gibberellin extraction procedures.	42
3	Materials for barley endosperm bioassay.	45
4	Summary of auxin extraction procedures.	47
5	Cutting block for *Avena* 1st internode sections.	48
6	The bioassay machine.	49
7a	The effect of Alar treatment on Gravenstein shoot growth.	51
7b	Comparison between initial and final shoot length after Alar treatment.	52
8	Internode length response to Alar treatment from shoots harvested on 24/10/67.	54
9	Internode length response to Alar treatment from shoots harvested on 7/11/67.	54

Figures		Face Page
10	Internode length response to Alar treatment from shoots harvested on 28/11/67.	54
11	Internode length response to Alar treatment from shoots harvested on 19/12/67.	54
12	Internode length response to Alar treatment from shoots harvested on 2/1/68.	54
13	Leaf area response with time from Alar application.	57
14	Internode dry weight (average) with time from Alar application.	58
15	Leaf dry weight (average) with time from Alar application.	59
16	Petiole dry weight (average) with time from Alar application.	60
17	Fruit sub-sample selected from fruit harvested from Block 2.	61
18	Size and weight of fruit with Alar treatment.	61
18a	Gibberellin induced reducing sugar release from barley endosperm.	69
19	Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 24/10/67).	77

Figures		Face Page
20	Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 31/10/67).	77
21	Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 28/11/67).	77
22	Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 19/12/67).	77
23	Increase in Avena 1st internode section length at different auxin (IAA) concentrations.	83
24	Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 7/11/67).	84
25	Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 5/12/67).	84
26	Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 26/12/67).	84
27	Response of Avena 1st internode sections to factorial combinations of IAA, GA_3, and buffer or Alar.	92

A B S T R A C T

Application of the growth retardant succinic acid 2,2-dimethyl hydrazide as a foliar spray to seven year old Gravenstein apple trees at full bloom and eleven days after, reduced extension growth in comparison to that on untreated trees. This retardation was characterized by a reduction in internode length and node number without formative effects on leaf area or observable leaf chlorophyll. Shoot and petiole dry weight was decreased with Alar treatment; leaf dry weight increased. Fruit size and weight was increased at 1000 and 2000 ppm Alar treatment; the converse relationship occurred at 4000 ppm Alar. Alar treatment improved apple keeping quality and enhanced fruit skin colouration.

Acidic gibberellin-like substances extracted from shoot apices decreased with Alar treatment and this reduction was accompanied by an increase in 'abnormal' gibberellin-like substances. Acidic, neutral, and basic auxins extracted from shoot apices also decreased with Alar treatment although evidence was not as conclusive as that shown by gibberellin-like substances.

A study of the interaction between Alar, auxin (IAA) and gibberellin (GA_3) on Avena 1st internode sections was used as the basis for a suggested mode of action of Alar on apple extension growth.

Chapter 1

REVIEW OF LITERATURE

A. INTRODUCTION

The discovery by Mitchell et al. (1949) that lanolin paste application of some nicotinium compounds could retard growth without apparent toxic effects, initiated the search for further compounds; namely the growth retardants. Cathey (1964) defines the term growth retardant as any chemical which slows cell division and cell elongation in shoot tissues and regulates plant height physiologically without formative effects.

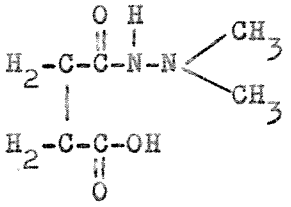
Plants within the Dicotyledonae appear to be affected to a great extent while variable responses have been obtained among the Monocotyledonae (Cathey 1964). No response to growth retardants has been reported from plants within the Gymnospermae or Pteridophyta although Larson et al. (1966) reported that Alar could limit growth of some micro-organisms.

The division between retardants on the basis of chemical structure, as made by Cathey (1964), characterizes six groups; the nicotiniums; the quaternary ammonium carbamates, e.g. AMO 1618; the hydrazines, e.g. β-hydroxyethyl hydrazine; the phosphoniums e.g. phosphon; the substituted cholines, e.g. CCC; and the succinamic

acids, e.g. Alar.

Riddel et al. (1962) established that maleamic acid 2,2-dimethyl hydrazide (CO11) and succinic acid 2,2-dimethyl hydrazide (Alar) exhibited growth retardant effects on a wide range of herbaceous species. These authors reported that like activity could not be shown in analagous compounds derived from phthalic acid although tetra- and hexa-hydrophthalic acids showed moderate growth regulation. The instability of CO11 in aqueous solution due to the intramolecular hydrolysis of the acid (Dahlgrew et al. 1963) has led to far wider usage of the stable succinic derivative.

Succinic acid 2,2-dimethyl hydrazide (Alar) is a free, ionizable acid, containing the C-C-N-N system found in β-hydroxyethyl hydrazine.



B. THE EFFECTS OF ALAR ON WHOLE PLANT SYSTEMS

I. (a) Stem growth

Growth retardant suppression of stem internode expansion has been shown for a wide range of seedling and mature plants. Recent literature contains reports of Alar induced growth retardation on apple: (Batjer et al. 1964a, 1964b, Batjer 1965; Edgerton 1964, Edgerton et al. 1965, 1967; Emerson et al. 1966; Greenhalgh et al. 1967; Luckwill et al.

1965, Luckwill 1966; Ryugo 1966 and Stahly *et al.* 1966); pear: (Batjer *et al.* 1964, Batjer 1965; Brooks 1964; Stahly *et al.* 1966); cherry: (Batjer *et al.* 1964, Batjer 1965; Stahly *et al.* 1966); plum: (Stahly *et al.* 1966); pea: (Reed 1965b); and cucumber: (Moore 1967). CO11 has been shown to be active on bean (Bukovac 1964).

(b) Stem girth

Increased stem girth has been achieved with Alar treatment of apple (Batjer 1964b; Edgerton 1964, Edgerton *et al.* 1965) and pear (Brooks 1964) although Stahly *et al.* (1966) showed that Alar had no effect on stem girth of apple, pear, cherry and plum.

(c) Leaf growth

Variable reports have been made concerning the effect of retardants on leaf shape and growth. Increased leaf growth on terminal shoots of apple (Batjer *et al.* 1964) and pear (Brooks 1964) have been obtained with Alar treatment. However, Edgerton (1964) and Edgerton *et al.* (1965) have reported that apple leaf growth and shape is unaffected by Alar. CO11 treatment of bean (Bukovac 1964) showed similar effects. Leaf thickness and colour have been increased with Alar treatment in apple (Edgerton *et al.* 1965). Increased leaf colour has also been shown for CO11 treated beans (Bukovac 1964) although petiole expansion was markedly reduced.

(d) Shoot apical dominance

Alar has been shown to enhance early cessation of growth in the terminal shoot meristem in apple (Edgerton *et al.* 1965, Greenhalgh

et al. 1967) without affecting apical dominance (Luckwill 1966). However, Brooks (1964), claims that apical dominance of pear shoot tips is reduced.

II. Plant Dry Weight

Little information is available in relation to the effect of Alar on changes in plant dry weight. Plaut et al. (1964) showed that Alar was without effect on the dry weight of bean plants. Similarly Bukovac (1964) found no significant differences between dry matter accumulation. Stem dry weight, however, was significantly different between 250 and 4000 ppm CO11 treatments (decreased) although neither differed significantly from the control. Significant differences between leaf dry weights from control and Alar treated apple (decreased) was reported by Greenhalgh (1967).

III. Flowering

(a) Flower bud initiation

Promotion of flower bud initiation by growth retardant treatment has been observed in a wide range of plant species. Application of CO11 to holly (Marth 1963), and lemon (Monselise 1964, 1966) resulted in increased flower number. Flower bud promotion with Alar treatment on apple (Batjer et al. 1964a, 1964b, Batjer 1965; Edgerton et al. 1965; Greenhalgh et al. 1967; Looney et al. 1967; Luckwill et al. 1965); pear and sweet cherry (Batjer et al. 1964b, Batjer 1965); and lemon (Monselise et al. 1964, 1965) is well documented in the literature. Batjer et al. (1964b) achieved a 2-12 times increase in flower number in the year following treatment.

(b) Delayed flowering

Pre-bloom application of Alar on apple resulted in 1-3 days delay in flowering (Edgerton et al. 1965). Delayed bloom time in apple has also been reported to occur in the year after application (Batjer 1965, Batjer et al. 1964b). 1000 and 3000 ppm Alar treatment to Starking Delicious delayed full bloom the following spring by 2 and 6 days respectively while pear and cherry were unaffected.

(c) Fruit set

Fruit set on lemon (Monselise et al. 1966) and apple (Edgerton et al. 1965; Looney et al. 1967) has been increased by Alar treatment. Batjer et al. (1964) however, reported that fruit set was unaffected in apple while Greenhalgh et al. (1967), found that apple fruit set was reduced with Alar treatment.

(d) Fruit size

The majority of the literature on growth retardant (Alar) effect on fruit size has been accumulated from studies of treated commercial crops. Golden Delicious (Batjer et al. 1964; Emerson et al. 1966; Looney et al. 1967), Jonathan (Emerson et al. 1966), McIntosh (Edgerton et al. 1965; Greenhalgh et al. 1967; Looney et al. 1967), Starking Delicious and Red King Delicious (Batjer et al. 1964), R.I. Greening (Edgerton et al. 1965), Delicious, Spartan, and Winesap (Looney et al. 1967) apple varieties all show a reduction in fruit size. Similarly, Bartlet and Anjou pears exhibit fruit size reduction with treatment (Batjer et al. 1964). Luckwill et al. (1965), showed fruit size reduction in two varieties although no significant differences occurred in crop weight.

C. GIBBERELLINS IN RELATION TO SHOOT GROWTH

I. Anatomical Considerations

A recent review by Sachs (1965) reiterates several points in relation to stem anatomy:

(i) The apical meristem functions in the organization of the developing shoot.

(ii) The subapical meristem is the site of formation of most of the cells that ultimately constitute the mature stem.

(iii) Cellular activity in the subapical meristem is largely independent of the apical meristem.

(iv) The rate of cell division decreases more rapidly in the basipetal direction than does that of cell elongation.

(v) Cell division in the subapical meristem can be modified by environmental factors.

A division of labour between the apical and subapical meristem appears to be present in many, if not all, plants. Although histological demarkations between the two meristems are gradual, their physiological differences are markedly well defined (Sachs 1965).

II. Physiological Considerations

(a) Gibberellins

In a review of the then current literature, Brian (1959) stated that exogenous gibberellin affected three characteristics of

shoot growth:

(i) Increased internode length.

(ii) Increased leaf growth - although increased leaf expansion was often coupled with changes in leaf shape.

(iii) Enhanced apical dominance in plants that normally branch.

Increased internode cell length appeared, at that time, to be the major factor responsible for increased growth. However, further histological studies on both normal caulescent plants and dwarf cultivars led to an increasing awareness that increased cell number, rather than increased cell length, was responsible for increased growth in stem length.

Application of gibberellins extracted from tall species to dwarf cultivars of the same species stimulated stem growth to the extent that treated dwarfs resembled the tall plants (Radley 1956). This criterion has been used as a basis for gibberellin bioassays; dwarf pea, bean and maize being common species used. Phinney (1961) proposed that mutant genes responsible for dwarf growth in Zea mays influenced endogenous gibberellin levels by causing an inhibition in their biosynthesis.

Gibberellin application to rosette plants results in a stimulation of the subapical meristem with concomitant increases in mitotic activity and zone size. The ultimate expression of this subapical meristem activation is stem elongation or "bolting".

Gibberellins are also considered to be active in the control of the subapical meristem of caulescent plants although exogenous gibberellin does not produce such marked effects as in dwarfs. This is considered to be due to the presence of near optimal amounts prior to application.

In a recent review Paleg (1965) considered that cell division in the terminal meristematic zone of the shoots may be the most important physiological site of gibberellin action. Although increased cell division leads to increases in stem length, cell elongation is also stimulated by gibberellin. Both effects have been well illustrated using dwarf cultivars of normal caulescent plants. Stems of gibberellin treated dwarfs contain more cells than untreated controls while cell length and total cell number are comparable with the normal plant.

Arney et al. (1966) applied gibberellin to whole pea shoots cv "Meteor". Considerable elongation occurred. A histological survey of cells in the cortex showed that cell division accounted for half the elongation in stem length. These workers considered that gibberellin stimulated cell division in apical and subapical meristems is more important than the effect on cell elongation. They proposed that increased cell division, with resulting amino acid metabolism, could release tryptophan for auxin synthesis. Increased auxin levels would be available for cell elongation.

(b) Auxins

Auxins, traditionally associated with cell elongation, also fill an important role as promoters of cell division. In the case of the stem apex, auxin acts not only to promote cell division of

meristematic cells but, once started, to also stimulate this activity further (Audus 1965). However, stem elongation in most plants is stimulated by gibberellin rather than auxin. Sachs (1965) reported that some evidence was available in relation to auxin involvement in the mechanism of shoot elongation. One such example, quoted in his review, is that of auxin mediated scape elongation. Although a great deal of evidence has been presented concerning the mechanism of action of auxin in isolated tissue, and on inherent cell elongation, little concrete evidence is available concerning the mode of action on shoot elongation.

(c) Auxin - Gibberellin interaction

Isolated sections of Avena coleoptile and etiolated pea epicotyl respond additively (frequently less than additive Galston et al. 1959) to auxin and gibberellin while some whole (and excised) plant systems exhibit a synergistic interaction (Galston et al. 1960). Several workers have proposed that auxin must be present before a gibberellin response can occur. Kefford et al. (1961) presented a review paper in which they proposed that auxin interacted with gibberellin in isolated tissue to control cell enlargement while auxin and kinin interacted in the initiation of cell division. These workers proposed that auxin acted as a predisposing agent to cause the production of a compound which would be essential to either action; in the presence of limiting auxin levels, competition for this factor for either reaction would be controlled by relative levels of gibberellin and kinin. However, Kefford et al. point out that this concept could not be readily applied to whole plant systems because cause and effect

relationships could not be readily established.

Gibberellin application to plants has been observed to increase endogenous auxin levels. This effect has been attributed to gibberellin action on auxin degredation. McCune et al. (1959) showed that dwarf -1 mutant corn and dwarf pea had higher levels of peroxidase enzyme than normal types. Gibberellin increased growth rate and decreased peroxidase levels in both plants although the decreased peroxidase level could not be related to increased growth. Gibberellin application did not alter peroxidase levels in normal pea and corn.

Galston et al. (1960) reported that gibberellin had similar effects on the IAA-oxidase enzyme. In this case, Hare (1964) argued that IAA-oxidase inhibitors tended to increase after gibberellin application.

However, Galston et al. (1960) also presented evidence which indicated that exogenous gibberellin had no influence on either enzyme system. Sachs (1965) concluded that both auxin and gibberellin were involved in stem elongation and that the mode of action of gibberellin was not solely one of increasing auxin levels.

III. Environmental Considerations

Plants grown in low light intensities contain fewer and shorter cells than those grown in darkness (Sachs 1965). In a series of papers, Lockhart (1957 - 1961) linked gibberellin-induced stem growth to photo-control. He showed that stem growth of dark grown dwarf pea could be inhibited by exposure to red light. Exposure

to far-red light after red light irradiation or application of gibberellin reversed this effect (Lockhart 1959). Further evidence, strengthened the case for photo-controlled stem elongation. Dwarf Zea mays plants (deficient in endogenous gibberellin) grown in shade, attained comparable growth to plants (normal and dwarf) treated with gibberellin and grown in full sun or 50 percent shade (Lockhart 1961a).

Analysis of 'Pinto' bean stem elongation under various light regimes indicated that both high intensity light and low energy red light inhibit stem growth. Saturation dosages of gibberellin reversed this red light inhibition (Lockhart 1961b). The interaction between light and gibberellin appeared to be the only mechanism involved in 'Pinto' stem growth as no interaction occurred between gibberellin and other factors such as water relations, photosynthesis, root activity, or temperature.

Lockhart proposed that light interference with gibberellin metabolism could occur as one (or more) of three possibilities:

(i) That light may inhibit endogenous gibberellin biosynthesis.

(ii) That light may stimulate gibberellin breakdown.

(iii) That light may render plant tissue less responsive to endogenous gibberellin.

Of these possibilities, Lockhart (1959, 1961a, 1961b) considered that light interfered with gibberellin biosynthesis. In this regard, far-red light or darkness would act to increase gibberellin levels which would explain the rapid stem growth experienced

under such conditions.

In a continuation of this line of thought, Lockhart (1964) removed the stem apex and all leaves distal to the second internode of 'Pinto' bean. Internode growth became independent of the photoreaction. If either the apex or the largest distal leaf were left, elongation of this internode responded to either red or far-red radiation. With saturation dosages of gibberellin, internode elongation became independent of the apex, or leaves, and was unresponsive to either red or far-red radiation. IAA had no effect on any system tested. Lockhart proposed that a gibberellin precursor, formed in young leaves and apical buds, moved to the region of elongation where it was converted to the active gibberellin. Red light irradiation would inhibit (far-red irradiation promotes) the conversion which could be phytochrome mediated. Red light irradiation, then, would lower the levels of active gibberellin necessary for stem elongation.

In order to verify Lockhart's hypothesis, Kende and Lang (1964) extracted endogenous gibberellins from both light and dark grown dwarf peas. These workers showed that no differences could be detected in the levels of the two gibberellin-like substances extracted which were tentatively identified as GA_1 and GA_5. However, substantial differences in their mode of action were shown to occur. Light appeared to lower plant tissue sensitivity to "GA_5" either by interfering with reactions leading from "GA_5" to the growth response proper or by inducing inhibitor production specific to "GA_5". Slight growth of dwarf peas did occur in the

light and this was attributed to the promotive effects of "GA_1". From a study of gibberellin addition to both tall and dwarf peas grown in light, these authors concluded that the light sensitive reaction in dwarf pea was about five times more sensitive to irradiation than that in tall peas.

It can be concluded, then, that light lowers the sensitivity of stem tissue to gibberellin rather than decreasing the endogenous gibberellin level.

D. THE INTERACTION BETWEEN GROWTH RETARDANTS AND GIBBERELLIN

I. The Interaction on Whole Plant Systems

Wittwer and Tolbert (1960b) reported that CCC suppressed gibberellin induced lettuce seed germination, vegetative extension of dwarf and normal plants, and the flowering of lettuce. It should be noted, however, that a synergistic growth rate was noted for tomato ovaries treated with CCC, gibberellin (GA_3), and auxin (IAA) which could not be ascribed to a GA_3 x IAA interaction. Similar results were reported by these workers for CCC (and 2 analogues) treatment of tomato (Wittwer and Tolbert 1960a). Gibberellin decreased stem diameter, increased plant and flower cluster height, and decreased observable leaf chlorophyll content. (2,3-n-propylene) trimethyl ammonium bromide (PTAB) treatment resulted in the opposite for each response. Combination of 1×10^{-4} M GA_3 and 1×10^{-3} M PTAB resulted in plants of similar height and flowering behaviour as

that of controls. However, GA_3 effects lasted a mere 2-3 weeks compared to 8-12 weeks for PTAB.

The interaction between gibberellin and the red-far-red reaction on stem growth of plants (Lockhart 1959-61) was investigated further. Downs and Cathey (1960) treated dark grown bean seedlings with AMO 1618 and gibberellin. Plants which received red irradiation subsequent to chemical application showed a stimulated rate of elongation in the first internode. This apparent contradiction of Lockhart's results was explained when Downs et al. showed that red light hastened the maturity of the hypocotyl; so allowing an earlier expansion of the first internode than would have occurred in the dark grown controls.

Gibberellin was shown to be inactive in removing this light effect on the hypocotyl and these workers concluded that it controlled growth through pathways entirely separate from that of the photo-reaction. AMO 1618 was shown to operate independently of the photo-reaction but to interact with gibberellin such that correct selection of gibberellin molarity nullified growth retardation.

Halevy (1962) came to the same conclusion after studying the interaction between gibberellin and AMO 1618 on seedling Cucumis sativus (L) hypocotyls. Both compounds interacted within the subapical meristem.

In a series of kinetic experiments, Lockhart (1962) attempted to clarify the probable interaction between growth retardants and growth promoters. He proposed that one of two interactions could occur:

(i) A competitive interaction between gibberellin and retardants.

(ii) An independent mode of action (but in opposite directions) for either class of chemical.

In order to test either hypothesis, Lockhart further stated that two requirements must be satisfied before kinetic experiments could be validly used to determine the retardant mode of action, viz.

(i) That a steady state system must be used.

(ii) The promoting factor (gibberellin) must be varied from zero to saturation with, and without, the inhibitor (retardant).

Phosphon D, applied as a soil drench to Phaseolus vulgaris cv "Pinto" seedlings, was shown to be translocated to the stem apex and initiate its retarding effect in this area. Graphical expression of growth rate versus gibberellin concentration showed that both phosphon D and CCC acted competitively with added gibberellin (therefore true antigibberellins). However, added gibberellin had no effect on stem growth inhibition imposed by maleic hydrazide and Lockhart concluded that maleic hydrazide acted independently to gibberellin.

Modification of the vegetative development of Phaseolus vulgaris by CO11 (Bukovac 1964) indicated a pattern of growth opposite to that experienced with gibberellin. Accordingly, plants were treated with CO11 and gibberellin (GA_3) alone, and in combination, to determine if CO11 acted independently from, or reacted with, gibberellin. Bukovac concluded that a significant interaction occurred between CO11 and GA_3

on epicotyl elongation and that this interaction could possibly be manifest as an interference with endogenous gibberellin synthesis.

Simultaneous application of gibberellin and CCC to strawberry (Guttridge 1966) resulted in an enhanced stem growth rate which was greater than the promotion induced by gibberellin alone. Application of CCC, as a foliar spray or soil drench, shortened petiole length and decreased top and root growth. Guttridge concluded that CCC in no way antagonised applied gibberellin. Native growth promoters are inactive in strawberry stem (compact internodes as a result); then CCC does not inhibit the site of action of endogenous gibberellin. To explain the synergistic effect experienced with strawberry Guttridge proposed three possible reasons:

(i) Gibberellin could be conserved by a reduction of usage elsewhere.

(ii) Gibberellin breakdown could be reduced.

(iii) Decreased supply or availability of an inactive form of a growth promoter which would compete with exogenous gibberellin for the active site.

Of these possibilities the above worker favours the last (iii). However, any of these proposals would promote the availability, or effective supply, of exogenous gibberellin to explain the synergistic growth rate.

Cucumber hypocotyl elongation can be promoted by 6 auxins and 2 gibberellins. Moore (1967) used this criterion to test the kinetics of the interaction between growth retardants (AMO 1618, CCC, Alar) and

hormones (IAA, GA_3) on hypocotyl elongation of light grown cucumber seedlings. Simultaneous foliar application of GA_3 with AMO 1618 or Alar, or to plants growing in CCC treated media, reversed the expected growth retardation. IAA reversed CCC induced retardation but was without effect on AMO 1618 or Alar treatments. Application of AMO 1618 and CCC, in combination, indicated that both chemicals acted at the same site, namely on the gibberellin biosynthetic pathway. However, reversal of CCC inhibition by both GA_3 and IAA would imply that retardation was not solely due to an inhibition of gibberellin synthesis. Alar did not act additively with either AMO 1618 or CCC and Moore proposed that Alar did not inhibit gibberellin biosynthesis, even though gibberellin addition overcame hypocotyl retardation.

II. The Interaction on Cell Division and Elongation

Sachs et al. (1960) proposed that retardants would appear to act on the subapical meristem rather than the apical meristem because leaf growth, a function of the apical meristem, ultimately approaches that of control plants. These workers substantiated this hypothesis with detailed experimentation using Chrysanthemum as a representative caulescent plant. Median longitudinal sections of the stem apex were cut from plants treated with AMO 1618, gibberellin, or both and the number of transverse cell divisions, average pith cell length, and the number of cell divisions counted.

The subapical mitotic figures and the length of the zone of division were reduced by AMO 1618 treatment. Apical meristem mitotic figures showed an initial decrease although average cell size and number were unchanged. Leaf initiation remained normal

for 12 days with respect to water treated controls. Leaf growth, severely retarded at first, eventually approached that of controls. In comparison, plants treated simultaneously with gibberellin and retardant remained normal with respect to controls. AMO 1618 treated plants also exhibited inhibition of cell elongation in the subapical meristem. However, the reduction in cell elongation was considered by Sachs et al. to account for only a minor part of the total growth reduction.

Measurement of cellular changes in the first internode of bean plants treated with an ammonium phenylcarbamate (Scherff 1952) showed that, in comparison with controls, parenchymatous cortical cell length was reduced by 69 percent. Vessel size was reduced while both proto and metaxylem development were inhibited. However, the cambium increased in size and mature cortical cells underwent a size increase in both the radial and longitudinal plane.

Different classes of growth retardants show marked differences in chemical structure. Sachs et al. (1963) applied three different classes of retardant (AMO 1618, phosphon, CCC) to Chrysanthemum morifolium to see if growth retardation followed the pattern previously described for AMO 1618 (Sachs et al. 1960). In all treatments, retardant and gibberellin concentrations were selected to give the maximum response, i.e. the maximum stem retardation or stem elongation. Growth retardants and/or gibberellin application had little effect on leaf initiation. Stem length of untreated and treated plants was increased by 100 percent and 70-80 percent, respectively by gibberellin treatment. AMO 1618 and CCC

did not alter either stem or root dry weight although phosphon markedly inhibited dry weight increases in both stems and roots. Exogenous gibberellin had little to no effect in reversing this inhibition. Sachs et al. proposed that these results could be taken as evidence for the hypothesis that retardants act only on intact stem tissue.

Comparative cytohistological studies on apical regions of shoots showed that the zone of elongation occurred in the top 3 cm; the topmost 1 cm area being the most active. With all retardant treatments, elongation within this zone ceased 1-7 days after treatment.

Examination of pith cells to ascertain rates of cell elongation showed that cell length was largely unaffected with gibberellin treatment. Cell elongation fell to zero in shoot apices from retardant treatments. The fact that gibberellin did not promote apparent cell elongation was explained by these workers; cell elongation was increased by gibberellin, as was cell division, but when elongation was expressed as a net cell elongation, no difference from controls can be detected because of the increase in cell division. Cell elongation, induced by gibberellin, was responsible for 30 percent of the increased stem length.

All the retardants tested inhibited subapical cell division and reduced the length of the meristematic zone. Phosphon and AMO 1618 were more effective than CCC in this regard. However, all retardant treatments indicated that proportionally more transverse divisions occurred than longitudinal with the end result being increased stem diameter. The inhibition of longitudinal growth was shown to be opposite from gibberellin treatments and Sachs et al. proposed that

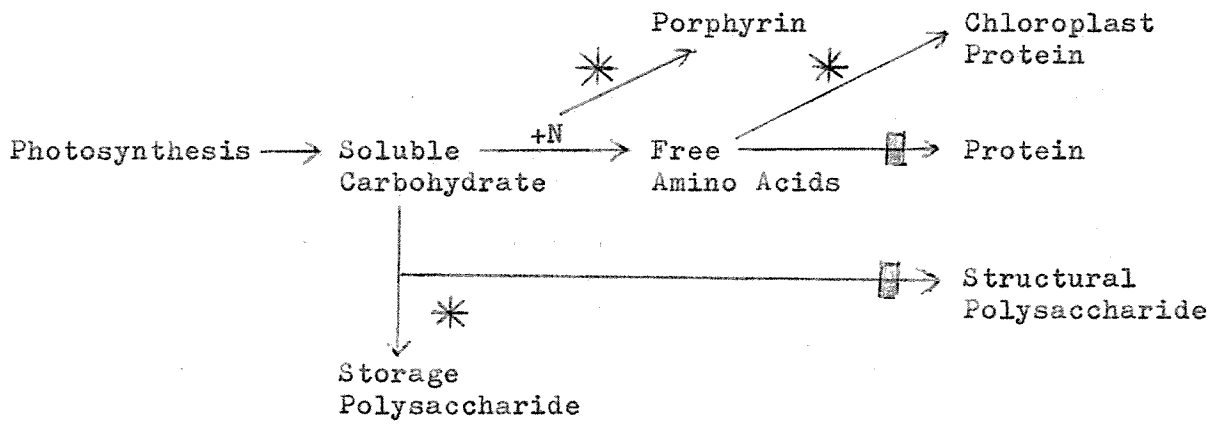
an inverse relationship occurred between transverse and longitudinal growth, such that if one was inhibited the other was promoted.

This work was carried further when Sachs et al. (1964) investigated retardant action at a cellular level. These workers grew retardant treated explants in the presence and absence of auxin and gibberellin. AMO 1618, CCC, and phosphon inhibited cell division and expansion in carrot, tobacco, and chrysanthemum explants grown in vitro. Simultaneous treatment of explants with GA_3 did not prevent retardant induced inhibition nor did addition of 2,4,D (as auxin source). These workers concluded that retardants act on growth processes other than the biosynthesis of auxin or gibberellin. However, addition of coconut milk to the basal media could well have introduced unknown quantities of both growth promoters and inhibitors; no growth of explants occurred when coconut milk and sugar were withheld. Further more, extrapolation of results from explant studies to whole plant systems has been shown by many workers to lead to incorrect assumptions.

III. Growth Retardant Influence on Cellular Metabolism

Lolium temulentum (L) plants, treated with CCC, showed that growth inhibition was accompanied by large increases in free sugar content (especially fructosan) and free ∝amino-N when nitrogen was in adequate supply. Crude protein content was increased under low nitrogen regimes and chlorophyll production was stimulated (Stoddart 1965). Photosynthesis and polymerization of sugars to fructosan were unaffected by CCC treatment as was the formation of amino sugars from carbohydrate.

Stoddart proposed the following metabolic scheme:



▯ Possible CCC induced metabolic block

✳ Diversion pathway

From J.Exp.Bot. 1965. p.611.

The author indicated two sites of CCC induced blockage although it was thought probable that CCC influenced a "single, cell growth-directing, system" which would exhibit effects as outlined above. It is of interest to note that Stoddart mentions that increased sugar levels could effect cold hardiness of plants.

A novel approach to the study of retardant mode of action in pea was carried out by Heatherbell et al. (1966) who incubated etiolated pea first internode, and root apex sections in the presence of CCC and Alar. Manometeric measurement of oxygen consumption allowed these workers to calculate changes in the respiration rate and coupled phosphorylation. CCC stimulated respiration in root apex sections, the stimulation increased with concentration. Alar was less active as a respiratory stimulant and, at 10^{-2} M, proved to be inhibitory.

These workers proposed that the rise in respiration could be due to the uncoupling of phosphorylation from respiration.

In order to test this hypothesis, mitochondrial suspensions (prepared from stem tissue) were incubated with CCC and Alar. Increasing the concentration (to 10^{-3} M) of both compounds had no effect on respiration rates but increasingly depressed phosphorus esterification. Again Alar showed lower activity than CCC. Concentrations greater than 10^{-3} M inhibited both respiration and phosphorus esterification although mitochondria did not completely cease to respire. Heatherbell et al. concluded that the effects of both retardants on plant growth were due to oxidative uncoupling of the respiratory chain which would result in decreased levels of adenosine triphosphate (ATP).

Brook et al. (1967) showed that changes occurred in various RNA fractions extracted from phosphon-S treated 'Alaska' pea. Levels of soluble RNA, endogenous RNA'ase, and specific activities of various nucleic acids all decreased while ribosomal RNA showed an increase. RNA'ase treated nucleic acids from treated plants showed a higher specific activity in the DNA-RNA fractions than controls. These authors presented some evidence for a possible phosphon-S-nucleic acid complexing and further suggested that changes induced in biochemical pathways caused by altered RNA levels could result in growth retardation.

E. GROWTH RETARDANT INDUCED INHIBITION OF GIBBERELLIN BIOSYNTHESIS

I. Studies on Fusarium moniliforme

Various strains of the fungus Fusarium moniliforme are known to produce gibberellins. Kende et al. (1963) introduced AMO 1618 and CCC into the culture medium of such a strain (Lilly M-45-399) in an attempt to distinguish between two possible modes of retardant action; namely, a competition with gibberellin for active sites or an inhibition of gibberellin biosynthesis. Both chemicals suppressed endogenous gibberellin biosynthesis without reducing the growth of the fungus. Neither retardant appeared to affect the site of action of the endogenous gibberellin. Phosphon D, tested in a like manner, appeared to be metabolised before it could act on the biosynthetic pathway.

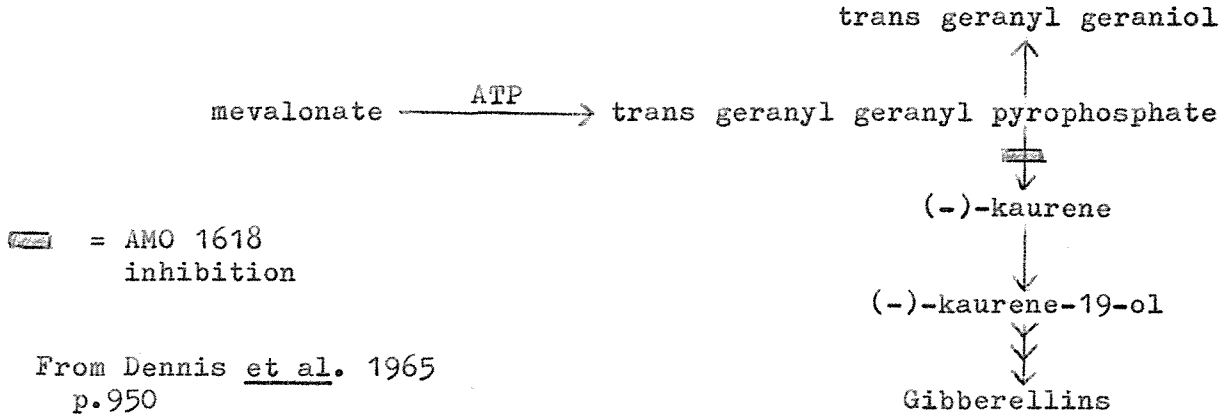
Subsequent work with the above fungus (Ninnemann et al. 1964) showed that the decreased level of gibberellin in the presence of CCC was due to a biosynthesis block rather than an enhanced rate of destruction. Alar was similarly tested and, although it was readily recovered from the fungal mycelia, it in no way decreased gibberellin production. Further work by Harada et al. (1965) with strain SS-1 of the above fungus again showed that CCC induced a biosynthetic block. In an attempt to discover the point of control in the biosynthetic pathway, (-)-kaurene, kaurenol, and steviol were added to culture media containing CCC. No increase in gibberellin levels were found. These workers concluded that the block could

occur at a point in the pathway subsequent to kaurenol formation.

II. Studies on Higher Plant Systems

Baldev et al. (1965) utilized the fact that developing seeds accumulate large amounts of gibberellin in order to test the effect of retardants on gibberellin synthesis. Young pea pods were grown on synthetic media containing variable levels of AMO 1618 for 10 days. Seeds were then assayed for gibberellin content. All AMO 1618 treatments decreased gibberellin production.

Further proof of a biosynthesis block in higher plants has been presented by Dennis et al. (1965) using enzyme preparations from Echinocystis macrocarpa (Greene) endosperm. Enzyme preparations were incubated with 2-C^{14}-DL-mevalonate in the presence of various growth retardants and the formation of labelled (-)-kaurene and (-)-kaurene-19-ol measured. Formation of both compounds was markedly inhibited by AMO 1618 although concomitant increases occurred in trans geranyl geraniol. These workers proposed that AMO 1618 inhibited cyclization of trans geranyl geranyl pyrophosphate to (-)-kaurene, so increasing the substrate level for hydrolysis to trans geranyl geraniol.



From Dennis et al. 1965 p.950

Similar incubations with phosphon and phosphon-S showed reduced (-)-kaurene levels. Alar (at 100 μg/ml) only reduced (-)-kaurene levels by 41 percent while CCC and β-hydroxyethyl hydrazine were not significantly effective. These workers consider that Alar is inactive in the prevention of cyclization to (-)-kaurene. However, this work does not exclude the possibility of Alar acting at a point closer to the gibberellin end point (cf Harada et al. 1965).

Steviol, a postulated intermediate in the biosynthetic pathway to gibberellins, acts as a substrate for gibberellin-like compounds produced by F. moniliforme (Ruddat 1966). When 2-C^{14}-sodium acetate and AMO 1618 were introduced into Stevia rubaudiana, a reduction in labelled steviol occurred in 24 hours. Furthermore, suppression of internode growth typical of AMO 1618 treatment was completely counteracted with exogenous gibberellin.

Treatment of Pharbitis nil with CCC prior to, and after, anthesis resulted in a reduction of gibberellin-like activity in the seeds (Zeevart 1966). Progeny from these seeds also had a lowered gibberellin-like content. Sufficient CCC accumulated in developing seeds to dwarf progeny growth and inhibit flowering. Gibberellin (A_3) application overcame both effects. Zeevart concluded that CCC blocked endogenous gibberellin synthesis.

Young leaves of sunflower apices serve as the primary site of gibberellin formation (Jones et al. 1966). CCC is effective in reducing stem growth of sunflower and this effect can be overcome with exogenous gibberellin. Jones et al. (1967) found that seedling sunflower apices, treated with CCC, contained significantly less

diffusible gibberellin than controls. This trend also held for diffusates from root apices. No measurable gibberellin-like compounds could be located in root exudates (or bleeding sap) of mature sunflower. These workers, however, argued that the lack of measurable gibberellin-like compounds in root exudates could have arisen as an artifact of lowered levels in stem apices rather than lowered levels in root apices.

This question was studied further by Reid et al. (1967) who assayed the bleeding sap of CCC treated Impatiens glandulifera (Royle) for gibberellin-like substances. Although CCC treatment significantly reduced gibberellin levels, conclusive evidence on the above problem could not be obtained. Accordingly, Pisum arvense (L) plants were decapitated just prior to CCC application. Bleeding sap from treated plants showed decreased gibberellin content although anomalous fractions appeared when compared to controls. Extraction of gibberellin-like substances from roots (24 hours after decapitation) showed a similar pattern to that of bleeding sap. Reid et al. concluded that CCC blocked the production of gibberellins normal for the species and caused a diversion of some precursor toward the production of "abnormal" gibberellins, which were active in both barley endosperm and 'Meteor' pea bioassays. This work adds further evidence to the hypothesis that CCC inhibits the gibberellin biosynthetic pathway at a point after cyclization to (-)-kaurere (Harada et al. 1965, Dennis et al. 1965).

F. GROWTH RETARDANT INTERACTION WITH GIBBERELLIN SITE OF ACTION

Gibberellin induces reducing sugar release from barley endosperm (Paleg 1960). Paleg et al. (1965) incubated various concentrations of growth retardants with endosperm halves and gibberellin. No retardant listed (AMO 1618, phosphon D, CCC, CO11, Alar, maleic hydrazide) interacted with the gibberellin induced response. As these workers point out, endosperm tissue neither synthesises gibberellin nor the gibberellin substrate as the tissue responds immediately to exogenous gibberellin. The response to gibberellin precluded the possibility of retardant enhanced gibberellin destruction. It can be concluded from this work that growth retardants do not exert an effect on the gibberellin site of action in barley endosperm.

G. INTERACTION BETWEEN GROWTH RETARDANTS AND AUXIN

Incubation of Avena coleoptilar sections with CCC (and 2 analogues) and IAA (Wittwer and Tolbert 1960) resulted in suppression of elongation, both in the presence and absence of IAA. Addition of gibberellin to the incubation media resulted in a reduced growth suppression by retardants. It should be pointed out, however, that Avena coleoptilar tissue, grown in the absence of growth retardants, may not elicit a full response to IAA when incubated in retardant

presence for 48 hours.

Auxin catabolism by IAA oxidase and peroxidase enzyme systems has been studied by several workers in recent years. Exogenous gibberellin has been shown to decrease high levels of both enzymes in dwarf plants and to decrease IAA oxidase levels in normal plants. Halevy (1963) determined levels of both peroxidase and IAA oxidase in cotyledons, hypocotyls, and radicles of dark grown cucumber seedlings treated with AMO 1618, carvadan, CCC, phosphon, and Alar. In all cases, a significant increase in enzyme levels was shown although IAA oxidase levels in hypocotyl tips and cotyledons were influenced to a greater extent. The reverse was shown to occur with gibberellin treatment although in radicles, peroxidase activity was unaffected and IAA oxidase only slightly decreased. Application of both AMO 1618 and GA_3 restored enzyme levels to about that of untreated seedlings. Halevy concluded that growth retardants exert their influence on plant growth by interacting with gibberellin in IAA oxidase (or its cofactors and inhibitors) activity such that auxin levels decrease.

Kuraishi and Muir (1963) showed that the inhibitory effect of CCC on both Avena coleoptile growth and 'Alaska' pea segment (5th internode) could be overcome with exogenous auxin (IAA). Phosphon showed a similar relationship with IAA on Avena coleoptiles. Gibberellin had no effect on the reversal of Avena retardation although a slight response was achieved with 'Alaska' stem tissue. Bioassay of diffusable auxin levels from CCC treated pea (6th internode) showed decreased auxin levels with increased retardant concentration. These workers concluded that the growth retarding effect of CCC was due to

reduced auxin levels and that the CCC x GA_3 interaction on pea stem tissue (5th internode) was due to increased auxin levels.

More recently Reed (1965), Reed et al. (1965) investigated the possibility that growth retardants acted at a point in the auxin biosynthetic pathway. Enzymes extracted from etiolated pea stem converted C^{14}-tryptamine to C^{14}-indoleacetaldehyde. β-hydroxyethyl hydrazide inhibited this reaction (Reed 1965). The reaction exhibited competitive enzyme inhibition if retardant and substrate were added simultaneously, non-competitive if the retardant was added prior to substrate. Alar, which inhibits shoot elongation in pea (Reed et al. 1965), was also investigated as a possible enzyme inhibitor in the above reaction. Tryptamine oxidation was shown to decrease. Similar results were shown with 1-1,-dimethyl hydrazine. Reed et al. calculated that hydrolysis of less than 0.1% of administered Alar to yield 1-1,-dimethyl hydrazine (Dahlgrew et al. 1963) could produce a 50% inhibition of tryptamine oxidation.

H. MOVEMENT AND FATE OF ALAR IN TREATED PLANTS

I. (a) Movement and Fate of Alar in Apple

Application of C^{14} - labelled Alar to apple seedlings cv. Delicious via petiole, stem, and root, resulted in rapid absorption and translocation of Alar. Areas of highest radioactivity corresponded to areas of highest plant density (Martin et al. 1964).

Recovery values of labelled Alar from whole plants treated from 0-24 hours showed that little to no metabolism had occurred. Injection of 40 μC Alar into the vascular system of 5 year old Red Delicious and subsequent leaf analysis for radioactivity indicated that breakdown only became significant about the 128th day after application. Martin et al. concluded that Alar was stable over the time period in which it was physiologically active.

Further work by Martin et al. (1966) showed that, after labelled Alar was injected into seedling Red Delicious stems, activity (in decreasing order) was located in the leaves, stems, roots, and root stock stem. The presence of radioactivity was also shown in soil water and the greater part of this excreted compound(s) occurred as unmetabolised Alar. However, the breakdown rate in soil was found to be faster than the rate found for the whole plant.

Extensive activity was shown to occur in the fruit skin, petal bundles, sepal bundles, fleshy pericarp and the cartilaginous pericarp. Radiological examination of fruit cellulose components showed that the predominant label fraction appeared in the acid extracted cellulose and the lignin polyuronide hemicellulose. Acid digestion of fruit holocellulose and chromatography of resulting sugars indicated that small levels of activity appeared at Rf values corresponding to 9 different component sugars. Reincorporation of released $C^{14}O_2$ from slow, but constant, breakdown of Alar (with time) was proposed to account for the low level of labelled cellulose components.

Edgerton et al. (1967) determined Alar levels in McIntosh

fruit (flesh and seed) and in dormant tissue (spurs and shoots) at various times after C^{14} - Alar application in the spring. Absorption from sprayed fruit surfaces occurred rapidly with measurable levels in seeds and flesh after 4 hours. Accumulation continued for 3 weeks before becoming constant. Flower buds from dormant wood showed highest levels of Alar with lower levels in vegetative buds, cluster bases, shoot bark, and shoot xylem. Similar tissue from adjacent untreated branches showed some activity in cluster bases and spurs. Analysis of shoot tissue formed the year after application showed that Alar was present in spur tissue, cluster bases and vegetative buds.

(b) Movement and fate in cherry

Alar, applied as a foliar spray to mature cherry trees in the spring and fall, was shown to be present in young leaf tissue the season after application (Ryugo 1966). This worker proposed that the retardant was mobilized from storage stem tissue and then translocated to the new expanding leaves. Fruit from trees treated with spring applications showed a continuous accumulation of Alar until fruit harvest.

II. Interaction and General Effects of Alar and Gibberellin on Apple Shoot Growth

Application of Alar (1000 ppm) to McIntosh shoots reduced shoot growth to about 45% of the control (Edgerton and Hoffman 1965). Lateral growth was unaffected. Simultaneous application of Alar (1000 ppm) and gibberellin (KGA 200 ppm) reduced shoot growth to less than 50% of the KGA treated controls. (This author calculates the reduction to be 57% and, furthermore, 1000 ppm Alar x 200 ppm KGA

increased shoot growth by 13% with respect to water controls while 200 ppm KGA showed a 160% increase).

In somewhat similar studies Greenhalgh and Edgerton (1966) showed that Alar and KGA application to McIntosh limbs induced opposite responses in shoot growth.

Luckwill (1966) treated maiden Scarlet Pimpernel trees with 2000 ppm Alar and 200 ppm gibberellin. Gibberellin treatment greatly reduced apical dominance, increased leaf number on lower shoots, and increased mean internode length on the lower shoots only - not on the leading shoots. Alar did not effect either of the first two responses but was effective in reducing mean internode length on all shoots.

I. DISCUSSION

The effect of Alar on stem elongation, and plant development as a whole, has been reported in considerable detail for several plant species. In the main, these reports have been restricted to descriptive accounts of changes in vegetative form (suppressed internode elongation, reduced extension growth, etc.) and plant yield (increased flower bud initiation, decreased fruit size in deciduous fruit trees, etc). Detailed studies on the physiological changes induced by Alar treatment have not been attempted on apple and only scattered evidence is available from studies on other plants. In order to obtain an indication of possible interactions between Alar and plant growth, it is necessary to examine evidence which has accumulated from other retardant studies.

In general, the growth response of plants treated with growth retardants is opposite to that obtained with exogenous gibberellin. Evidence has been presented that the primary site of retardant action occurs in the stem subapical meristem. Endogenous gibberellin promotes cell division and elongation in this area; growth retardants slow both processes. Several workers have proposed that growth retardant interaction with endogenous gibberellin could be manifest as one or more of the following:

(i) An inhibition of gibberellin biosynthesis.

(ii) An enhanced rate of gibberellin destruction.

(iii) An interaction at the site of gibberellin action or on a pathway which would lead to substrates contributing to the active site.

The weight of evidence favours the first possibility; namely that growth retardants inhibit the biosynthesis of endogenous gibberellin. However, evidence presented in the literature does not wholly support this claim in relation to the mode of action of Alar. The biosynthetic pathway leading to the gibberellins is only partially understood and, as a consequence, investigators have only tested for retardant induced inhibition on steps between geranyl geranyl pyrophosphate and (-)-kaurene-19-ol or steviol. It is possible that Alar inhibits at a point between (-)-kaurene-19-ol and the physiologically active gibberellin. Such an inhibition has been shown for CCC. Heatherbell et al. (1966) showed that Alar lowers ATP levels in treated stem tissue. The relationship between lowered ATP levels and gibberellin biosynthesis has not been shown although ATP is necessary for geranyl geranyl pyrophosphate formation from acetate.

The only evidence for, or against, the hypothesis that growth retardants and gibberellins could interact at the gibberellin site of action has been presented for the gibberellin mediated release of reducing sugar from barley endosperm. The fact that no interaction could be shown does not preclude the possibility that growth retardants could interact with the gibberellin site of action in the stem apex.

Endogenous auxins are also active in the control of cell division and elongation in stem tissues. However, conflicting evidence has been presented in relation to the possible interaction between auxin and growth retardants. Auxin (IAA) has only been shown to reverse CCC induced retardation (cucumber hypocotyl, Avena coleoptile, 'Alaska' pea) and that induced by phosphon (Avena coleoptile). Similarly, decreased auxin levels have only been shown in CCC treated pea. More substantial evidence has been presented from enzyme studies. Increased IAA oxidase and peroxidase levels have been shown for cucumber seedlings treated with growth retardants. However, it is open to conjecture whether or not increased IAA oxidase and peroxidase activity is due to a direct retardant effect or to lowered gibberellin levels in retardant treated tissue. A direct interaction with the biosynthetic pathway leading to auxin (IAA) has also been shown for β-hydroxyethyl hydrazine and Alar in pea.

Some evidence has been presented in regard to an interaction between gibberellin and Alar in apple stem tissue. Simultaneous application of selected gibberellin and Alar concentrations result in shoot growth which approaches that of controls. Alar has been shown to accumulate in the stem apex and young leaves; both organs have been

shown to produce auxins and gibberellins. It was considered, on the basis of evidence presented in this discussion, that an investigation into the possible interaction between Alar and endogenous auxin and gibberellin was warranted.

Chapter 2

MATERIALS AND METHODS

Chapter 2

M A T E R I A L S A N D M E T H O D S

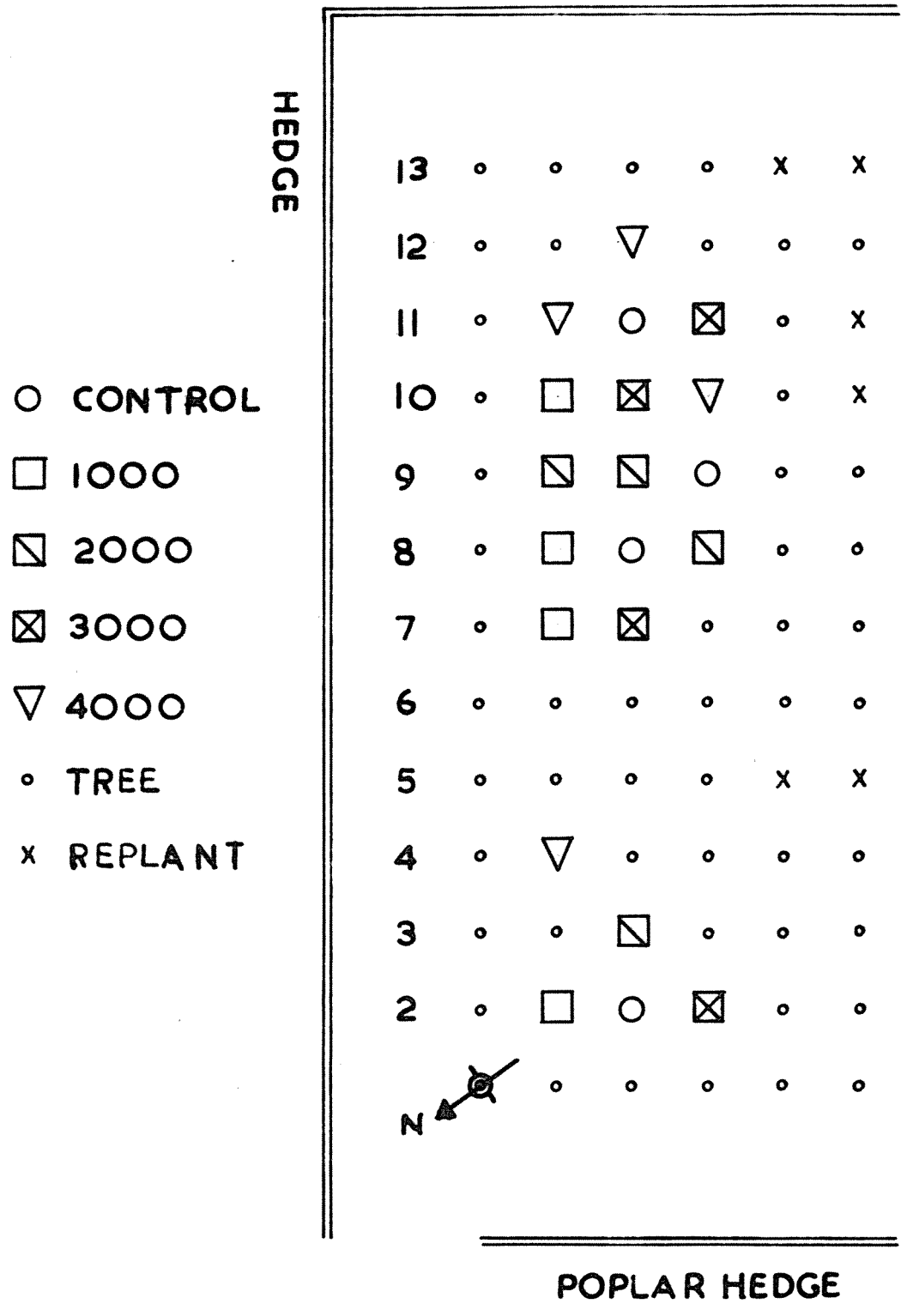
A. INTRODUCTION

Conflicting evidence has been presented in the literature concerning the necessity of a repeat Alar application to apple trees treated at full bloom. Several workers (Emerson et al. 1966 included) have reported that single applications gave variable responses and shoot growth often resumed in the same season as application. Williams (1967) confirmed the advisability of using a double spray application.

The literature also contains many schemes for both auxin and gibberellin extraction. Many of these schemes have been devised for non-photosynthetic tissue and, as such, are difficult to apply to the present study. Further more, very recent literature contains reports of suitable extraction and chromatographic procedures, involving sophisticated equipment which was unavailable for this study.

Accordingly, all methods used for the assessment of auxin and gibberellin levels in apple shoot tissue were selected as being the most suitable for the equipment available.

Figure 1. Experimental Gravenstein planting - Massey University Orchard.



B. EXPERIMENTAL PLOTS AND SAMPLING TECHNIQUES

I. Experimental Apple Planting

Experimental trees were selected from a 7 year old planting of Oratia Beauty (Gravenstein) growing in the Massey University Orchard. The planting was divided into two blocks on the basis of root stock type (M.M. 779); between rows 1-4 and 7-12 (Figure 1). Outside rows were discarded as guard rows. Trees left within each block were selected for treatment application with the use of random tables to give, in the case of Block 1, 5 individual treatments by 3 replications.

(a) Block 1 (Rows 7-12)

Trees were sprayed with a knapsack applicator to the point of runoff with Alar at 500, 1000, 1500, and 2000 ppm at full bloom (6/10/67) and 11 days after (17/10/67) to give a total treatment of 1000, 2000, 3000, and 4000 ppm Alar (Emerson et al. 1966). Control trees received water sprays on both dates. A wetting agent, Tween-20 (0.1%), was included with all sprays.

(b) Block 2 (Rows 1-4)

Trees were sprayed to the point of runoff with Alar at 1000, 2000, 3000, and 4000 ppm when extension growth was well advanced (22/11/67). Application methods and wetting agent concentration were as for Block 1.

II. Sampling Technique

(a) Block 1

Experimental trees were divided into quadrants along the axes of the block and each quadrant further divided into upper, middle, and lower sectors. Shoot samples were taken from each quadrant once every 4 weeks; starting from the north west quadrant, and then from each quadrant in rotation. Four shoots were selected, at random, from the upper sector; one from the middle sector; and two from the lower sector. The seven shoots from each replicate were bulked and immediately transferred to a deep freeze.

Each harvest, then, consisted of twenty-one randomly selected shoots from each of the five treatments.

(b) Harvest dates

(i) Shoot harvest from Block 1 began on 24/7/67 and continued each week until the 2/1/68 by which time, terminal bud formation was well advanced and extension growth had ceased.

(ii) All fruit from both blocks were harvested on 15/1/68.

III. Treatment of Each Harvest

(a) Stem and leaf measurement

Fifteen shoots from each treatment were selected at random, and the shoot apex excised. The fifteen apical samples (2.0-3.5 cm long) were immediately returned to deep freeze storage.

The leaf laminae from the decapitated shoots were removed

at the petiole junction and laminae area measured on an air flow planimeter. Leaf laminae were then discarded.

Individual shoots were measured for internode length. The distance between the point of petiole junction to the stem and the beginning of the next most distal petiole base was taken as a measure of internode length. After measurement, shoots were returned to the deep freeze.

(b) Dry weight measurement

Various apple shoot harvests were removed from cold storage and dry weight assessments made. The fifteen shoots which had been previously measured for internode length were transferred to an 80°F oven for three days.

At the time of sampling for internode length assessment, six intact shoots had been returned to cold storage. From these intact shoots, a further sub-sample of three shoots was taken. Thirty leaf laminae were selected at random from the sub-sample and subjected to the same drying treatment as the stems.

(c) Fruit harvest

All trees carried a light crop and, as a consequence, were not thinned. Fruit from Block 1 were weighed and manually assessed for size (diameter) with standard orchard sizing rings. The size group falling into the range 2.75-3.25 inches was stored for ten days at 35°F and then at packing shed temperatures for a further eleven days. After this storage period fruit were weighed, cut in two along the

transverse diameter and visually assessed for development of bitter pit, mouldy core, bruising, and fruit deterioration (softness).

(d) Photography

(i) Shoot photography

Two shoots of approximately equal size were selected, and tagged, on 12/12/67 from the north west quadrant of each experimental tree in Block 1. These tagged shoots were harvested on 12/2/68 and total internode number recorded for each sample. Each sample contained at least one shoot with thirty-two internodes and random selection within this grouping was used for photographic material.

In order to compare initial to final shoot size, three shoots were selected from the stored harvest of 24/10/67. A similar selection procedure, as above, was used although in this instance, an internode number of ten was used as the basis of selection.

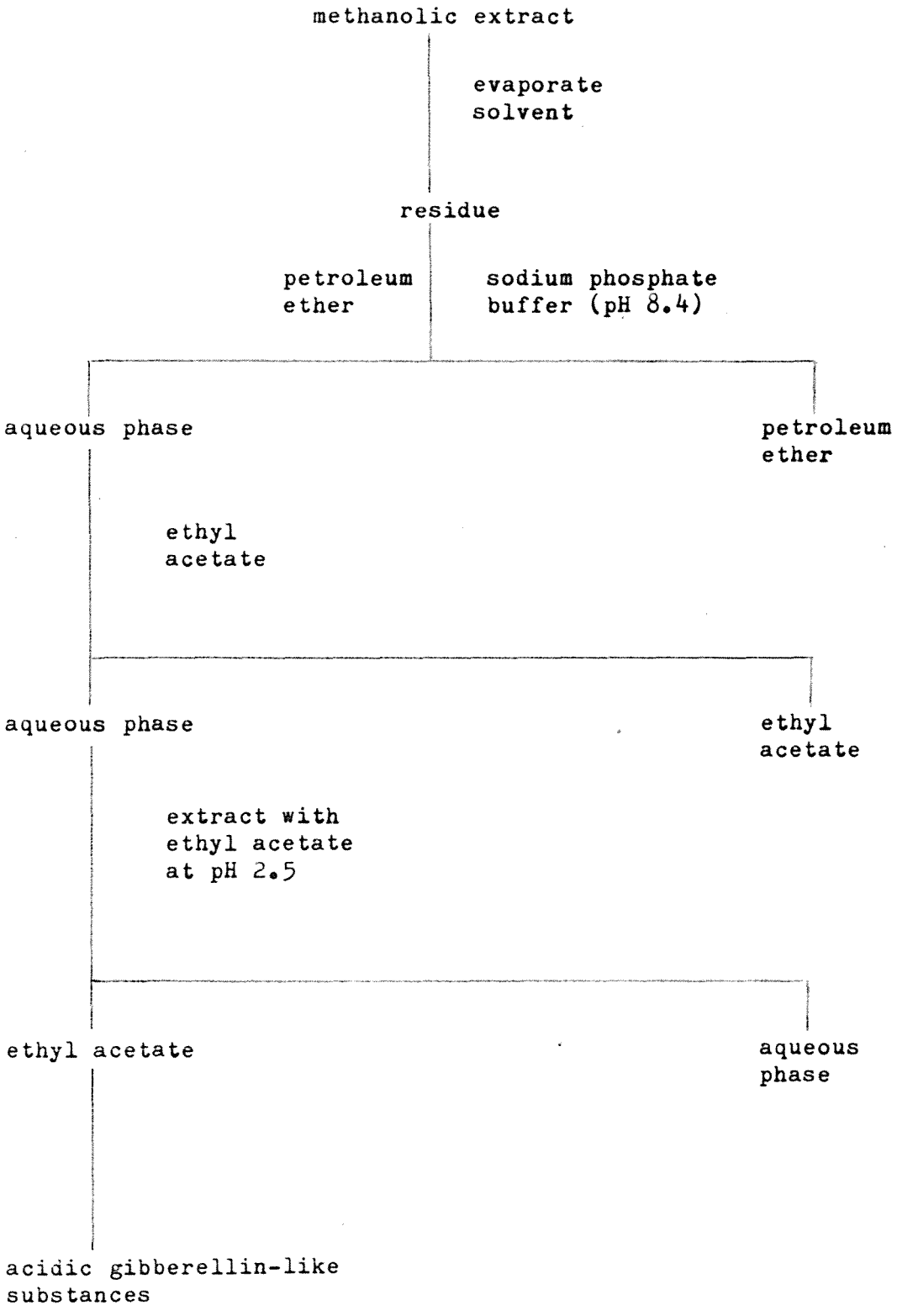
(ii) Fruit photography

Fruit from Block 2 were stored at packing shed temperatures for four weeks. Twenty fruit were selected at random from each treatment. A further sub-sample from these fruit was used for photographic material.

(e) Anthocyanin determinations

Anthocyanin levels in fruit skin were determined immediately after photography. Each fruit was peeled with an ordinary potato

Figure 2. Summary of gibberellin extraction procedures.



peeler and the surface area of the peel measured on an air flow planimeter.

Anthocyanins were extracted from the bulked peel with 50 ml of methanolic HCl (40:60 v/v; 1% HCl) for 24 hours at 5°C. The extract was decanted off, and made up to constant volume (50 ml) with methanolic HCl. 1 ml of solution was tested for anthocyanin content after Swain and Hillis (1959). Optical densities of all tubes were determined before and after addition of the peroxide reagent and the difference between the readings was taken as a measure of anthocyanin content.

A standard curve for cyanidin chloride in methanolic HCl (40:60 v/v; 1% HCl) was also erected, using the above method.

C. GIBBERELLIN

I. Extraction of Gibberellin-like Substances

Shoot apices and young unfolded leaves, which had been deep frozen, were further cooled with dry ice just prior to freeze drying. Dried tissue was weighed, macerated, and then extracted overnight at 5°C with methanol (approximately 1 gm/20 ml). The extract was filtered and the residue re-extracted for 6 hours at 5°C. The residue was removed from the extract by filtration, washed several times, and the washings and two extracts bulked and evaporated to dryness in a rotary evaporator.

Extraction of acidic gibberellin-like substances was carried out after Kende and Lang (1964) (Figure 2). The following modifications were made to the isolation procedure:

(i) 0.1 M sodium phosphate buffer (pH 8.4) (Appendix I) was used rather than the specified 1.0 M buffer (pH 8.4) which precipitated from aqueous solutions during the extraction.

(ii) The bulked ethyl acetate extracts were evaporated to dryness without first drying over sodium sulphate.

II. Thin Layer Chromatography

Thin layer chromatography was used to separate the gibberellin-like substances from the crude extracts. Plates (20 x 20 cm) were poured using Pleuger equipment loaded with silica gel (Kieselgel H) : water (1:2) which had been thoroughly combined in a blender for 2 minutes. Poured plates (0.05 mm thick) were dried in a cool air stream and activated at 100°C for 60 minutes.

The dried acidic fraction was taken up in small quantities of ethyl acetate and applied as a streak to activated plates. Plate development was as for MacMillan and Suter (1963) using di-isopropyl ether : acetic acid (95:5 v/v). Developed plates were dried in a cool air stream and divided into 10 Rf zones. Each zone was scraped and eluted with four successive additions of water saturated ethyl acetate (Reid and Carr 1967).

Where more than one plate was used for the chromatography of an extract, the scrapings from each Rf zone were bulked and eluted as above. All scrapings were centrifuged after the third elution to reduce silica gel contamination of bioassay incubation vials. Silica gel controls were scraped from each plate and eluted as above.

The combined eluates from each treatment were evaporated to a small volume, distilled water (1 ml) added and the remainder of the solvent evaporated. The extract was made up to 3 ml with distilled water and bioassayed.

III. Barley Endosperm Bioassay

The barley endosperm bioassay used to determine GA_3 equivalents from each Rf zone was based on the method used by Jackson (1967).

(a) Materials

(i) Barley cv 'Research Barley' obtained from Crop Research Division, Lincoln.

(ii) Seed was dried over concentrated sulphuric acid and stored at 5°C.

(iii) Plastic vials (3 x 6.5 cm) were used for all incubations.

(iv) Somogyi's Reagent (Appendix I).

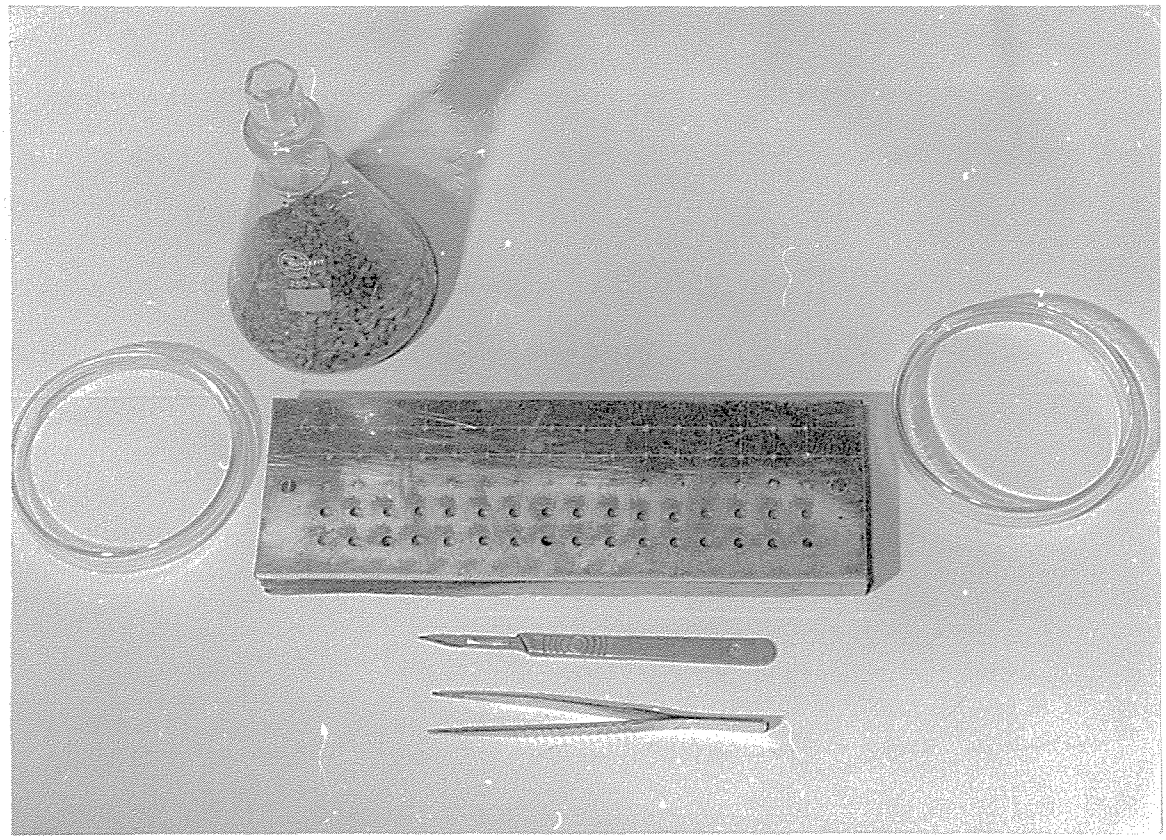
(v) Nelson's Reagent (Appendix I).

(b) Method

(i) A uniform sample was selected from the dried seed and soaked for 3 hours in 50% H_2SO_4 at room temperature. In all cases, more seed was selected than would be needed.

(ii) The acid was removed by decantation and seed washed with distilled water (10 times); each washing being discarded. The seed sample was washed a further 5 times with sterile distilled water and then soaked for 21 hours in sterile distilled water.

Figure 3. Materials for barley endosperm bioassay.



Cutting block dimensions, 3 x 9½ x ¾".

Placement holes, 3/16" wide x ⅜" deep.

(iii) The water was decanted off and seed transferred to a petri dish lined with filter paper (Figure 3). Individual seeds were removed from the petri dish with forceps and placed, embryo down, in the cutting block. Care was taken at all times to select an even seed line; all broken seeds were discarded. Seeds were cut into two by holding the endosperm with forceps and sliding a thin bladed scapel along the cutting block surface (Figure 3). Endosperm pieces were selected on the basis of cut surface colour. Cut surfaces which appeared to be "soft" and "grey-white" in colour were discarded; endosperm pieces with "pearly white" cut surfaces were stored in a petri dish lined with streptomycin sulphate (10 mg/ml) treated filter paper. Sterile conditions were observed at all times; flame sterilization for forceps, scapel, and cutting block. In most bioassays, the endosperm pieces were used immediately. In some instances, endosperm pieces were stored at 5°C for 30 minutes before use.

(iv) Each incubation vial was loaded with 1 ml of extract to be tested, 0.05 ml streptomycin sulphate (10 mg/ml) and two, randomly selected, endosperm pieces. Vials were incubated for 24 hours in a 30°C water bath.

(v) At the completion of incubation, vials were either assayed immediately or deep frozen and stored for up to 3 days before assaying (Coombe et al. 1967).

(c) Reducing sugar estimation

Reducing sugar was assayed after Nicholls and Paleg (1963).

However, the following modifications were made to the methods:

(i) Tubes were shaken continually for 5 minutes.

(ii) Solutions were only filtered if silica gel contamination had occurred.

(iii) Only 1.0 ml of solution was assayed for reducing sugar.

(iv) Boiling tubes were heated for 15 minutes exactly.

(v) Absorbance was measured at 550 mμ.

Some of these modifications were suggested by Jackson (1967).

IV. Standard Curves

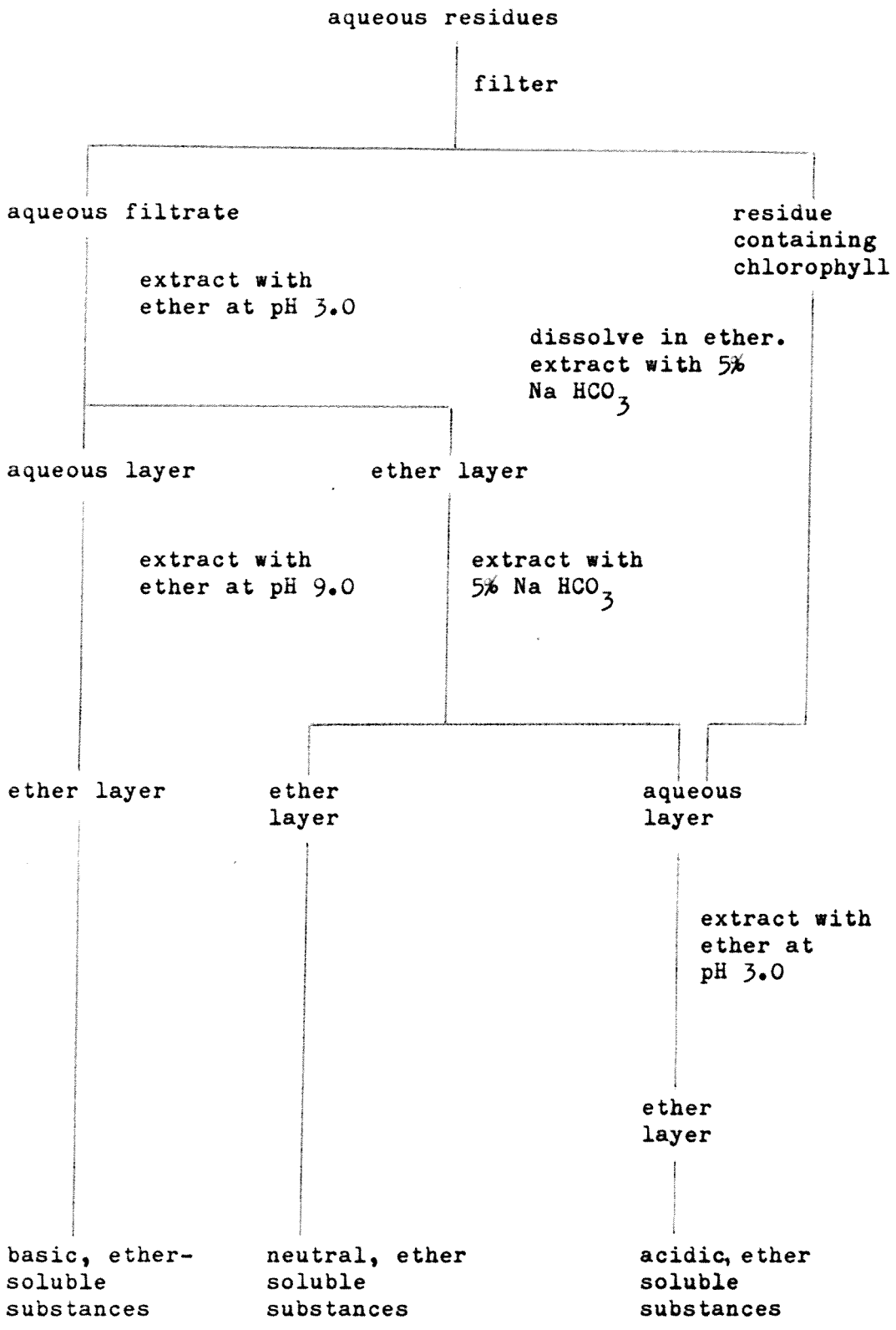
Several standard curves were erected within the concentration range 1×10^{-7} to 1×10^{-11} gm/ml gibberellin (GA_3). 1 ml of GA_3 (x 3 replications) from each concentration used was incubated with endosperm pieces and assayed as above. In order to relate optical density readings to reducing sugar equivalents a standard curve using 0-3.5 mg/ml glucose was also erected.

D. AUXIN

I. Auxin Extraction

Plant material for auxin extraction was prepared in a similar fashion to that for gibberellin determinations. The dried stem tissue was macerated in a M.S.E. homogenizer, extracted with methanol (20 ml/gm)

Figure 4. Summary of auxin extraction procedures.



for 24 hours in the dark at -10°C. The plant debris was removed from the methanolic extract by filtration, washed several times, and discarded. The combined washings and initial filtrate were evaporated to dryness under reduced pressure. The dried filtrate was taken up in 50 ml of distilled water and acidic, basic, and neutral auxins extracted (Kefford 1959) (Figure 4). The following modifications were made to Kefford's method:

(i) 1 N Na OH and 50% HCl were used to correct pH levels to the stated values.

(ii) All separations and extractions were repeated twice.

(iii) The final extracts for basic, neutral, and acidic ether soluble substances were reduced to a small volume.

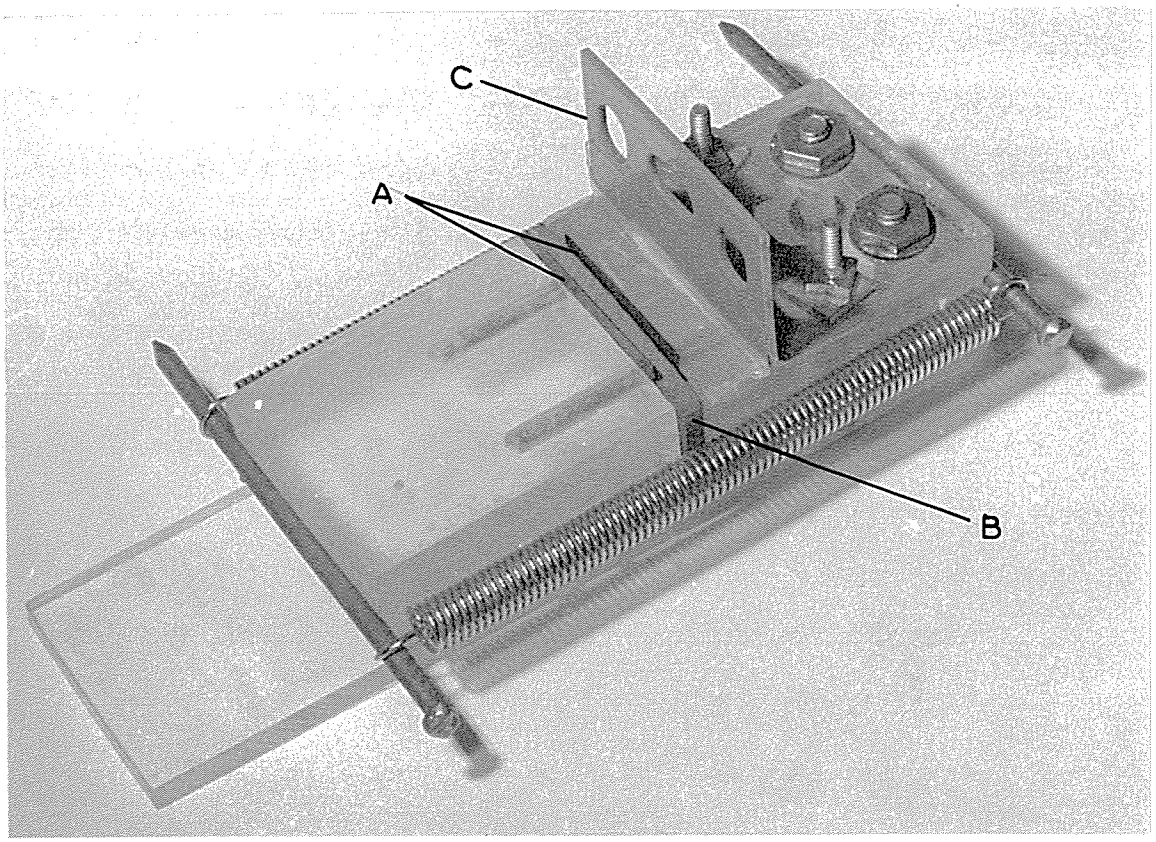
II. Chromatography

Ascending paper chromatography was used for all extracts. Strips of Whatman No.1 paper (23 x 12 cm) were pre-run in solvent (Burnett et al. 1965) to remove chemical substances active in Avena bioassays. Extracted substances were applied as a 0.5 cm wide streak and papers run in isopropanol : water : ammonia (27%) (10:1:1) (Kefford 1959) for a distance of 15 cm in the dark. Chromatograms from harvest date 26/12/67 were run for 20 cm.

The chromatograms were dried under a cool air stream and cut into 10 strips on the basis of Rf. Each strip was cut into very small pieces and transferred to pyrex tubes (75 x 8 mm) for bioassay.

Control strips were taken at Rf 1.1 and treated as above.

Figure 5. Cutting block for *Avena* 1st internode sections.



A, razor blades; B, spacer (5 mm); C, stop (7 mm from razor blade).

Overall dimensions, 5 x 2 x 1¼".

III. Auxin Bioassay

Auxins, separated by chromatography, were bioassayed by the Avena first internode test, essentially after Nitsch and Nitsch (1956). However, several modifications were made to their method by this author.

(i) Oat seeds cv (Garton) were soaked in tap water for 2 hours in the dark. Seeds were washed 3 times with tap water and planted in plastic trays (27 x 39 cm) containing about 4 cm of moistened pumice. The seed was covered with a further 1 cm of moist pumice and germinated in the dark at 25°C. All manipulations from this point onwards were carried out in dim green light (Ilford filter G No.907).

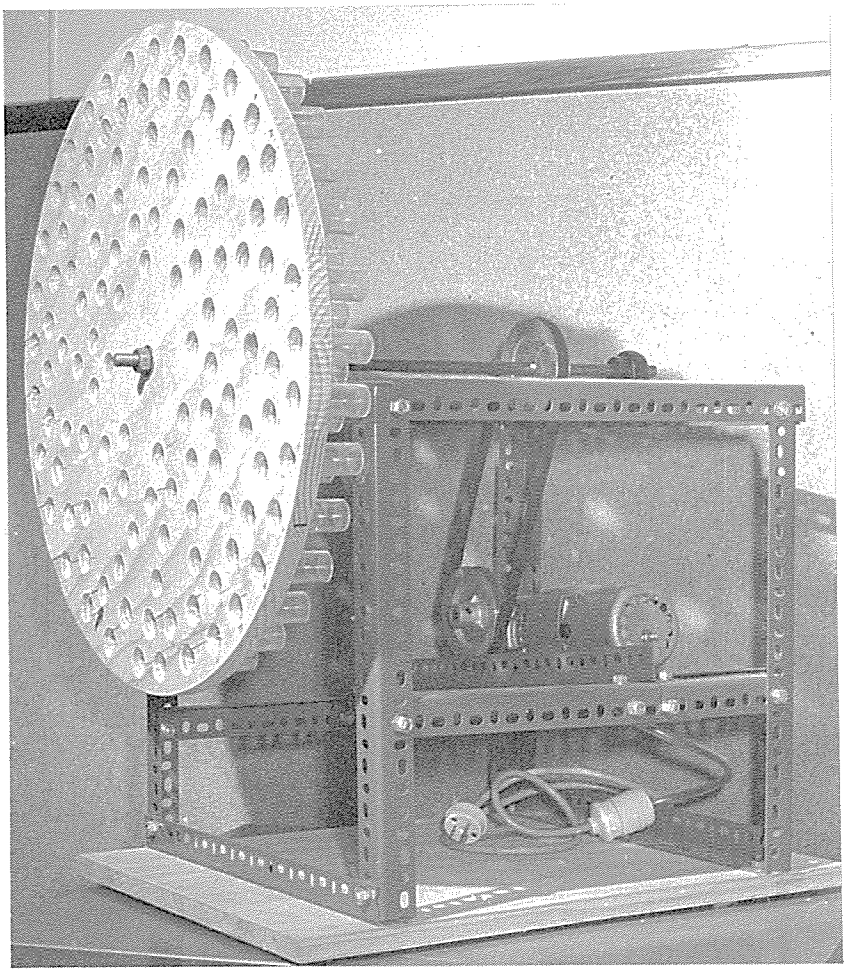
(ii) Seedlings were harvested 90-96 hours after planting, at which time they measured 2.5-3.5 cm in length; the coleoptile being about 0.5 cm long. Seedlings were selected about 3 cm long, placed on the cutting block, (Figure 5), and first internode sections, 5 mm long, were cut 2 mm from the coleoptilar node.

(iii) Sections were floated on glass distilled water for 1 hour (Nitsch and Nitsch 1956).

(iv) Five sections were placed in each pyrex tube which contained 2 ml of buffer (pH 5.0) in 2% sucrose (Appendix I) and the chromatographic strips.

(v) Tubes were rotated at 1 r.p.m. for 20 hours in the dark. (The bioassay machine (Figure 6) was constructed in similar fashion to one at F.R.D. Auckland).

Figure 6. The bioassay machine



Frame dimensions, 18 x 18 x 18". Turntable, 12" diam.

Plastic vial inserts, 1 x 2". Motor geared to 1 r.p.m.

(vi) At the completion of rotation, the sections were killed with the addition of ethyl alcohol and measured against a mm scale with the use of a binocular microscope (x 10.8).

IV. Standard Curve

A standard curve was constructed using auxin (IAA) concentrations in the range 5×10^{-5} - 1×10^{-9} M. The stock solutions (5×10^{-4} M) were made up in distilled water, stirred for 4 hours at 30°C, and stored in the dark at 5°C. Dilutions from the stock solution were made using buffer (pH 5.0) in 2% sucrose and used immediately.

E. HORMONE INTERACTION WITH ALAR ON AVENA FIRST INTERNODE TISSUE

Gibberellin (GA_3) and auxin (IAA) interaction on Avena tissue, grown in the presence of Alar, was investigated using techniques previously described for auxin bioassays.

(i) Oat seeds cv Garton were imbibed (in the dark) for 2 hours in water, 2000 or 4000 ppm Alar. Seeds were then planted in pumice moistened with water (or appropriate Alar solutions) and grown as previously described.

(ii) Seedlings were grown for 105.5 hours and sampled (as for the bioassay). After sampling, seedlings were grown for a further 14.5 hours and total length measured to assess the degree of retardation.

(iii) Factorial combinations of auxin (5×10^{-7}, 5×10^{-5}) gibberellin (1×10^{-7}, 1×10^{-5}) and Alar (2000, 3000, 4000 ppm) were added to pyrex tubes and incubated with 5 sections per tube for 24 hours. Auxin and Alar solutions were made up in buffer (with 2% sucrose) (Nitsch and Nitsch 1956); gibberellin solutions were made up in distilled water. In each case 0.7 ml of buffer, hormone, or retardant were added to each tube to give a total volume of 2.1 ml.

(iv) Sections were killed and measured as previously described.

Chapter 3

THE EFFECT OF ALAR TREATMENT ON APPLE SHOOT GROWTH

Figure 7a. The effect of Alar treatment on Gravenstein shoot growth.
(Left to right; 4000, 3000, 2000, 1000 ppm Alar and control).

Chapter 3

THE EFFECT OF ALAR TREATMENT ON APPLE SHOOT GROWTH

A. SHOOT PHOTOGRAPHY

Shoot growth of Gravenstein apple trees was severely reduced by Alar treatment (Figure 7A). The reduction in shoot length is a reflection of retarded internode elongation as all shoots photographed had an equal number of internodes. Further more, the degree of retardation is related to, but not proportional to, Alar concentration (Table 1).

TABLE 1

	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
Total internode length (cm)	70.3	52.9	50.1	38.4	28.0
Reduction in length (%)		24.7	28.7	45.3	60.1

Alar treatment increased foliage density, an effect due to an increased number of leaves per unit length (Figure 7A). It should be noted, however,

Figure 7b. Comparison between initial and final shoot length after Alar treatment.
(Left to right; 4000, 3000, 2000, 1000 ppm Alar and control).

that the uneven appearance in leaf distribution of photographed shoots was caused by wind damage between 7/11/67 - 14/11/67.

The reduction in internode length with Alar treatment is more effectively shown in Figure 7B. Comparison between initial and final shoot length (Table 2) substantiates the point that Alar treatment markedly decreases the growth of apple shoots.

TABLE 2

	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
Total internode length (cm) 24/10/67	5.98	5.32	5.49	4.28	4.41
Total internode length (cm) 12/2/68	70.3	52.9	50.1	38.4	28.0
Growth increase (cm)	64.32	47.58	44.61	34.12	23.59

B. THE EFFECT OF ALAR ON SHOOT NODE NUMBER

On any one harvest date, differences in shoot internode number occurred within treatments as well as between treatments. These differences can be attributed to the following:

(i) Sampling technique

Shoots selected from the upper sector, with a growth habit near the vertical plane, were more vigorous than those of the

TABLE 3

Total internode number from fifteen decapitated samples

Harvest Date	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
24/10/67	157	155	158	157	164
31/10/67	171	173	173	171	161
7/11/67	187	189	196	187	181
28/11/67	268	262	257	252	247
19/12/67	345	338	338	304	313
2/1/68	395	376	353	375	368

Total internode number from fifteen samples

Harvest Date	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
4/5/68	491	480	479	470	456

lower sector, which were more horizontal in aspect. The difference in growth rates explains internode differences between shoot samples of any one treatment.

(ii) Alar effects

With increasing Alar concentration, internode and leaf expansion were progressively delayed. Delayed leaf expansion reduced the number of possible internodes to the point of decapitation. Furthermore, Alar treatment reduced internode number (Table 3). This effect became apparent about 19/12/67 and is clearly shown in results from the harvest on 5/4/68.

For these reasons, results of every internode measurement from each treatment have not been presented. The lowest internode number recorded for any of the fifteen shoot samples was assessed for each treatment. The lowest internode number from each of the five treatments of any one harvest date was taken as an arbitrary level, below which all treatments would consist of an equal number of measurements.

C. THE EFFECT OF ALAR TREATMENT ON SHOOT INTERNODE LENGTH

In order to equate the variability in internode length between the fifteen individual shoots of any one treatment, the mean internode length was assessed for each internode number. Graphical expression of these mean values (corrected to the second decimal place) for each harvest date showed that Alar-induced retardation of internode length

Figure 8. Internode length response to Alar treatment from shoots harvested on 24/10/67.

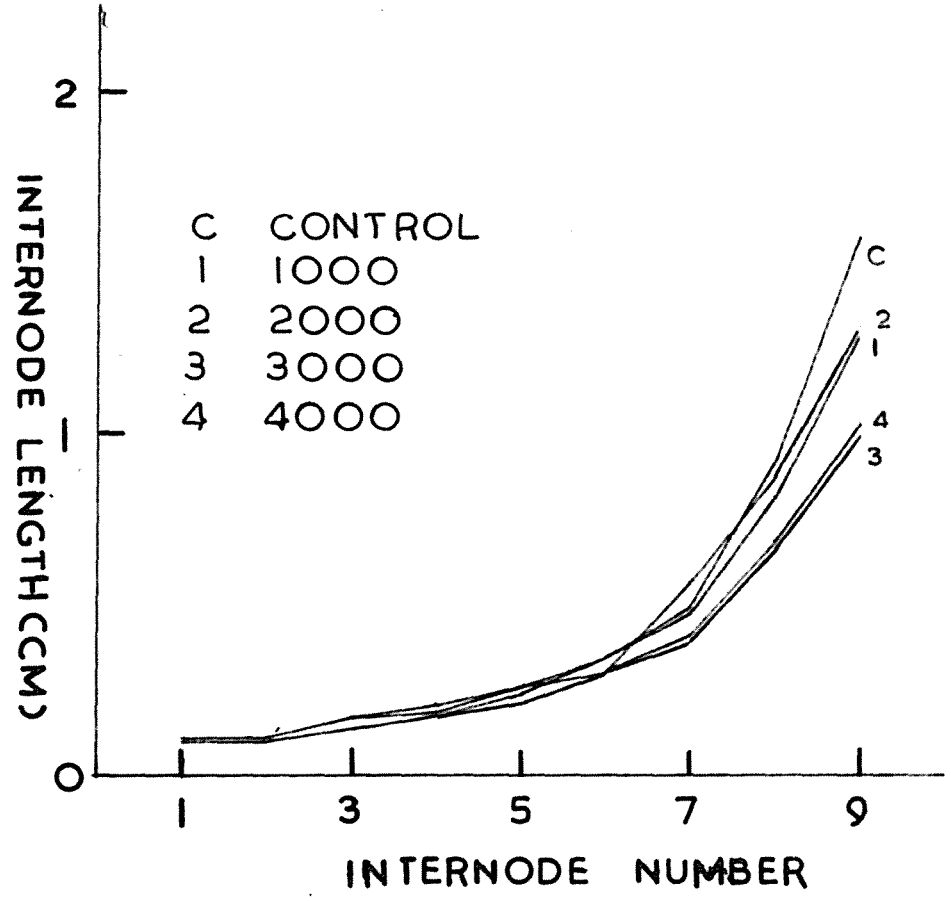


Figure 9. Internode length response to Alar treatment from shoots harvested on 7/11/67.

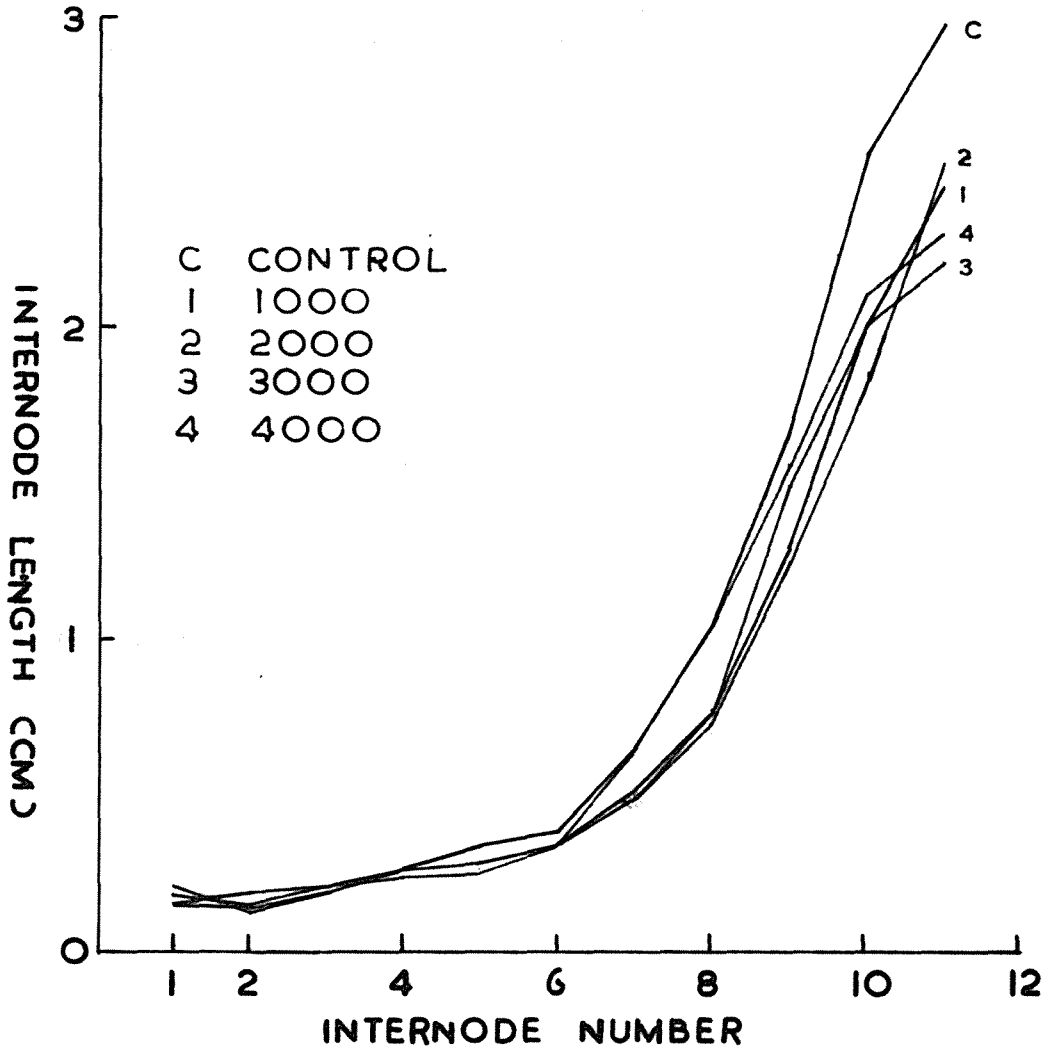


Figure 10. Internode length response to Alar treatment from shoots harvested on 28/11/67.

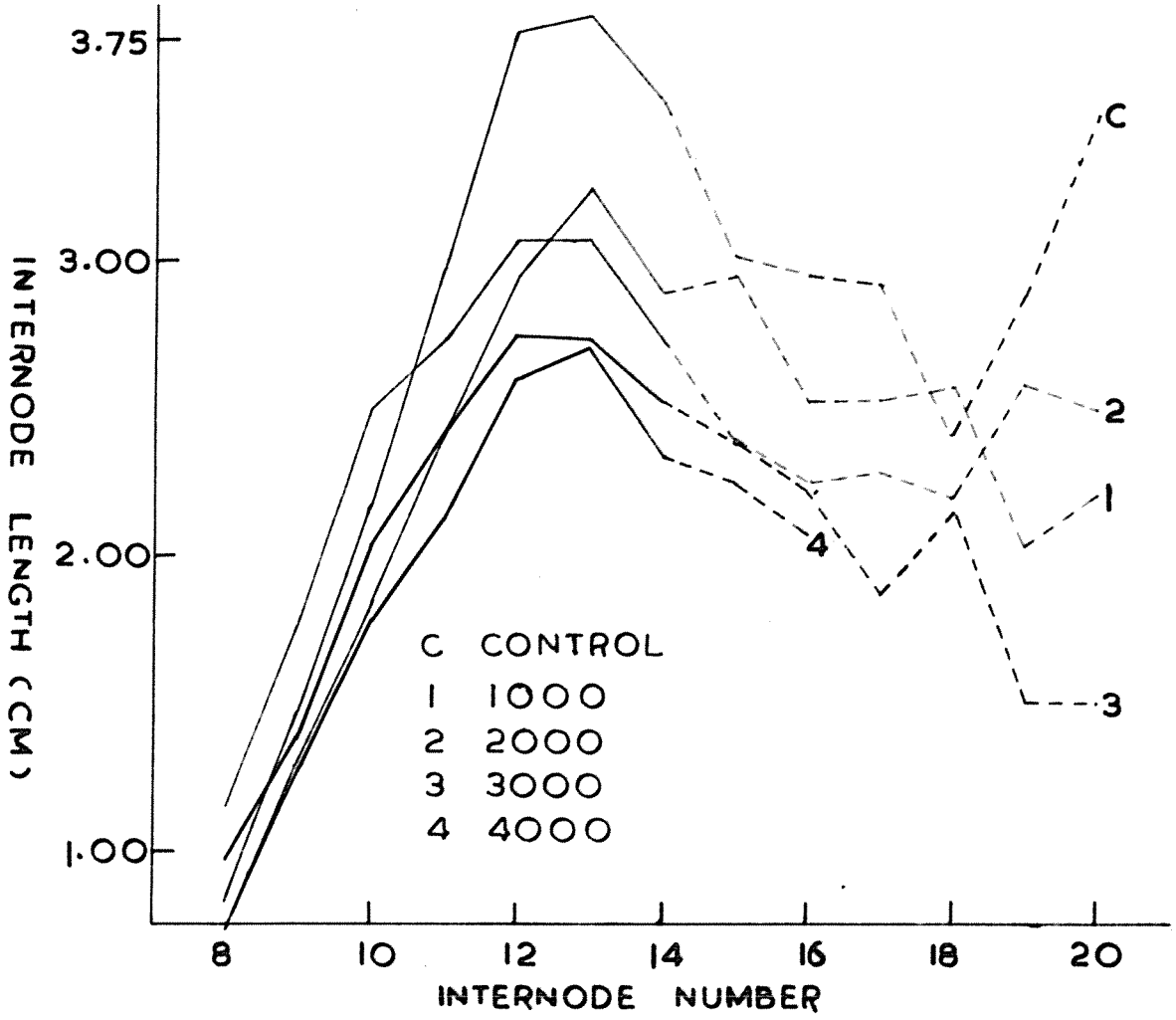


Figure 11. Internode length response to Alar treatment from shoots harvested on 19/12/67.

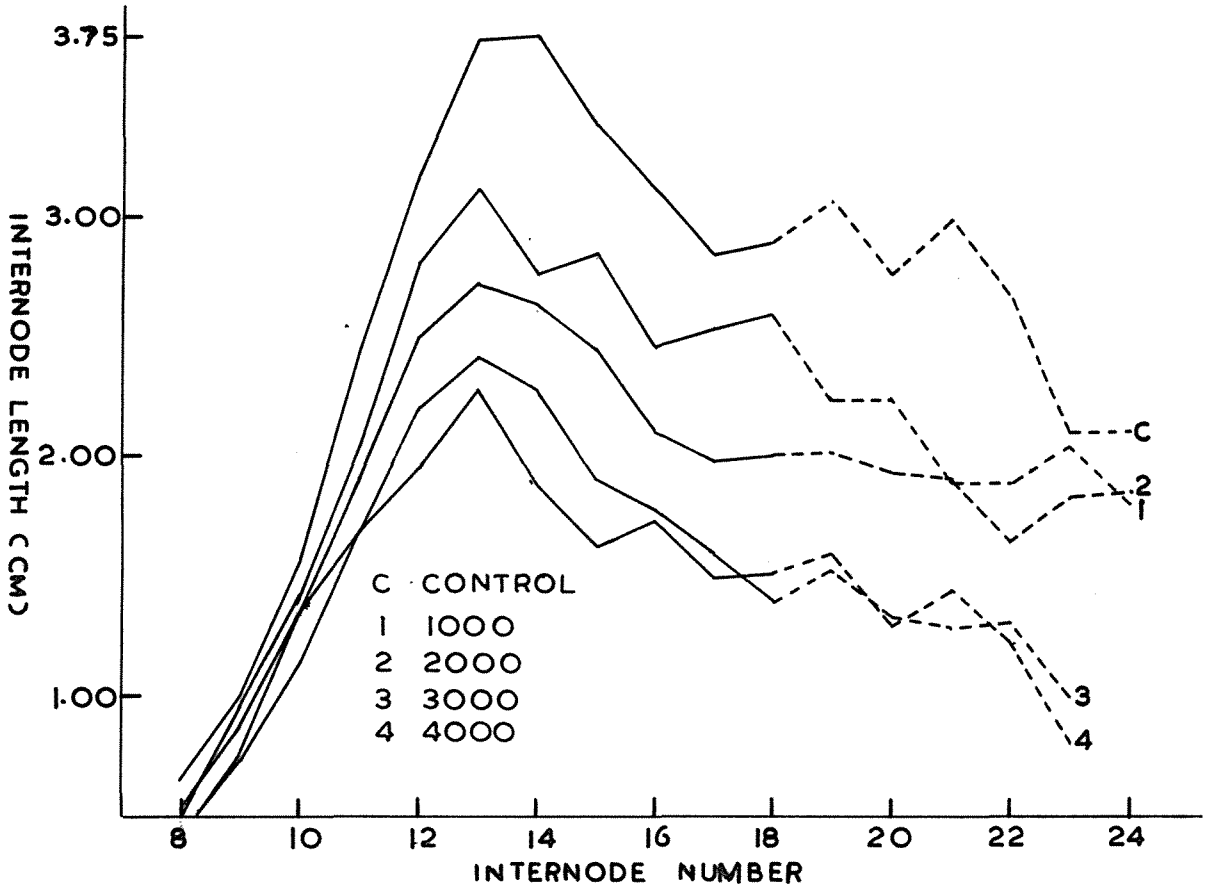
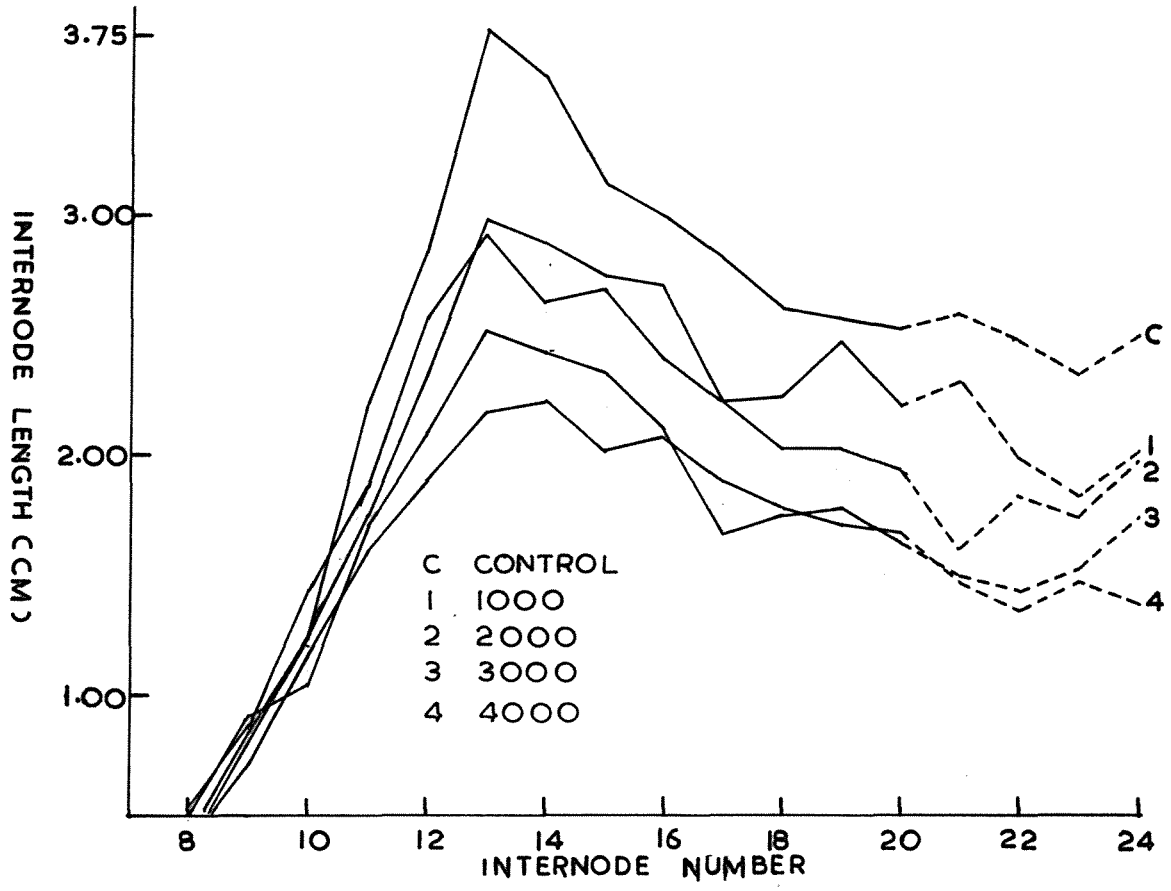


Figure 12. Internode length response to Alar treatment from shoots harvested on 2/1/68.



exhibited the same overall pattern in each case. Accordingly, only those graphs which are considered to illustrate the pattern of Alar retardation are included. Tables of mean internode lengths used to construct graphs (Figures 8, 9, 10, 11 and 12) can be found in Appendix II.

The plot of mean internode length versus internode number (Figure 8-12) showed similar sigmoid growth curves for each harvest date. Little internode elongation occurred between internodes 1-6. Internode elongation becomes apparent after the 7th internode and increases rapidly to a peak which occurred at internode 13 for all but two treatments in harvests after 21/11/67. The slope of this area of the curve tended toward linearity. Comparison between treatments for any internode number shows, in the majority of harvests, that increasing Alar concentration decreased internode length. Variable results are shown to occur between internodes 7-9 and it is probable that these internodes had begun to elongate before the Alar treatment. Furthermore, Luckwill et al. (1965) reported that the effect of Alar treatment on apple shoot growth became apparent 2-3 weeks after spraying. It would appear, then, that a lag period occurs between application and observable effects. However, comparison between individual internodes from the same treatment, but from successive harvests, shows that internode length apparently decreases during the 7 day interval.

Exceptions to this apparent decrease can be shown (as for 3000 ppm treatment Figure 9; 3000 and 4000 ppm treatments Figure 12) but in most cases the observed decrease in internode length with time holds true. This decrease in internode length can be attributed to progressive harvesting of terminal shoot growth from the same experimental trees and comparing

TABLE 4

Mean internode length (cm)

Harvest Date		Alar Treatment (ppm)				
		Control	1000	2000	3000	4000
24/10/67	Total	4.04	3.74	3.87	3.05	3.21
	% Reduction	-	7.42	4.20	24.50	20.54
	S.D.	-	N.S.	N.S.	*	*
31/10/67	Total	4.16	4.44	4.71	4.66	4.43
	% Reduction	-	-	-	-	-
	S.D.	-	N.S.	N.S.	N.S.	N.S.
7/11/67	Total	10.44	8.42	8.25	8.30	9.01
	% Reduction	-	19.34	20.97	20.49	13.69
	S.D.	-	*	*	*	N.S.
28/11/67	Total	20.32	16.74	18.76	16.34	14.96
	% Reduction	-	17.61	7.67	19.58	26.37
	S.D.	-	N.S.	N.S.	*	*
19/12/67	Total	29.78	25.05	21.82	18.48	17.97
	% Reduction	-	15.88	26.72	37.94	39.65
	S.D.	-	N.S.	*	*	*
2/1/68	Total	32.60	26.55	27.24	23.52	22.19
	% Reduction	-	18.55	16.44	27.85	31.93
	S.D.	-	N.S.	N.S.	*	*
12/2/68	Total	70.3	52.9	50.1	38.4	28.0
	% Reduction	-	24.7	28.7	45.3	60.1
	S.D.	-	N.S.	*	*	*

S.D. = Denotes significant differences at the 5% level about the treatment means.

N.S. = Not significantly different from the control means.

* = Significantly different from the control means.

individual internode lengths with those of preceding harvests.

Constant removal of the more vigorous terminal shoot growth in the early harvests meant that shoot harvests in the latter part of the season comprised of shoots which, in many cases, had been derived from terminal buds which had "burst" in the late spring. Such shoots were not as vigorous as those from the leader tips and, to confound the effect, the exceptionally dry season opens the possibility of late developing shoots suffering water deficits.

When comparisons between treatments of individual harvests are made the effect of Alar treatment becomes obvious. Total internode length was calculated from mean values for each internode (Appendix II) and the results are presented in Table 4. The percent reduction in growth was calculated for each harvest date and an analysis of variance put across mean values (as presented in Appendix II).

When significant differences were detected between treatments the significant difference at the 5% level was calculated for treatment means. Significant differences between means, when they occurred, are shown in Table 4. Results from harvest dates 24/10/67 - 7/11/67 show no constant trends. This is not surprising in view of the fact that only the first 9 and 11 internodes respectively were considered in the analysis of variance.

For harvest dates 28/11/67, 19/12/67, 2/1/68, significant differences at the 5% level were detected between control means and 3000, 4000 ppm Alar treatment means. The non-significant difference detected between control means and 1000 ppm Alar treatment means appears to be true of all harvest dates from 28/11/67. Further support on this

TABLE 5

Average leaf area (x 10 cm^2) from five shoot samples

Harvest Date	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
24/10/67					
Total	17.12	18.02	15.04	16.17	15.94
Mean	3.42	3.60	3.00	3.23	3.18
31/10/67					
Total	21.69	19.99	17.84	19.38	21.70
Mean	4.33	3.99	3.56	3.87	4.34
7/11/67					
Total	20.58	20.79	20.65	19.20	21.89
Mean	4.11	4.15	4.13	3.84	4.37
14/11/67					
Total	23.95	18.87	20.63	18.33	22.05
Mean	4.79	3.77	4.12	3.66	4.41
21/11/67					
Total	18.69	19.37	19.75	19.85	17.32
Mean	3.73	3.87	3.95	3.97	3.46
28/11/67					
Total	20.44	19.89	20.78	19.69	20.21
Mean	4.08	3.97	4.15	3.93	4.04
5/12/67					
Total	20.29	21.14	19.81	18.29	20.80
Mean	4.05	4.22	3.96	3.65	4.16
12/12/67					
Total	19.37	19.79	20.62	19.36	19.66
Mean	3.87	3.95	4.12	3.87	3.93
19/12/67					
Total	20.60	18.46	17.95	18.50	20.37
Mean	4.12	3.69	3.59	3.70	4.07
2/1/68					
Total	15.31	15.46	16.85	15.94	15.02
Mean	3.06	3.09	3.37	3.18	3.00

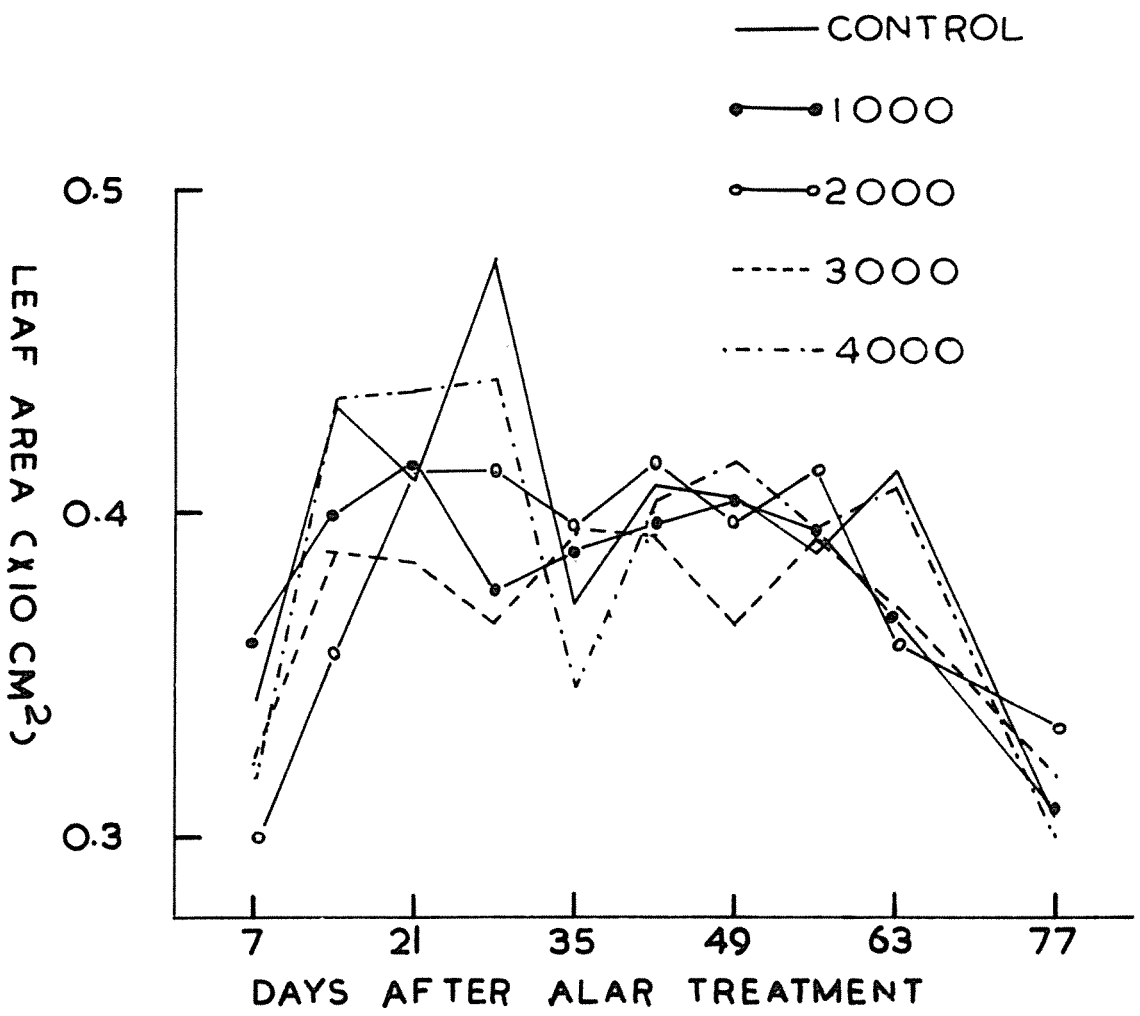
point is shown when data of internode measurements from photographed shoots (Harvest date 12/2/68) are tested for variance (Appendix II). However, it should be noted that these results have been derived from only one sample in each treatment. Analysis of 2000 ppm Alar treatment means shows somewhat conflicting results. Depending on harvest date selection the mean internode length either becomes significantly different or non-significantly different from control values.

Comparison between control and Alar treated shoots (2000 ppm) (Figures 10, 11 and 12) for internode numbers greater than those selected for analysis, indicates that if more internode measurements had been included in the determination of variance a probable difference would have been obtained. This point gains some support from results obtained from analysis of harvest date 12/2/68.

D. THE EFFECT OF ALAR TREATMENT ON SHOOT LEAF AREA

Leaf number differed between samples of any one treatment (Section B). Accordingly, total leaf area of each shoot was divided by the leaf number for that shoot and the value obtained expressed as the average leaf area per sample. Wind damage to leaf laminae (Figure 7A) on some shoot samples from harvests after 7/11/67 was so extensive that leaf area measurements were not attempted. As a result, treatments after 7/11/67 contained a variable number of leaf area measurements. In order to express leaf area results in a form which would lend itself to statistical analysis, the first five leaf area

Figure 13. Leaf area response with time from Alar application.



recordings from any one treatment were selected for presentation (Appendix II). The average leaf area from each of the 5 shoot samples was totalled (Appendix II) and the results presented in Table 5. From these results the mean leaf area was calculated for each treatment (Table 5) and expressed graphically (Figure 13).

From Table 5 and Figure 13 it can be seen that, after an initial increase, the mean leaf area per shoot reaches a more or less stable level. During the early stages of shoot growth the magnitude of the mean leaf area shows more marked fluctuations than results obtained from later measurements.

In order to test for differences between treatments, analyses of variance were put across results from 14/11/67, 5/12/67, 19/12/67, and 2/1/68 harvest dates. The harvest dates tested represented one showing the most marked difference between treatments (14/11/67), the final harvest date (2/1/68) and two intermediate points. In all cases, no significant differences were obtained between treatments of any one harvest. It can be concluded, then, that Alar treatment of apple shoots does not significantly affect leaf area.

E. THE EFFECTS OF ALAR TREATMENT ON SHOOT DRY WEIGHT

I. Shoot Dry Weight

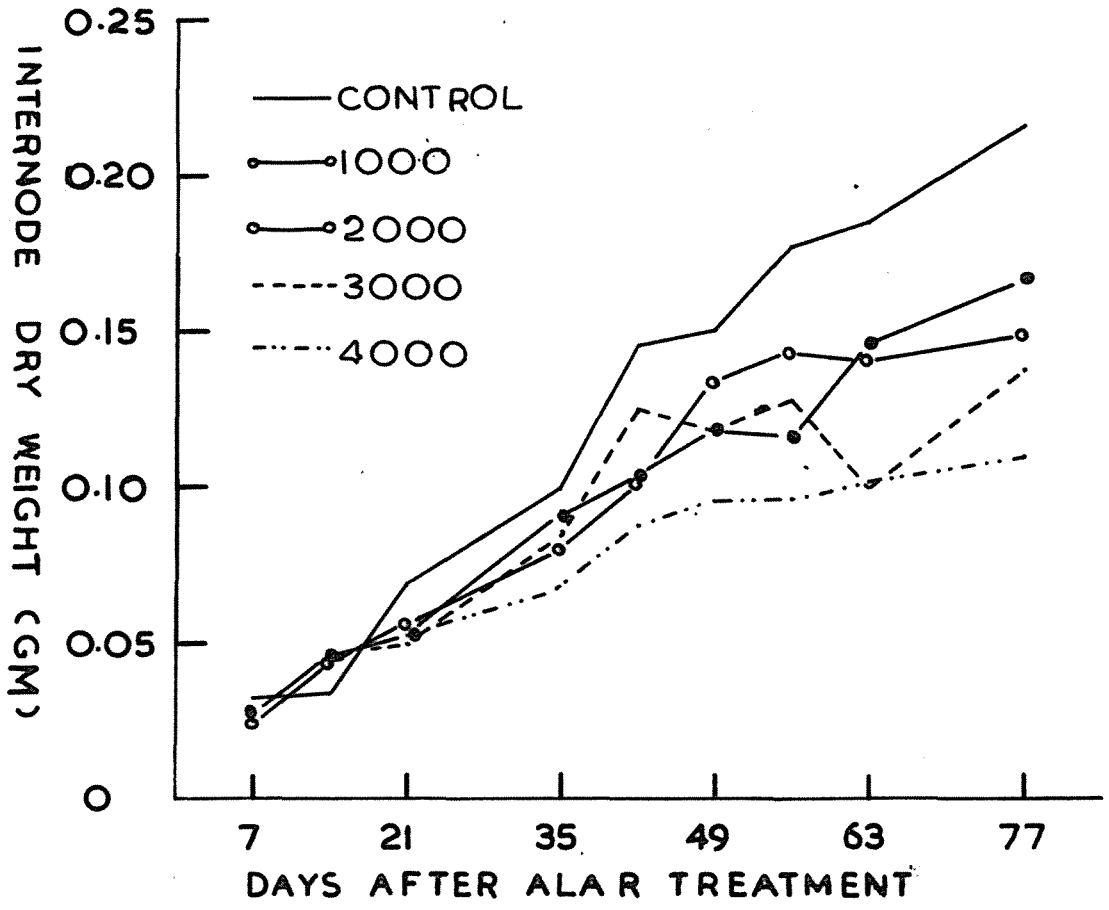
Results from dry weight measurements of the 15 shoot samples of any one treatment are presented in Table 6. For reasons previously outlined, the total dry weight has been divided by the total internode

TABLE 6

Dry weight (gm) from fifteen shoot samples

Harvest Date	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
24/10/67					
Dry weight	5.04	4.12	3.93	4.29	3.88
Total internode No.	157	155	158	157	164
Average internode weight	0.032	0.027	0.025	0.027	0.024
31/10/67					
Dry weight	5.70	7.90	7.57	7.65	7.65
Total internode No.	171	175	173	171	161
Average internode weight	0.033	0.046	0.044	0.045	0.048
7/11/67					
Dry weight	12.83	9.82	11.01	9.39	9.50
Total internode No.	187	189	196	187	181
Average internode weight	0.069	0.052	0.056	0.050	0.052
21/11/67					
Dry weight	22.56	20.27	17.79	20.09	14.97
Total internode No.	226	222	222	231	220
Average internode weight	0.099	0.091	0.080	0.086	0.068
28/11/67					
Dry weight	38.89	27.10	25.90	31.61	21.37
Total internode No.	268	262	257	252	247
Average internode weight	0.145	0.103	0.101	0.125	0.087
5/12/67					
Dry weight	44.38	33.42	37.01	34.00	26.49
Total internode No.	295	283	278	288	276
Average internode weight	0.150	0.118	0.133	0.118	0.095
12/12/67					
Dry weight	57.97	36.17	46.06	38.80	28.98
Total internode No.	326	310	320	305	301
Average internode weight	0.177	0.116	0.143	0.127	0.096
19/12/67					
Dry weight	63.72	49.47	47.44	30.20	31.69
Total internode No.	345	338	338	304	313
Average internode weight	0.185	0.146	0.140	0.099	0.101
2/1/68					
Dry weight	85.51	62.37	52.33	51.42	40.47
Total internode No.	395	376	353	375	368
Average internode weight	0.216	0.166	0.148	0.137	0.110

Figure 14. Internode dry weight (average) with time from Alar application.



number of the 15 dried shoots. Graphical expression of average internode dry weight (Figure 14) shows that dry weight decreases with increasing Alar concentration. This effect is more marked with time. When dry weights and internode length from harvest date 2/1/68 are compared (Table 7) a decrease in internode dry weight per unit length occurs with increasing Alar concentration.

TABLE 7

	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
Average internode length (cm)	1.63	1.32	1.36	1.17	1.10
Average internode weight (gm)	0.21	0.16	0.14	0.13	0.11
Dry weight/unit length (gm/cm)	0.128	0.121	0.102	0.111	0.100

This leads some support to the observation that stem diameter decreased with increasing Alar concentration although no direct measurements were made on fresh shoot tissue.

II. Leaf Dry Weight

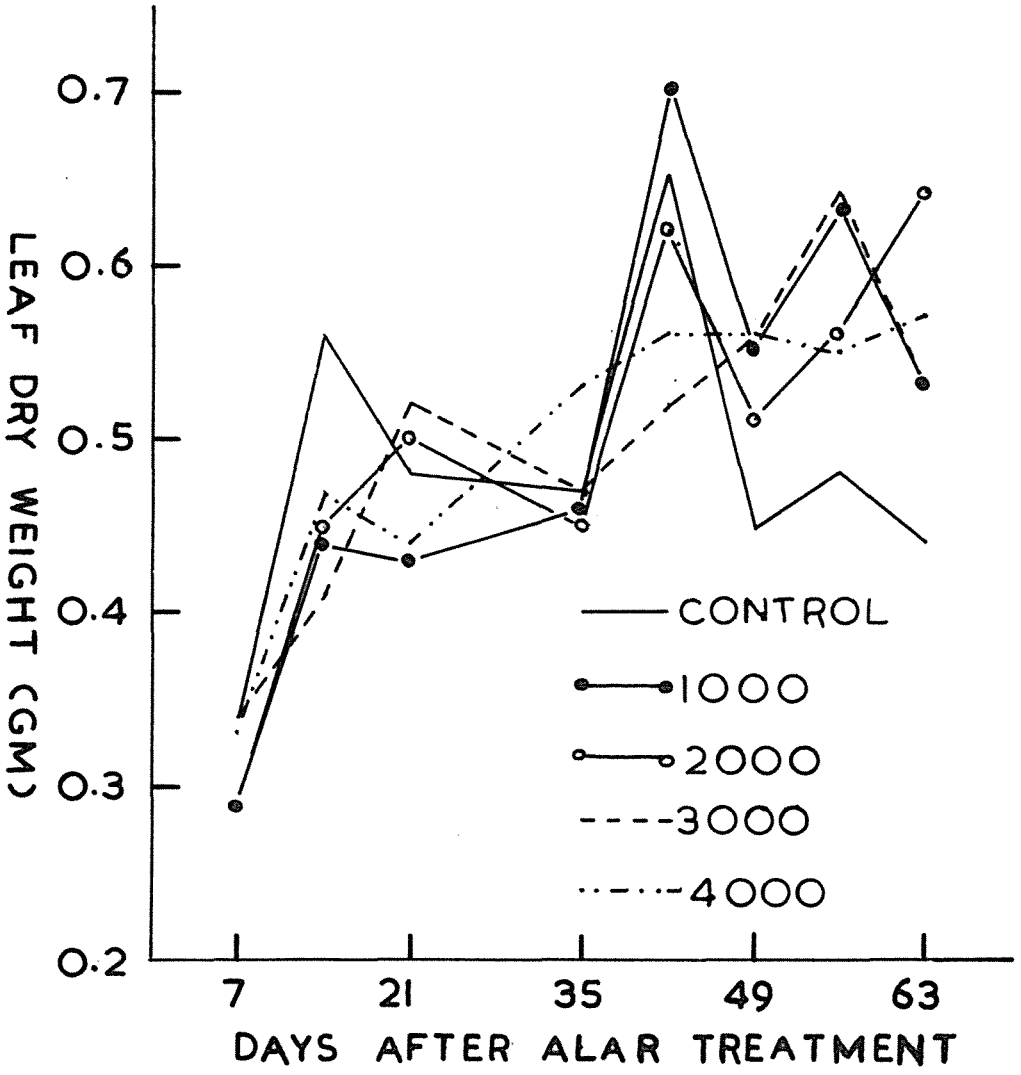
The dry weight of 30 randomly selected leaves from each treatment (wind damage in later harvests precluded the possibility of a larger sample) was assessed, the mean dry weight calculated, (Table 8) and

TABLE 6

Mean 1st 1 day weight (gm) from entire leaf samples

Variates	Mid Treatment (ppm)				
Date	Control	1000	2000	3000	4000
14/10/87	0.34	0.29	0.25	0.34	0.33
21/10/87	0.44	0.44	0.49	0.41	0.47
27/10/87	0.46	0.43	0.36	0.36	0.44
04/11/87	0.47	0.46	0.43	0.47	0.39
10/11/87	0.62	1.20	0.82	0.36	0.36
17/11/87	0.4	0.58	0.54	0.40	0.36
24/11/87	0.46	0.44	0.36	0.36	0.33
01/12/87	0.44	0.39	0.04	0.39	0.31

Figure 15. Leaf dry weight (average) with time from Alar application.



results expressed graphically (Figure 15). From this graph it can be seen that leaf dry weight from control shoots is initially greater than that of Alar treated shoots. This response does not hold true for all harvest dates. Leaf dry weight from Alar treated shoots eventually becomes greater than the leaf dry weight of control shoots (Harvest dates, 5/12/67, 12/12/67, 19/12/67). However, no constant relationships can be shown between various Alar treatments from these later harvest dates. Increased leaf dry weight does not appear to be proportional to increasing Alar concentration.

Leaves from 4000 ppm Alar treatments and, to a lesser extent, leaves from 3000 ppm Alar treatments show a gradual increase in dry weight accumulation with time. A similar relationship cannot be shown for other treatments. From a comparison between leaf dry weights from control and Alar treated shoots it can be concluded that the retardant increases leaf dry weight and that the increase is not apparent until the 49th day after application.

III. Petiole Dry Weight

Petioles were removed from dried shoot samples, counted, and weighed. The results from each treatment are presented in Table 9. The average petiole weight of each treatment was calculated from the total dry weight in order to compensate for differences in node number between treatments of any one harvest date.

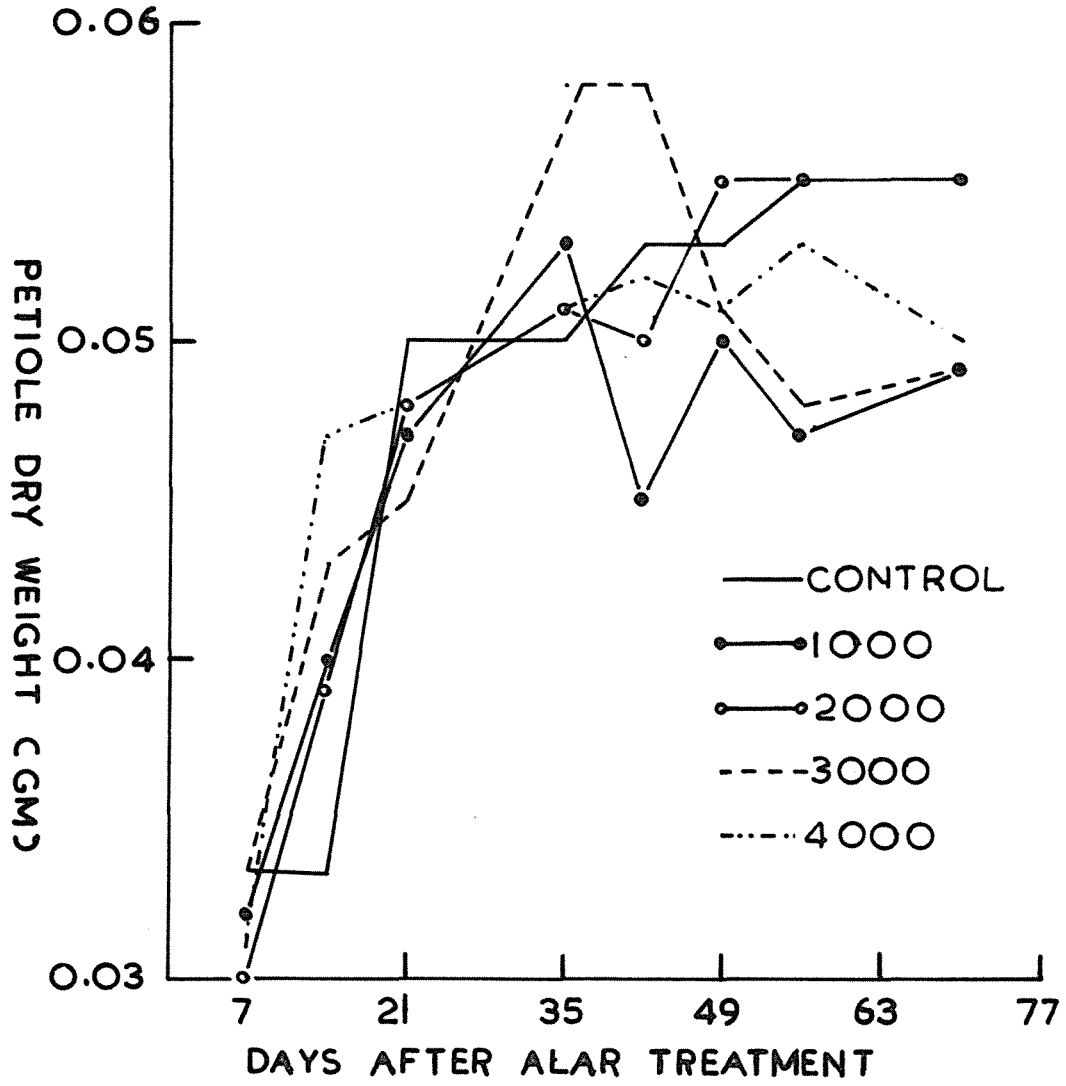
Graphical expression of these average petiole dry weights (Figure 16) shows that dry weight accumulation initially increases for all treatments and eventually reaches a near stable level.

TABLE 9

Petiole dry weight (gm) from fifteen shoot samples

Harvest Date	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
24/10/67					
Petiole weight	5.38	4.90	4.81	5.33	5.07
Petiole No.	157	155	158	157	164
Average petiole weight	0.034	0.032	0.030	0.034	0.031
31/10/67					
Petiole weight	5.81	6.90	6.82	7.33	7.58
Petiole No.	171	173	173	171	161
Average petiole weight	0.033	0.040	0.039	0.043	0.047
7/11/67					
Petiole weight	9.26	8.79	9.46	8.23	8.52
Petiole No.	187	189	196	184	177
Average petiole weight	0.050	0.047	0.048	0.045	0.048
21/11/67					
Petiole weight	10.66	10.59	10.23	11.98	9.53
Petiole No.	212	200	198	205	187
Average petiole weight	0.050	0.053	0.051	0.058	0.051
28/11/67					
Petiole weight	13.70	11.52	12.73	14.40	12.16
Petiole No.	257	255	256	248	232
Average petiole weight	0.053	0.045	0.050	0.058	0.052
5/12/67					
Petiole weight	13.68	12.08	13.62	12.94	11.61
Petiole No.	255	242	247	252	228
Average petiole weight	0.053	0.050	0.055	0.051	0.051
12/12/67					
Petiole weight	14.42	11.10	13.67	11.99	13.09
Petiole No.	258	237	247	246	244
Average petiole weight	0.055	0.047	0.055	0.048	0.053
26/12/67					
Petiole weight	15.90	14.01	15.81	12.97	13.01
Petiole No.	288	284	288	261	258
Average petiole weight	0.055	0.049	0.055	0.049	0.050

Figure 16. Petiole dry weight (average) with time from Alar application.



Comparison between treatments from harvests prior to 5/12/67 shows that petiole dry weights, other than those from the 3000 ppm Alar treatment, do not markedly differ from controls. Any effect of Alar treatment is lost among the fluctuations of petiole dry weight between harvest dates. No reason can be advanced at present to explain the anomalous results obtained for the 3000 ppm Alar treatment (Harvest dates 21/11/67 and 28/11/67). However, in harvest dates after 5/12/67, petiole dry weights from Alar treatments show a decrease when compared with controls. The decrease in petiole dry weight does not appear to be proportional to Alar concentration. In fact, the close parallel shown between results from control and 2000 ppm Alar treatments places considerable doubt on the conclusion that Alar treatment induces a decrease in petiole dry weight. However, it is also possible that the results from the 2000 ppm Alar treatments are not typical of the norm and that Alar does decrease petiole dry weight. Of these two possibilities it is considered that the latter is the more likely as anomalous results have been demonstrated for the 3000 ppm treatment.

F. EFFECTS OF ALAR TREATMENT ON FRUIT SIZE, WEIGHT, AND STORAGE

I. Fruit Photography

Fruit samples were selected from mature fruit from Block 2. These fruit had received only one Alar application 47 days after full bloom. It can be seen (Figure 17) that fruit colour is increased with increasing Alar concentration. However, these fruit had been

Figure 17. Fruit sub-sample selected from fruit harvested from Block 2.
(Left to right and from top to bottom; control, 1000, 2000, 3000, and 4000 ppm Alar).

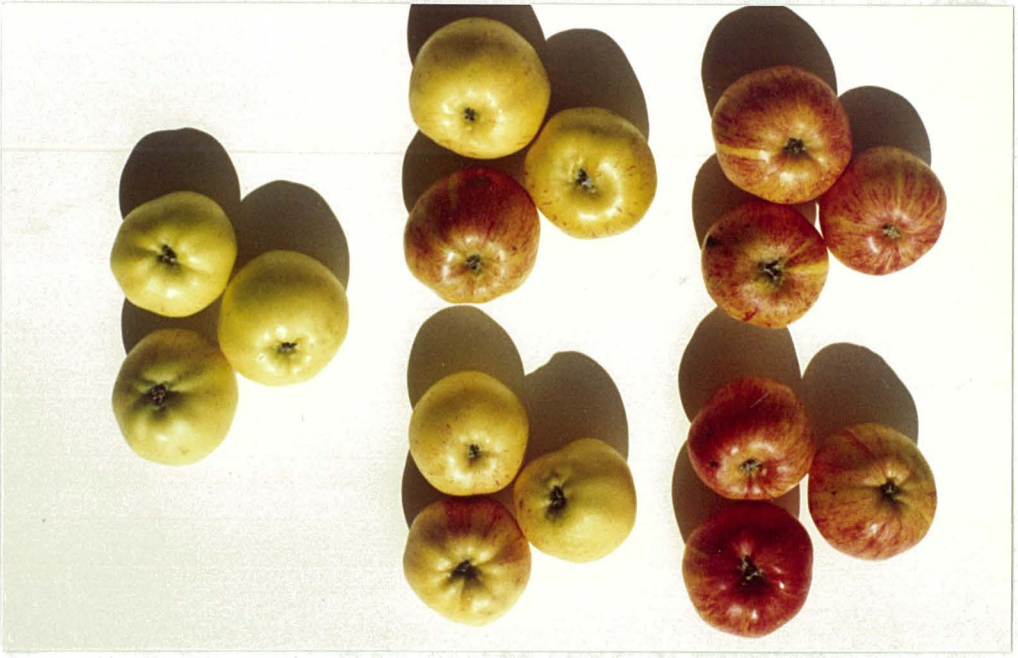
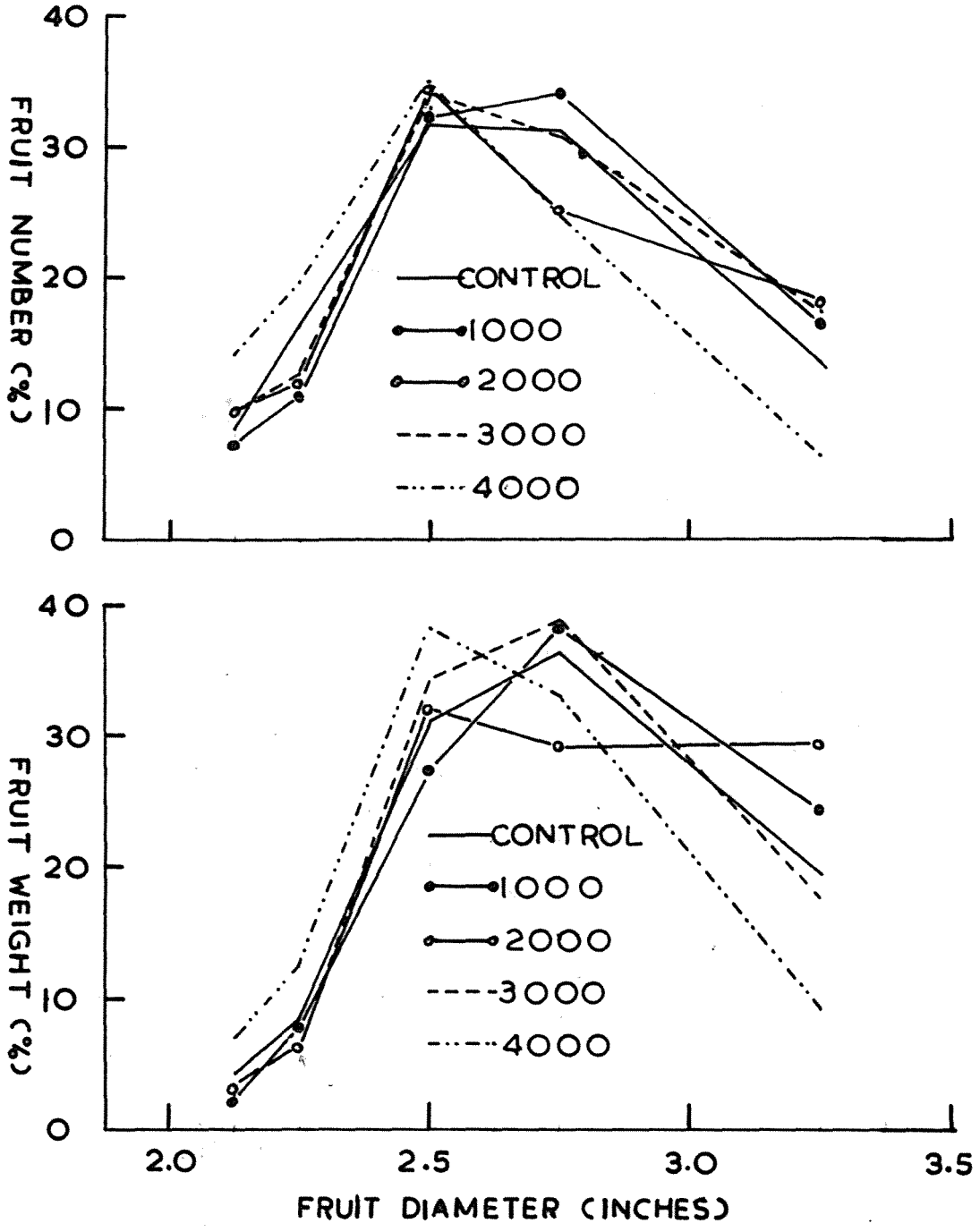


Figure 18. Size and weight of fruit with Alar treatment.



stored at under two temperature regimes and some colour developed during storage, especially the yellow-green background which tended to enhance fruit colour.

II. The Effects of Alar Treatment on Apple Fruit

Fruit were harvested at the point of "tree ripeness", graded into size distributions, and the weight of each size distribution recorded. In order to locate changes in size distribution and fruit weight, all results have been presented as a percentage of the total.

(a) Fruit size

Graphical expression of the percentage fruit number occurring within any one treatment (Figure 18) shows the effect of Alar treatment more clearly than in Table 10. With 1000 ppm Alar, the percentage number of small fruit (< 2.25" diameter), decreases when compared with controls. This effect is compensated for by an increase in the number of fruit falling in the large size distribution (> 2.75" diameter). In 4000 ppm treatments, an increased number of fruit falls in the small fruit range (< 2.50" diameter) while a sharp reduction occurs in the larger fruit range (> 2.75" diameter). The 2000 and 3000 ppm Alar treatments fill the intermediate range between these two extremes. From these results it can be concluded that Alar treatment, at low levels (1000 ppm), increases the percent number of large fruit, while at higher levels (4000 ppm) a decrease in the percent number of large fruit occurs with a concomitant increase in the number of small fruit.

(b) Fruit weight

Results from fruit weight measurement (Table 10) show that

TABLE 10

Fruit number and weight (oz) from Alar treated apple trees

	Alar treatment (ppm)																			
	Control				1000				2000				3000				4000			
Fruit diameter (inch)	Fruit No.	%	Fruit weight (oz)	%	Fruit No.	%	Fruit weight (oz)	%	Fruit No.	%	Fruit height (oz)	%	Fruit No.	%	Fruit weight (oz)	%	Fruit No.	%	Fruit weight (oz)	%
2.125	34	8.39	65	4.39	50	7.07	60	2.12	51	9.96	63	3.22	40	9.70	45	3.03	119	14.25	203	6.90
2.125 - 2.25	66	16.29	125	8.45	76	10.74	222	7.87	62	12.10	124	6.34	53	12.86	93	6.26	165	19.76	373	12.69
2.25 - 2.50	128	31.60	460	31.12	227	32.10	775	27.49	177	34.57	625	31.98	140	33.98	507	34.14	289	34.61	1122	38.18
2.50 - 2.75	122	30.12	539	36.46	239	33.80	1076	38.16	129	25.19	570	29.17	127	30.82	575	38.72	208	24.91	970	33.01
2.75 - 3.25	55	13.58	289	19.55	115	16.26	686	24.33	93	18.16	572	29.27	52	12.62	265	17.84	54	6.46	270	9.18
Total	405		1478		707		2819		512		1954		412		1485		835		2938	

Alar treatment induces considerable changes in fruit weight within any one size classification (Figure 18). Comparison between treatments indicates that at 1000, 2000, 3000 ppm Alar there is little change in fruit weight from controls for the small size ranges (< 2.25" diameter). At 4000 ppm, Alar increases fruit weight within this size distribution.

When the larger fruit size ranges are considered it can be seen that at 1000 and 2000 ppm Alar fruit weight is increased; this increase in fruit weight is most pronounced at 2000 ppm. In contrast, 4000 ppm Alar causes a marked reduction in fruit weight over the same size range, while Alar at 3000 ppm results in fruit weights which are intermediate between the two extremes and, as such, compares with the control.

III. Fruit Storage

Fruit from the size distribution 2.50-2.75 inches diameter were assessed for quality after a period of storage at two temperature levels (Table 11). Due to a misunderstanding, some fruit from the 1000, 2000 and 4000 treatments were lost from storage and, as a consequence, fruit numbers for the size distribution do not exactly correspond between Tables 10 and 11. In order to determine weight loss in storage, the mean fruit weight of each of the original weighings were calculated and then multiplied by the number of fruit in each of the storage treatments. The loss of fruit weight in storage was determined by subtraction of the final weighing from the calculated harvest weight. In all Alar treatments except for 2000 ppm the loss in fruit weight was greater than that of the controls. This loss was most pronounced at 1000 and 4000 ppm.

TABLE 11

Apple fruit quality after storage - fruit from size distribution 2.50-2.75 inches

Alar treatment (ppm)	Sample No.	Sample weight (oz)	Weight loss in storage (oz)	% Bruised	% Soft	% Mouldy core	% Bitter pit	% Undamaged
Control	120	485	46	9.2	1.7	17.5	1.7	70.0
1000	98	383	59	12.2	-	9.2	4.1	74.5
2000	92	377	30	4.3	-	6.5	4.3	84.8
3000	126	524	48	5.6	0.8	12.7	3.2	77.8
4000	119	482	73	0.8	-	21.0	-	78.2

In comparison to controls, the percentage of bruised fruit was considerably reduced in 2000, 3000, 4000 ppm Alar treatments, the effect being most pronounced at 4000 ppm. However, fruit from 1000 ppm Alar treatments showed an increased percentage of bruised fruit. Soft fruit were only detected in control and 3000 ppm treatments and on a percent basis the controls contained twice as many soft fruit as did the 3000 ppm treatment.

All treatments yielded some fruit in which the disease "mouldy core" was present. Fruit from 1000, 2000 and 3000 ppm Alar treatments exhibited lower levels of this disease than controls. However, a promotion in the incidence of "mouldy core" was found in fruit from the 4000 ppm treatment. The converse was true of the incidence of the physiological disorder "bitter pit". Fruit from 1000, 2000 and 3000 ppm Alar treatments had a considerably greater percentage of infected fruit than controls. No evidence of bitter pit was shown in fruit from the 4000 ppm Alar treatments.

When compared with controls an increased number of fruits which were commercially acceptable was shown for all Alar treatments. The 2000 ppm treatment exhibited the greatest increase; the 1000 ppm treatment, the least.

G. ANTHOCYANIN DETERMINATIONS

Anthocyanin levels were determined in fruit peel taken from photographed fruit (Figure 17). Extracted anthocyanins were related to a standard curve of known anthocyanin content (Figure I, Appendix III)

TABLE 12

Anthocyanin levels extracted from photographed fruit

	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
Mean surface area of peel $(cm)^2$	1250	1276	1576	1296	1383
Final extract volume (ml)	50	50	50	50	50
Optical density change/ml	0.009	0.022	0.029	0.143	0.087
mg cyanidin chloride/apple	0.100	0.240	0.315	2.0	0.95
mg cyanidin chloride/1000 cm^2	0.08	0.18	0.20	1.54	0.68

and the amount of cyanidin chloride per 1000 cm^2 of peel was assessed (Table 12).

It can be seen from results presented in Table 12 that anthocyanin levels increase with increasing Alar concentration. The optical density change recorded for the 3000 ppm treatment fell outside the range of the standard curve and has been estimated. It can be concluded that the increased fruit colour achieved with Alar treatment (Figure 17) is a direct reflection of increased anthocyanin formation in the fruit skin and this increase can be shown to occur in fruit which were well developed at the time of application.

H. DISCUSSION

The degree of Alar induced retardation of Gravenstein apple shoots was related to, but not proportional to, Alar concentration. This finding is in agreement with Batjer et al. (1964). Alar induced suppression of shoot elongation is primarily a function of retarded internode elongation. This effect is well documented in the literature, not only for apple but also for a wide range of plant species. However, a secondary effect was shown to occur. Node number did not approach ultimately that of control shoots as has been previously reported in the literature. Decreased node number occurred with increasing Alar concentration (Table 3) and this effect became more apparent with time from application. The only reason that can be suggested is that the longer growing season under New Zealand conditions allows more time for Alar, or its metabolic by-products,

to react with the apical meristem. This effect may not become apparent under short growing seasons of the northern hemisphere.

Reductions in shoot growth with 3000 and 4000 ppm Alar were significantly different (at the 5% level) from controls. No significant differences were detected between controls and 1000 ppm Alar. With 2000 ppm Alar treatments, both significant and non-significant differences were obtained with respect to controls. However, a significant difference was obtained between control and 2000 ppm treatments of the final harvest (2/1/68) and it must be concluded that the reduction in shoot internode length is significant for 2000, 3000, and 4000 ppm Alar.

Shoot dry weight showed a corresponding decrease with increasing Alar concentration. No evidence was obtained to substantiate the findings of Batjer (1964b), Edgerton (1964), Edgerton et al. (1965) who claimed that stem girth increased with Alar concentration.

Alar treatment did not significantly alter leaf area. This finding is in accordance with reports by Edgerton (1964), Edgerton et al. (1965). Considerable evidence has been presented by several workers to show that leaf colour is increased with Alar treatment. No visual differences were detected between leaf colour from control and Alar treated leaves. However, leaf dry weight from Alar treated shoots showed an eventual increase over that of controls. This increase in leaf dry weight suggests that leaf thickness increased with Alar treatment although no direct measurements were made. The difference, if any, was not apparent from visual assessment of leaves from controls and treated shoots. However, decreased leaf dry weight was reported by Greenhalgh (1967) for McIntosh apple. Petiole dry weight appears

to decrease with Alar treatment although the decrease is not proportional to concentration. This effect is not immediately apparent and does not become so until the 49th day after treatment. It should be noted however, that both petiole and stem dry weights show a decrease with Alar treatment while leaf dry weight increases.

Results from the distribution of fruit size and weight show that an increased number of large fruit occur at low Alar concentrations (1000 ppm) while the converse is true of high Alar concentrations (4000 ppm). Fruit weight within these groupings shows a similar relationship. These results have possible commercial use; fruit size and weight could be increased, or decreased, to suit market preferences. The literature contains several reports of reduced fruit size with all Alar concentrations tested and Luckwill et al. (1965) showed that size reduction was not accompanied by differences in crop weight. No reports have been made concerning the increase in either crop size or weight with low Alar concentrations.

Analysis of fruit storage results to obtain the percentage number of fruit which would be commercially acceptable shows that the percentage increases with Alar treatment. Breakdown of these overall results shows that fruit bruising and deterioration (softness) is reduced in Alar treatments (except for 1000 ppm). However, the incidence of "mouldy core" increases with treatment to reach a level which is greater than that of the control. This evidence would be important to varieties such as Delicious which exhibit late closing of the calyx. Increased levels of "bitter pit" over that occurring for control fruit are evident in the 1000, 2000, and 3000 ppm treatments.

The fact that high levels of Alar (4000 ppm) show no evidence of bitter pit could be taken to mean that Alar may reduce this disorder in New Zealand pip fruit varieties. However, Williams (1967) reported that this possibility had been investigated and that no evidence could be found to support the claim.

Chapter 4

ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES EXTRACTED FROM ALAR TREATED APPLE SHOOTS

Chapter 4

ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES EXTRACTED FROM ALAR TREATED APPLE SHOOTS

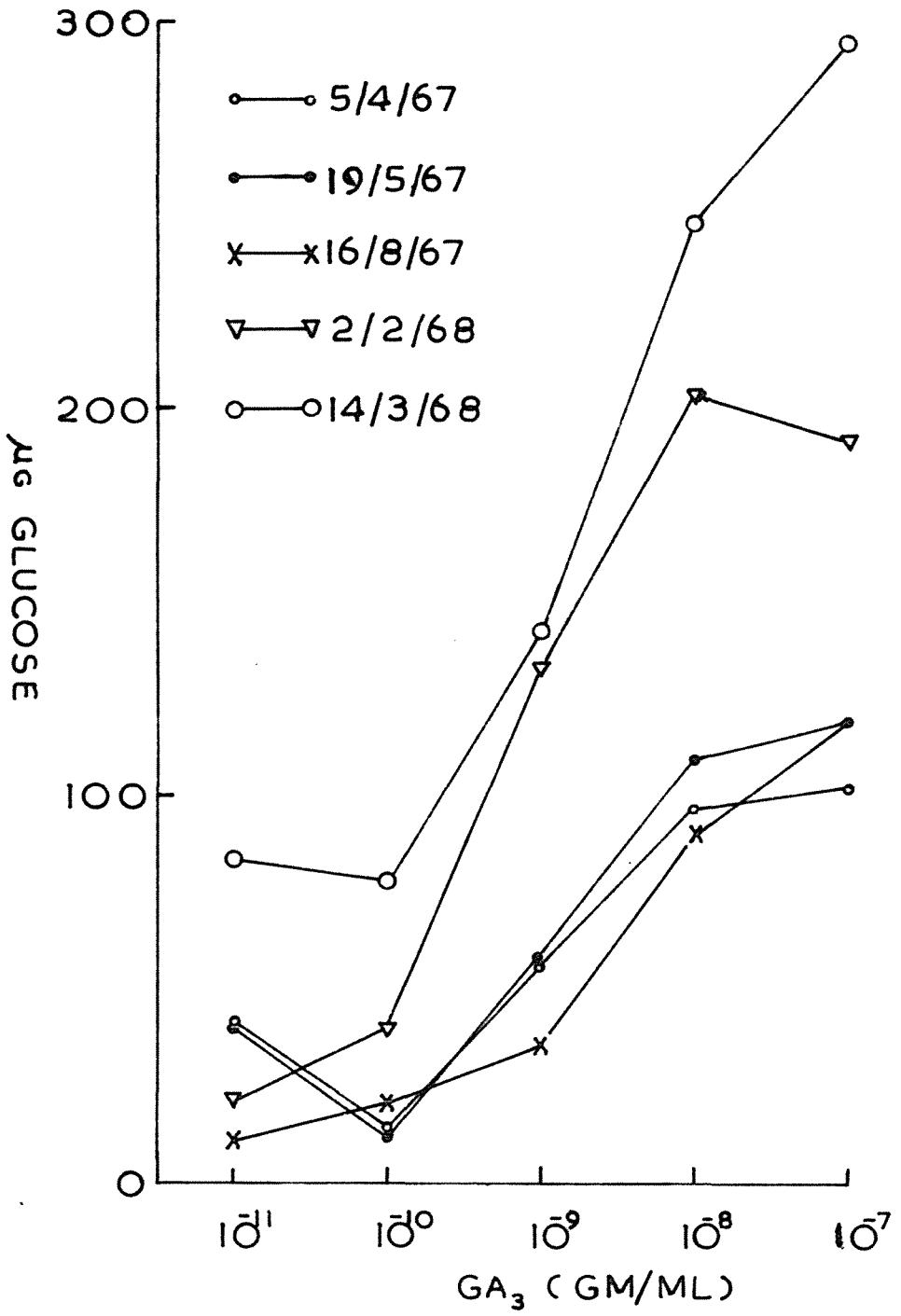
A. INTRODUCTION

Gibberellin treatment liberates reducing sugar from barley endosperm. With increasing gibberellin concentrations, increasing amounts of reducing sugar are liberated (Paleg 1960). Further work by Paleg (1960b, 1961) Paleg *et al.* (1962) led to the development of a reliable gibberellin bioassay based on the gibberellin induced release of reducing sugar (Nicholls and Paleg 1963). Minor modifications to this bioassay procedure have been made by Coombe *et al.* (1967) and Jackson (1967) in order to reduce the time taken to bioassay large numbers of samples.

B. DERIVATION OF THE STANDARD CURVE

Barley endosperm pieces were incubated with known amounts of gibberellin (GA_3) to determine a standard curve of gibberellin concentration versus reducing sugar release. Controls were run with distilled water. Three replicates were used for each gibberellin concentration and mean values used to construct the standard curves.

Figure 18a. Gibberellin induced reducing sugar release from barley endosperm.



However, reducing sugar released at each gibberellin concentration was measured in units of optical density. In order to equate optical density units to μg glucose (reducing sugar) a standard graph of optical density versus μg glucose was erected (Figure II, Appendix III) by the method used to determine reducing sugar in bioassays. Expression of reducing sugar in terms of μg glucose enabled a measure to be made of the amount of reducing sugar present in each incubation vial.

Standard bioassays were done on freshly harvested seed (5/4/67, 19/5/67; Figure 18a). A sample of the seed was then dried over concentrated H_2SO_4 and stored at 5°C in polythene bags. Further estimations of the GA_3 induced reducing sugar release were made on this seed (16/8/67, 2/2/68, 14/3/68) (Figure 18a). Measurement of reducing sugar was done immediately after the addition of Nelson's reagent in bioassays 5/4/67, 19/5/67. It was noted, however, that colour development tended to increase after reagent addition. In order to determine the extent of the colour development, reducing sugar measurement from bioassay 16/8/67 was made at 30, 60, and 90 minutes after reagent addition. Comparison between optical density values from each measurement showed that colour development stabilized after 60 minutes. Accordingly, all subsequent reducing sugar determinations were made 60 minutes after the addition of Nelson's reagent.

Expression of the standard curves (Figure 18a) shows that reducing sugar increases with increasing gibberellin concentration (between 1×10^{-10} - 1×10^{-8} gm/ml). Anomalous results with 1×10^{-11} gm/ml GA_3 (5/4/67, 19/5/67, 14/3/67) have also been shown by other workers (Jackson 1967). The decrease in reducing sugar

release between 1 x 10^{-8} - 1 x 10^{-7} gm/ml GA_3 for bioassay 2/2/68 is not typical of the norm. Results from other bioassays (Figure 18a), and from bioassays not presented, all show an increase in reducing sugar release over this concentration range.

More important, the magnitude of the reducing sugar release is not constant for any one gibberellin concentration. This effect is more pronounced with time from seed harvest. Comparison between bioassay dates (5/4/67, 19/5/67) shows increased (diverging) reducing sugar levels between 1 x 10^{-9} - 1 x 10^{-7} gm/ml GA_3.

When bioassays 5/4/67 and 16/8/67 are compared it can be seen that storage appears to have lowered seed sensitivity to low concentrations of gibberellin (1 x 10^{-11} - 1 x 10^{-9}). Differences between individual bioassays 5/4/67 - 16/8/67 are minor in extent when compared with differences between bioassays 5/4/67, 2/2/68 and 14/3/68. Prolonged cold storage appears to either enhance the gibberellin induced sugar release or to cause hydrolysis of starch to reducing sugar. Further information on this point can be obtained from an analysis of water controls (Table 13).

TABLE 13

Bioassay Date	5/4/67	19/5/67	16/8/67	11/9/67	2/2/68	14/3/68
Water Control (μg glucose)	1	21	3	22	110	41

It can be seen from Table 13 that reducing sugar levels increase between 5/4/67 - 19/5/67 (before drying and cold storage), decrease with short term cold storage (16/8/67) and show increased values with further cold storage (11/9/67 - 14/3/68). The reducing sugar value for water control 2/2/68 appears to be higher than the norm. Bioassays of endogenous gibberellins from Alar treated shoots attempted over the period December 1967 - January 1968, gave water control values (μg glucose) of 27, 28, and 82, 66, respectively while a further bioassay (5/4/68) yielded a water control of 68 μg glucose. Although reducing sugar levels from water controls show an increase with cold storage time, and that these increases tend to reduce differences between bioassays, it is evident that some further factor influences sugar release after prolonged storage time (shown by an increase in the slope of the graphs). This additional factor is in no way related to the quality of the distilled water. For every bioassay, incubation of water controls without endosperm gave optical density readings equivalent to, or less than, reagent blanks.

C. ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES

I. Purification of Ethyl Acetate Extracts

Eluates from Rf zones were bioassayed and reducing sugar levels measured as optical density units. In all cases, eluates from uncontaminated silica gel were used as water controls. Optical density units were converted to μg glucose (Figure II, Appendix III). Total reducing sugar levels (μg glucose) from each Rf eluate are expressed in histogram form (Figures 19 - 22).

Quantitative comparisons cannot be made between treatment histograms of any one harvest date, or between harvest dates, for two reasons:

(i) The dry weight of extracted shoot tissue varied between treatments and between harvest dates.

(ii) Thin layer chromatograms were contaminated with coloured compounds at Rf values 0-0.1.

When final ethyl acetate extracts, which contained acidic gibberellin-like substances were reduced to dryness a yellow precipitate formed. Although the precipitate was readily soluble in fresh ethyl acetate, some difficulty was experienced in application to thin layer plates. Some of the coloured compounds moved into the Rf zone 0-0.1 during plate development and subsequent bioassays of eluates from this zone were yellow in colour. Dilution of bioassay vials prior to the removal of 1 ml aliquots and further dilution during reducing sugar estimation decreased the levels of coloured compounds. However, some interference with the somogyi's reagent did occur with concomitant intensification of colour.

It was found that interfering compounds in ethyl acetate extracts could be precipitated by the addition of excess petroleum ether. To test the practicability of this precipitation process the ethyl acetate extracts from harvest date 19/12/67 were treated in the following manner:

(i) Coloured compounds were precipitated with excess petroleum ether and removed by filtration.

(ii) Residues were washed with petroleum ether and then resuspended in ethyl acetate.

(iii) The precipitation and filtration steps were repeated twice more.

(iv) The combined filtrates and washings were reduced to dryness and treated as previously described.

(v) Recovery values were estimated on the residues.

The residues from filtration were resuspended in 30 ml of water, centrifuged and 3, 1 ml aliquots, (x 2 replicates) were assayed for gibberellin-like substances. It was found that residues from the 4000 ppm Alar treatment contained measurable levels of substances active in the reducing sugar release. Control values showed no such response (Table 14).

TABLE 14

Harvest Date	Treatment	Mean sugar release/3ml (µg glucose)
19/12/67	Control	133
	Alar (4000 ppm)	181
	Water control	153

Dilution to 30 ml in order to reduce the degree of interference with somogyi's reagent may well have obscured activity in control bioassays. However, it was considered that higher activity

located in bioassays from the 4000 ppm Alar treatment were indicative of gibberellin-like substances removed from ethyl acetate extracts during the precipitation process.

II. Recovery Values

Petroleum ether and ethyl acetate residues from initial steps in the extraction procedure were assayed for activity by the barley endosperm bioassay. In each case, residues were taken to dryness, made up to 2 ml with distilled water and bioassayed. Results from these bioassays are presented in Table 15.

TABLE 15

Harvest Date	Treatment	Residue source	Total reducing sugar release (2 ml) (µg glucose)
19/12/67	Control	Pet. Ether	102
		Ethyl Ac.	85
		Water control	138
	4000	Pet. Ether	72
		Ethyl Ac.	114
		Water control	138

Higher reducing sugar levels were found from water controls than from either ethyl acetate or petroleum ether residues.

Recovery values were also estimated from silica gel residues (Harvest date 19/12/67). Control and 4000 ppm residues were shaken

with distilled water, deep frozen for seven days, and brought to room temperature. Ethyl acetate (5 ml) was added to each Rf residue, shaken, and stored at 5°C for five days. The ethyl acetate-water mixture was decanted off, solvent evaporated and solution made up to 2 ml with distilled water. Two aliquots of 1 ml were bioassayed and sugar levels determined. Results (as μg glucose) have been multiplied by a factor of 1.5 to enable comparison to be made with initial results (Figure 22). Comparison between histograms of initial estimations and those derived from recovery values reveals several points.

(i) Water controls from recovery values are much lower than those of the initial estimations. Further more, water controls from other bioassays attempted on, or about, the same date show water control levels of 125, 148, 143, and 153 μg glucose.

(ii) Presupposing that the water control is an accurate estimate, it can be seen that the peak which occurs at Rf 0.3-0.4 in both control and 4000 ppm Alar treatments does not occur in either of the recovery histograms. This can be taken as a measure of adequate elution of gibberellin-like substances. Ethyl acetate has found wide usage as an elutant of gibberellins from silica gel (Ried and Carr 1967) although Jackson (1967) found that adequate elution could only be obtained after freeze drying (two hours).

(iii) Anomalous fractions were shown to occur in eluates from treated silica gel (Figure 22). The difficulty in assessing the importance of these anomalous fractions stems from the

TABLE 16

Gibberellin (GA_3) equivalents from Alar treated shoots

Harvest Date	Treatment	Sample dry weight (gm)	GA_3 equivalents (10^{-10} gm)		GA_3 equivalents (10^{-10} gm/gm D.W.)	
			Rf 0-1.0	Rf 0.1-1.0	Rf 0-0.1	Rf 0.1-1.0
24/10/67	Control	1.757	4.95	3.73	2.82	2.12
	4000	2.101	6.07	2.75	2.89	1.31
31/10/67	Control	5.901	27.9	6.80	4.74	1.15
	1000	5.014	9.20	2.30	1.83	0.46
	3000	6.626	57.9	0.80	8.76	0.12
	4000	6.614	13.4	0.30	2.03	0.04
28/11/67	Control	7.448	1.50	0.20	0.20	0.03
	1000	6.644	2.00	0.20	0.31	0.03
	2000	6.220	2.20	1.10	0.35	0.18
	4000	8.524	4.90	1.10	0.58	0.13
19/12/67	Control	7.675	2.00	0.40	0.26	0.05
	4000	9.748	1.80	0.10	0.19	0.01
	4000	correction	4.5	-	0.46	-

D.W. = Dry Weight

fact that the water controls are of doubtful accuracy. It can be concluded that incomplete elution by ethyl acetate probably occurred although complete elution of the gibberellin-like compounds at Rf 0.3-0.4 (Figure 22) in both control and 4000 ppm treatments lends support to the proposal that possible errors in elution were standard for all treatments.

III. Gibberellin-like Activity

In order to demonstrate changes, if any, between gibberellin-like substances extracted from control and Alar treated shoots, histogram peaks were converted to GA_3 equivalents. Histogram peaks, which represented total sugar levels from three estimations, were read against a standard bioassay curve (2/2/68) (Figure 18a) which had been constructed from total sugar levels obtained from three replicates. These results have been presented in Table 16 as total GA_3 equivalents (Rf 0-1.0) and as GA_3 equivalents from Rf zones 0.1-1.0. Division by sample dry weight enables quantitative comparisons to be made between treatments and between harvest dates.

Comparison between GA_3 equivalents (10^{-10} gm/gm dry weight) estimated from Rf zones 0.1-1.0 shows that gibberellin levels decrease with increasing Alar concentration in harvest dates 24/10/67 - 31/10/67. However, the converse relationship occurs with harvest date 28/11/67. Comparison between total GA_3 equivalents (Rf 0-1.0) shows that no difference occurs between control and Alar treatments from harvest date 24/10/67, while increasing Alar concentrations decreased GA_3 equivalents in harvest date 31/10/67. The result from the 3000 ppm Alar treatment is probably erroneous as all other estimations follow

Figure 19. Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 24/10/67).

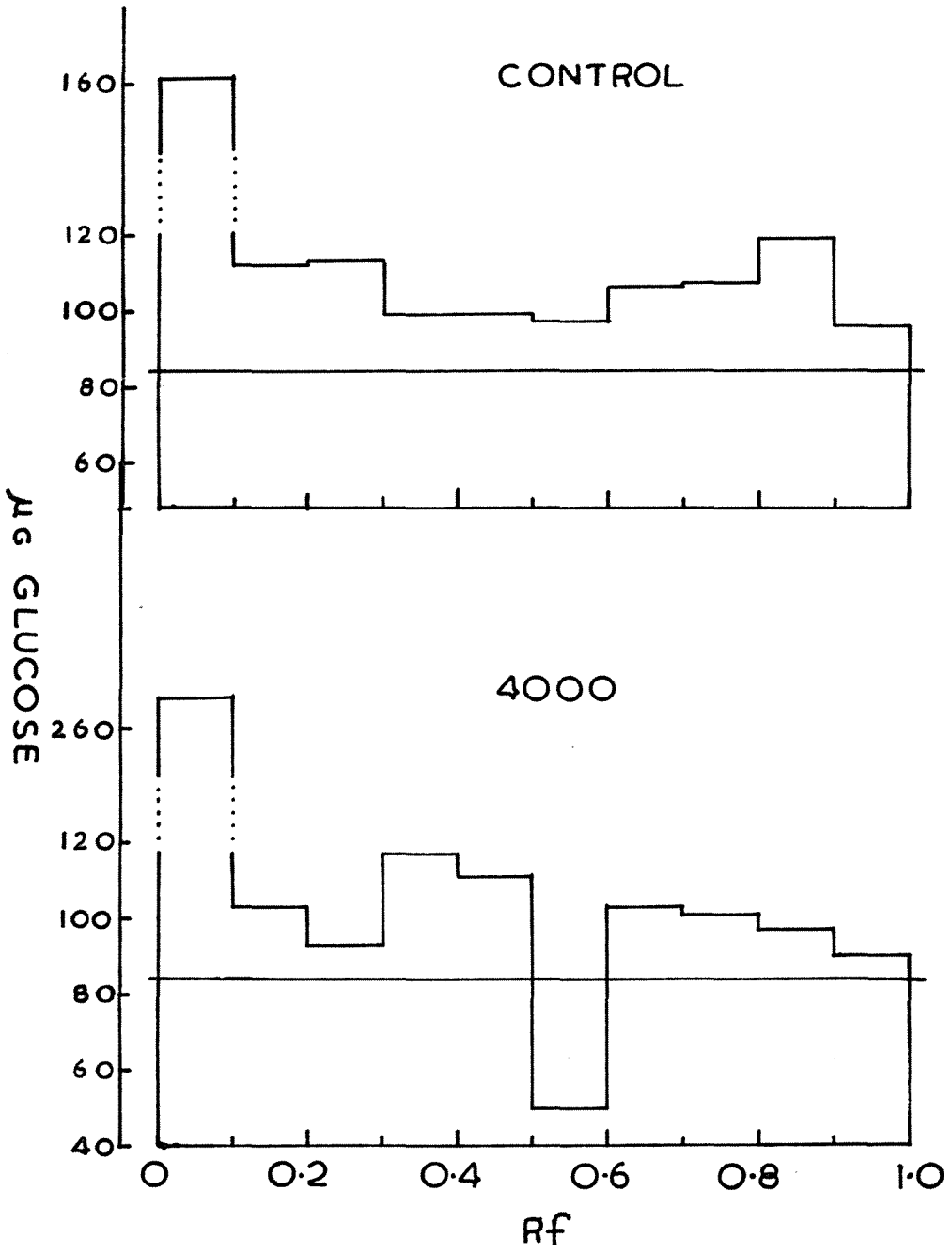


Figure 20. Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 31/10/67).

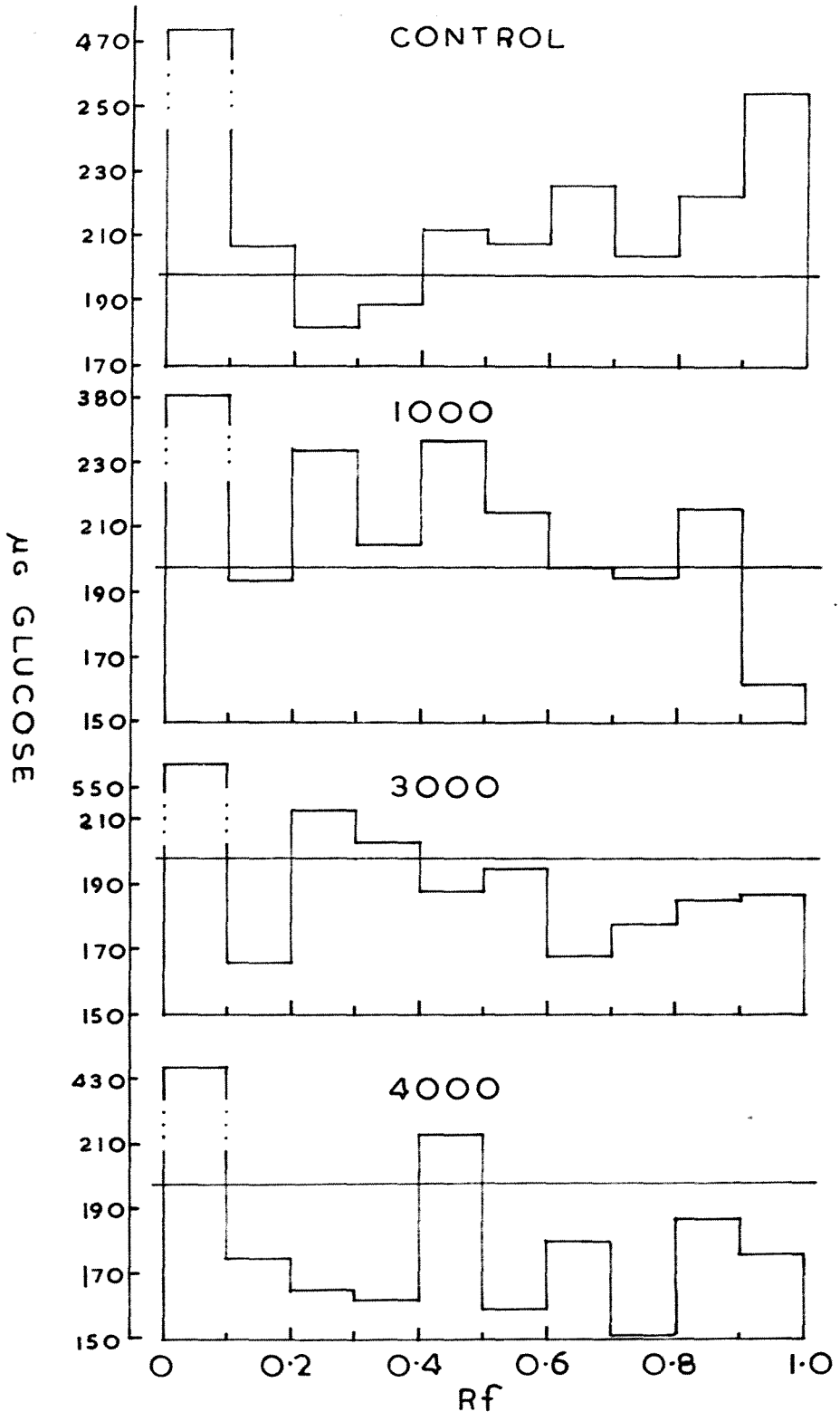


Figure 21. Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 28/11/67).

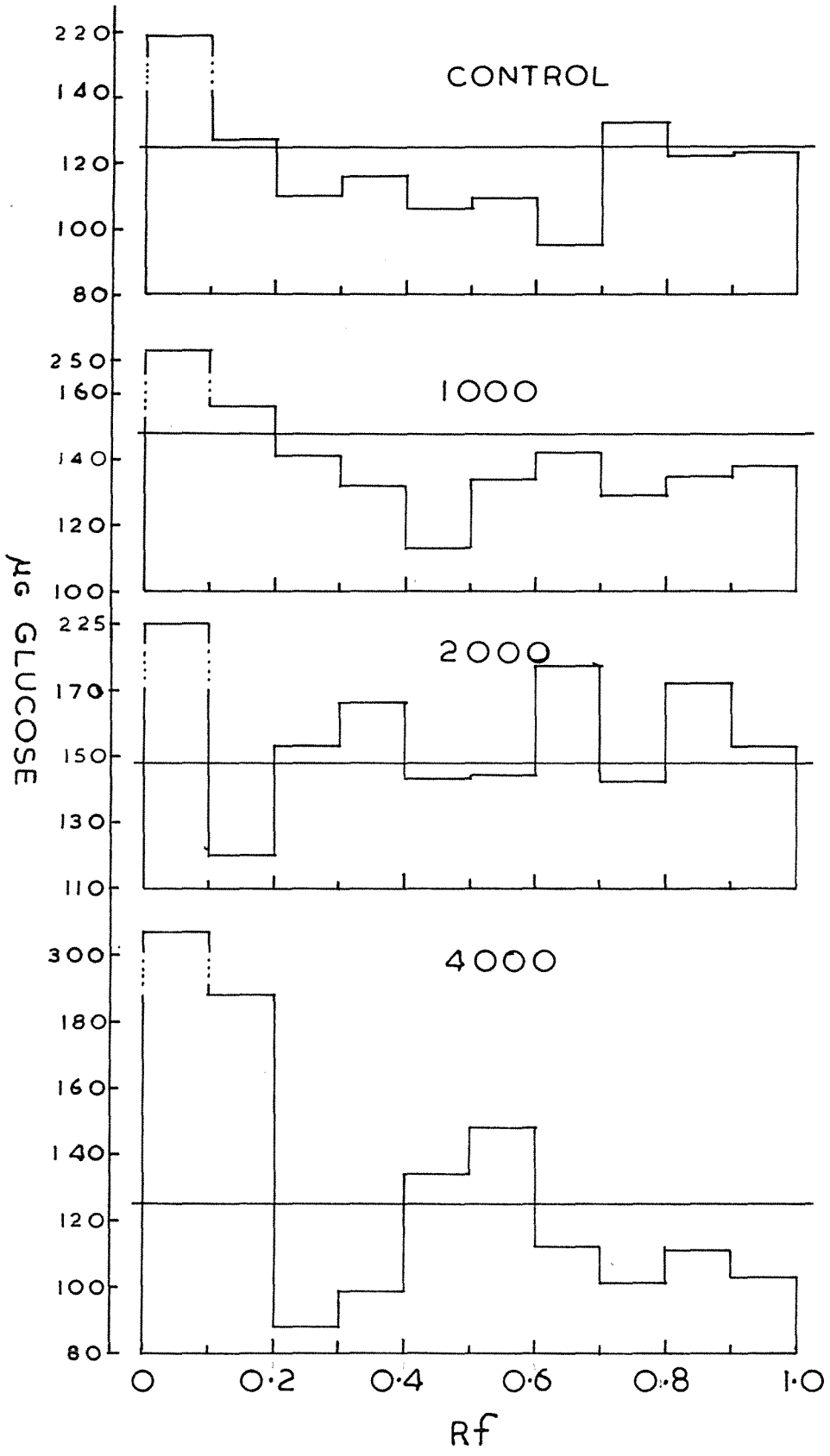
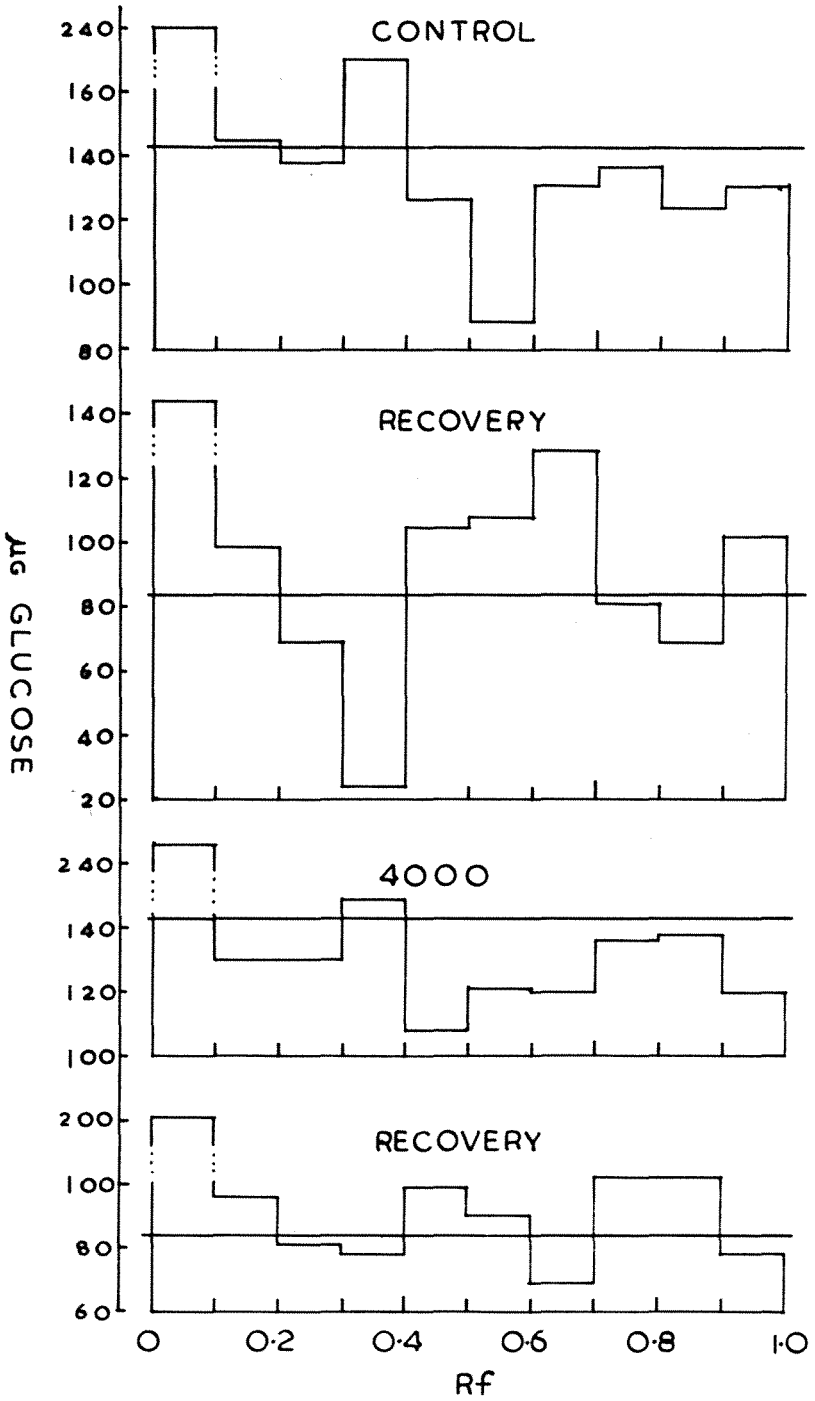


Figure 22. Reducing sugar release from barley endosperm incubated in the presence of eluates from thin layer chromatography (Harvest date 19/12/67).



the overall trends set by Rf 0.1-1.0 estimations. GA_3 equivalents show increased levels with Alar concentration in harvest 28/11/67.

Although comparisons between treatments from harvest date 19/12/67 show decreased gibberellin levels with increasing Alar concentration, the results from recovery values (previously described) suggest that a loss of gibberellin-like substances occurred during extraction procedures. Expression of sugar values, obtained from recovery bioassays, as GA_3 equivalents (4000 correction, Table 16) shows that overall levels increase with Alar treatment.

It can be concluded from this quantitative appraisal of endogenous gibberellin-like substances that Alar treatment initially decreases, and later increases, the levels of gibberellin-like substances.

In order to understand the implications of this Alar effect it is necessary to assess histograms (Figures 19 - 22) in the qualitative, rather than quantitative sense. Histogram peaks show little correlation between treatments (on a Rf basis) from any one harvest date or between individual treatments from different harvest dates. Comparison between control and 4000 treatment histograms (Figure 19) shows that the general peak at Rf 0.6-1.0 decreases with Alar treatment. This decrease is accompanied by the concomitant development of an anomalous peak at Rf 0.3-0.5. This peak is also present in the 1000 and 4000 treatments of Figure 20, although somewhat decreased in magnitude. Comparison between control treatments (Figures 19 - 20) shows that the peak at Rf 0.6-1.0 (Figure 19) fractionates into two peaks at Rf 0.6-0.7 and 0.8-1.0 (Figure 20). Both of these latter

peaks decrease in magnitude with increasing Alar concentration. A further anomalous peak occurs at Rf 0.2-0.3 in 1000 and 3000 ppm Alar treatments although this peak is absent from the 4000 ppm Alar treatment.

Comparison between results from harvest dates 28/11/67 and 19/12/67 (Figures 21 - 22) bear little relationship to the preceding histograms (Figures 19 - 20). This is to be expected if the changing ontogenetic development of the plant is considered. However, comparison between treatments from harvest date 28/11/67 (Figure 21) shows that anomalous peaks occur with Alar treatment although little correlation occurs between Rf values of these peaks. At a cursory glance, it appears that histogram peaks shift to the left (decreased Rf values) with increasing Alar concentration. This general observation also holds true for treatment histograms from harvest date 31/10/67 (Figure 20). In each case, the shift in Rf is approximately 1 Rf unit for 1000 and 2000 ppm Alar treatments and 2 Rf units for 3000 and 4000 ppm Alar treatments.

Qualitative assessment of histograms (Figure 22) shows that peaks occur at Rf 0.3-0.4 in both the control and Alar treatments. However, further comparisons cannot be made because of the probable loss of gibberellin-like substances during initial purification steps.

D. DISCUSSION

Extraction procedures for gibberellins and gibberellin-like substances appear to be almost traditionally associated with non-photosynthetic tissue (seeds, etiolated seedlings) or plant exudates

(bleeding sap). Similar extraction procedures from photosynthetic material have been reported in more recent literature although the plant material on which such extractions are based is invariably of the "soft" herbaceous type (pea, bean, etc). Extraction of gibberellin-like substances from woody plant tissue (e.g. apple shoots) has not been subjected to similar investigations.

The presence of interfering compounds occurring in gibberellin-rich ethyl acetate extracts (as previously described) confounds the interpretation of results presented in this chapter. High reducing sugar levels detected in eluates from Rf 0-0.1 show fluctuations in magnitude between treatments and between harvest dates. Fluctuations between treatments of any one harvest date shows trends similar to those previously discussed for GA_3 equivalents from Rf 0.1-1.0. However, when trends between harvest dates are considered it is apparent that reducing sugar levels/gm dry weight decrease with time of shoot harvest. This precludes the possibility that precipitate formation is a consequence of Alar treatment and lends support to the fact that data presented may represent, in a semi-quantitative fashion, levels of gibberellin-like substances present at Rf 0-0.1.

With this in mind, what changes do Alar induce in endogenous gibberellin levels? Comparison between treatments from harvest dates 24/10/67 and 31/10/67 shows that levels of endogenous gibberellin-like substances decrease with increasing Alar concentration. Such a response could be expected; recent literature (Baldev et al. 1965, Dennis et al. 1965, Ruddat 1966, Zeevart 1966, Jones et al. 1967, Reid et al. 1967), contains reports of similar responses induced by

other growth retardants on a range of species. However, the increase in endogenous gibberellin-like substances found for harvest dates 28/11/67 and 19/12/67 is an apparent contradiction of results from earlier extractions (24/10/67, 31/10/67). This increase in gibberellin-like substances precludes the possibility that initially lowered levels of gibberellin-like substances in Alar treated apple tissue were due to enhanced gibberellin destruction.

Reid and Carr (1967) showed that both quantitative and qualitative changes occurred in gibberellin activity from bleeding sap, and roots, of CCC treated pea. These workers concluded that CCC blocked the production of gibberellins normal to that species and caused a diversion of precursor(s) toward the production of 'abnormal' gibberellins active in the barley endosperm bioassay.

Results presented in this study showed that anomalous fractions occurred with Alar treatment. It is possible that these anomalous fractions are due to variations in chromatography. However, the fact that the observed Rf shift of 0.1 unit in 1000 and 2000 treatments and 0.2 units in 3000 and 4000 treatments occurs for harvest dates 24/10/67, 31/10/67 and 28/11/67 can be taken as evidence that these peaks are the result of Alar treatment and not due to variations in chromatography. Other investigators (Dennis *et al.* 1965) showed that Alar did not significantly inhibit the cyclization of trans geranyl geranyl pyrophosphate to (-)-kaurene. This could be expected if Alar acted at a point later in the biosynthetic scheme (as shown for CCC). Evidence from *F. moniliforme* studies (Kende *et al.* 1963) showed that Alar was inactive in inhibiting gibberellin biosynthesis. This

negative result could be due to insufficient incubation time (3-4 days) to elicit a response as Alar was readily recovered from fungal mycelia and appeared to be unmetabolised. The finding that Alar decreased ATP production in pea stem mitochondria (Heatherbell et al. 1966) may be important in that ATP could be required for precursor conversion to the active gibberellin although there is no evidence to support this proposal.

In summary, it appears that Alar treatment of apple shoots inhibits the production of gibberellin-like substances and that this inhibition is evident 18 days after spray application. Production of 'abnormal' gibberellin-like substances becomes apparent about the same time and this could be taken as evidence for an Alar induced block in the latter stages of gibberellin biosynthesis. The fact that these 'abnormal' gibberellin-like substances accumulate suggests that they are inactive in shoot growth.

Chapter 5

ENDOGENOUS AUXINS EXTRACTED FROM ALAR TREATED APPLE SHOOTS

Chapter 5

ENDOGENOUS AUXINS EXTRACTED FROM ALAR TREATED APPLE SHOOTS

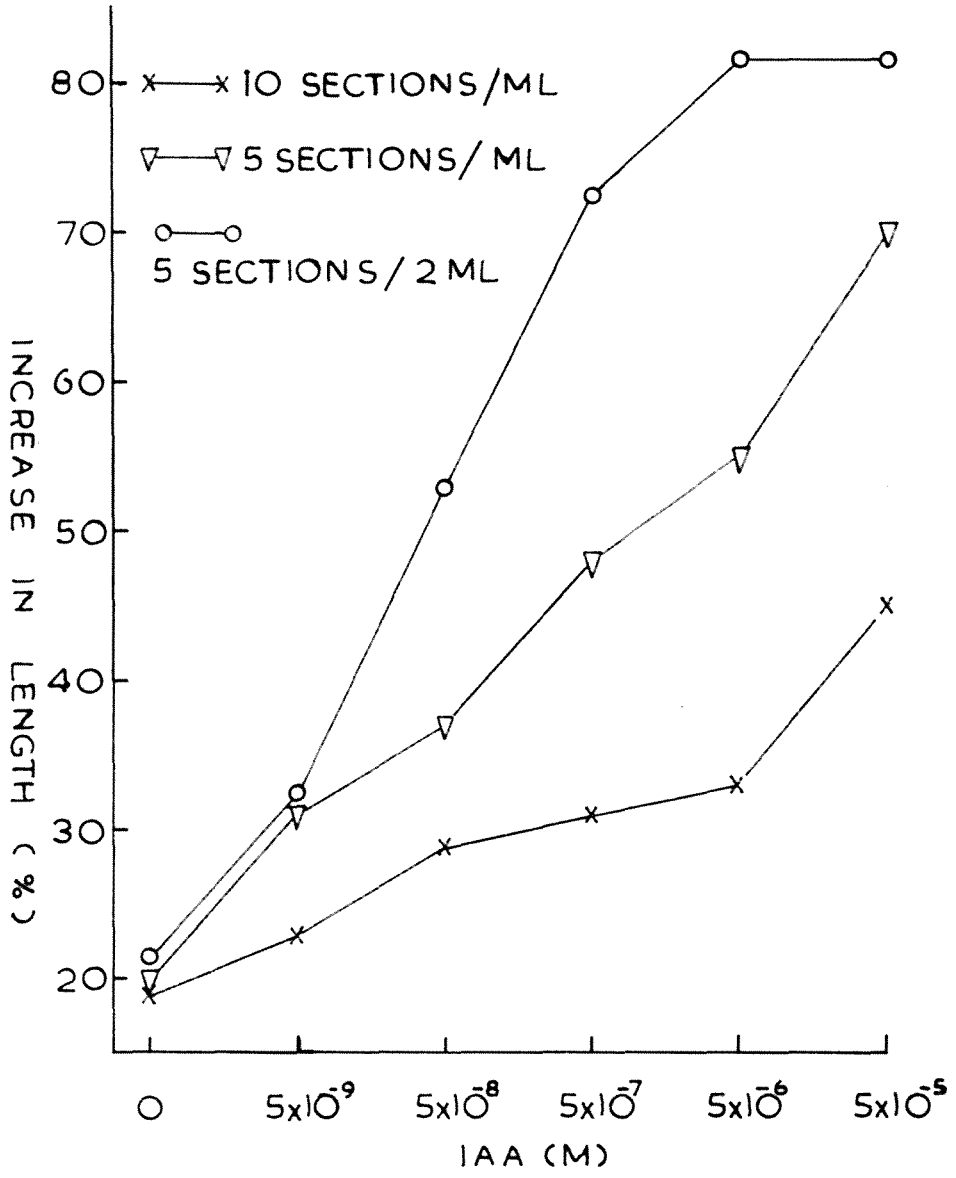
A. INTRODUCTION

Evidence has been presented in the literature of both direct and indirect interactions between growth retardants and auxins. A direct interaction was shown between Alar and β-hydroxy ethyl hydrazide and tryptamine oxidation leading to IAA biosynthesis (Reed 1965, Reed et al. 1965). Similarly, Kuraishi and Muir (1963) showed that diffusible auxin levels from pea 6th internode decreased with increasing CCC concentration. Indirect interactions between growth retardants and auxin metabolism have been shown by Halevy (1963) to be manifest via decreased gibberellin levels. Gibberellin is active in the control of IAA-oxidase activity such that high gibberellin levels decrease IAA-oxidase activity and hence increase auxin levels.

B. DERIVATION OF STANDARD CURVE

In order to determine the effect of various auxin (IAA) concentrations on Avena 1st internode sections (cv Garton), ten sections were bioassayed against known auxin concentrations by the method outlined

Figure 23. Increase in Avena 1st internode section length at different auxin (IAA) concentrations.



by Nitsch and Nitsch (1956). At the completion of incubation, segment length from each of three replicates was totalled, the mean length calculated, and results expressed as a percent increase in section length (Figure 23). Although increases in section length followed increases in auxin concentration, the slope of the graph did not approach that obtained by Nitsch and Nitsch with 'Brighton' oat. Furthermore, it was considered that the sheer mechanics of cutting the large number of sections required made the method impracticable with respect to the numbers of bioassays that had to be attempted in the time available. Accordingly, standard bioassays were attempted with 5 sections per ml of auxin solution.

Results from one such bioassay (treated as above) are presented in Figure 23. A more uniform response was obtained to increasing auxin concentration than was shown by the curve derived from 10 sections per ml. However, adequate elution from chromatographic strips could not be obtained with 1 ml aliquots of buffer. As a consequence, standard bioassays were attempted with 5 sections per 2 ml auxin solution (Figure 23). A near linear response was obtained between 5×10^{-9} - 5×10^{-7} M IAA although at higher concentrations (5×10^{-7} M IAA) auxin reached saturation levels.

Application of known quantities (40 μl) of 5×10^{-5} M IAA to chromatograms and subsequent bioassay of chromatograph strips against 2 ml buffer (in 2% sucrose) showed that a major peak occurred between Rf 0.3-0.4 (Figure 24). Smaller peaks at Rf 0.2 and 0.5 were considered to be associated with the main IAA peak (Rf 0.3-0.4). The small peak at Rf 0.7-0.8 is considered to be due to IAA breakdown products; the

Figure 24. Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 7/11/67).

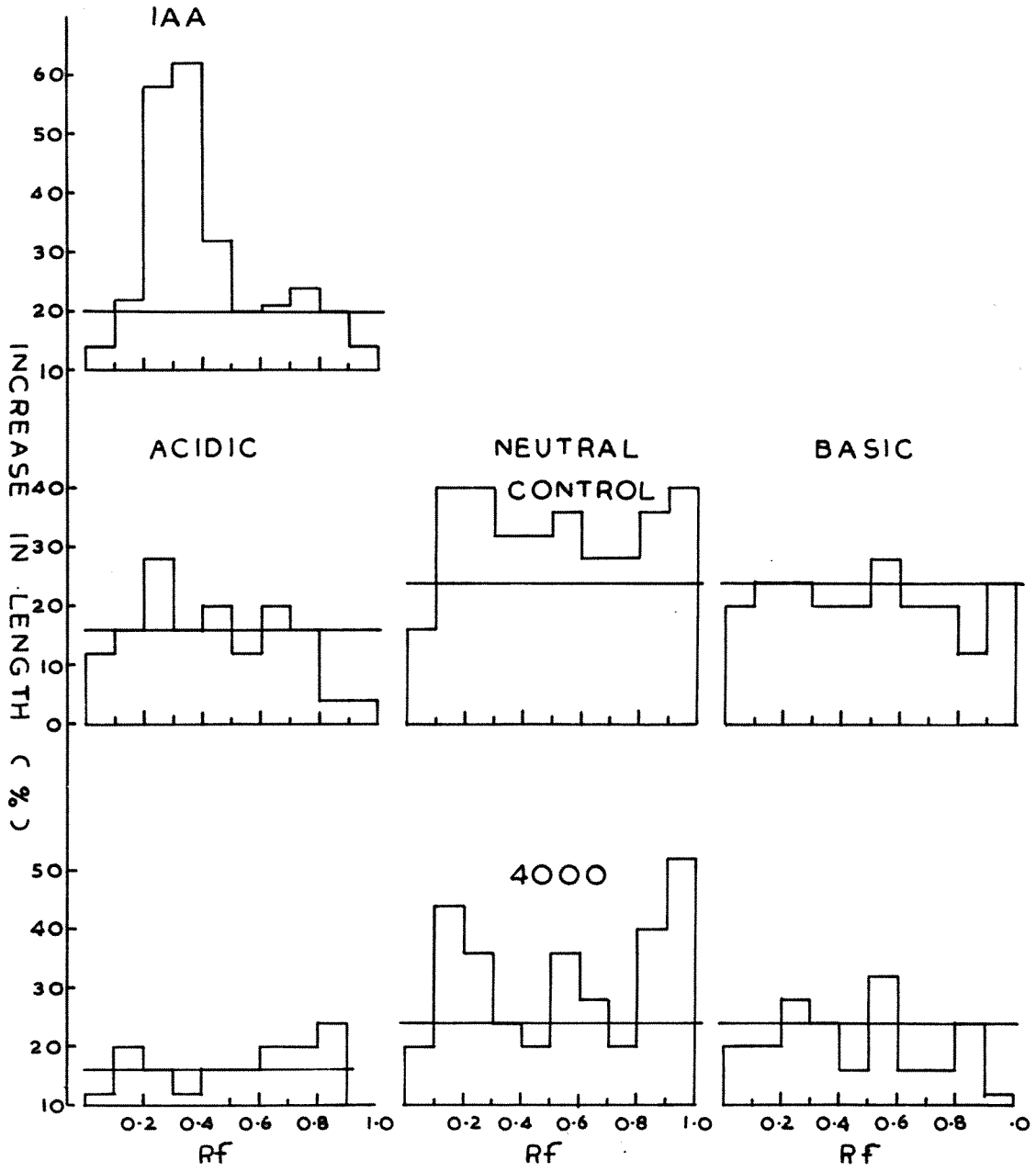


Figure 25. Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 5/12/67).

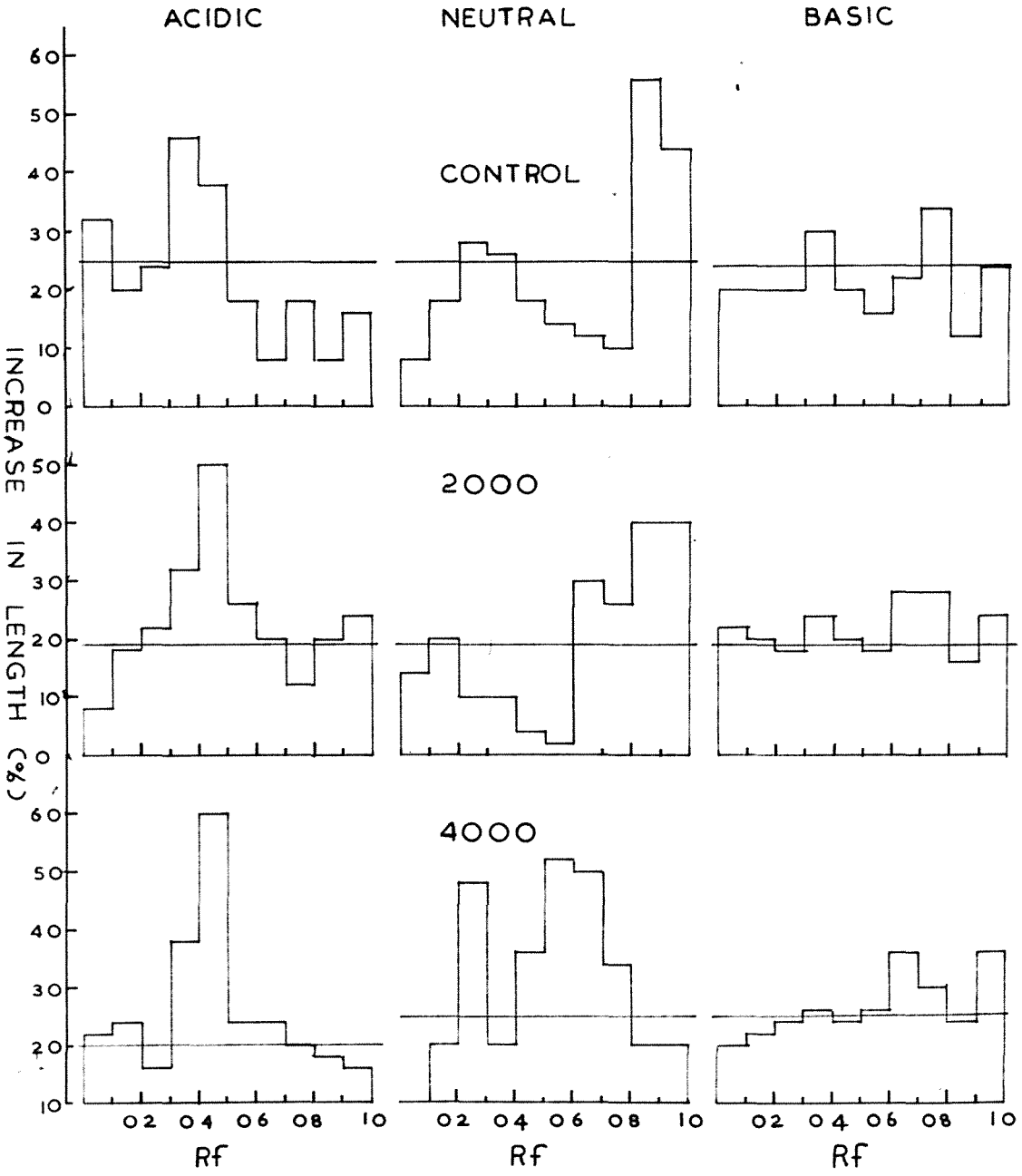
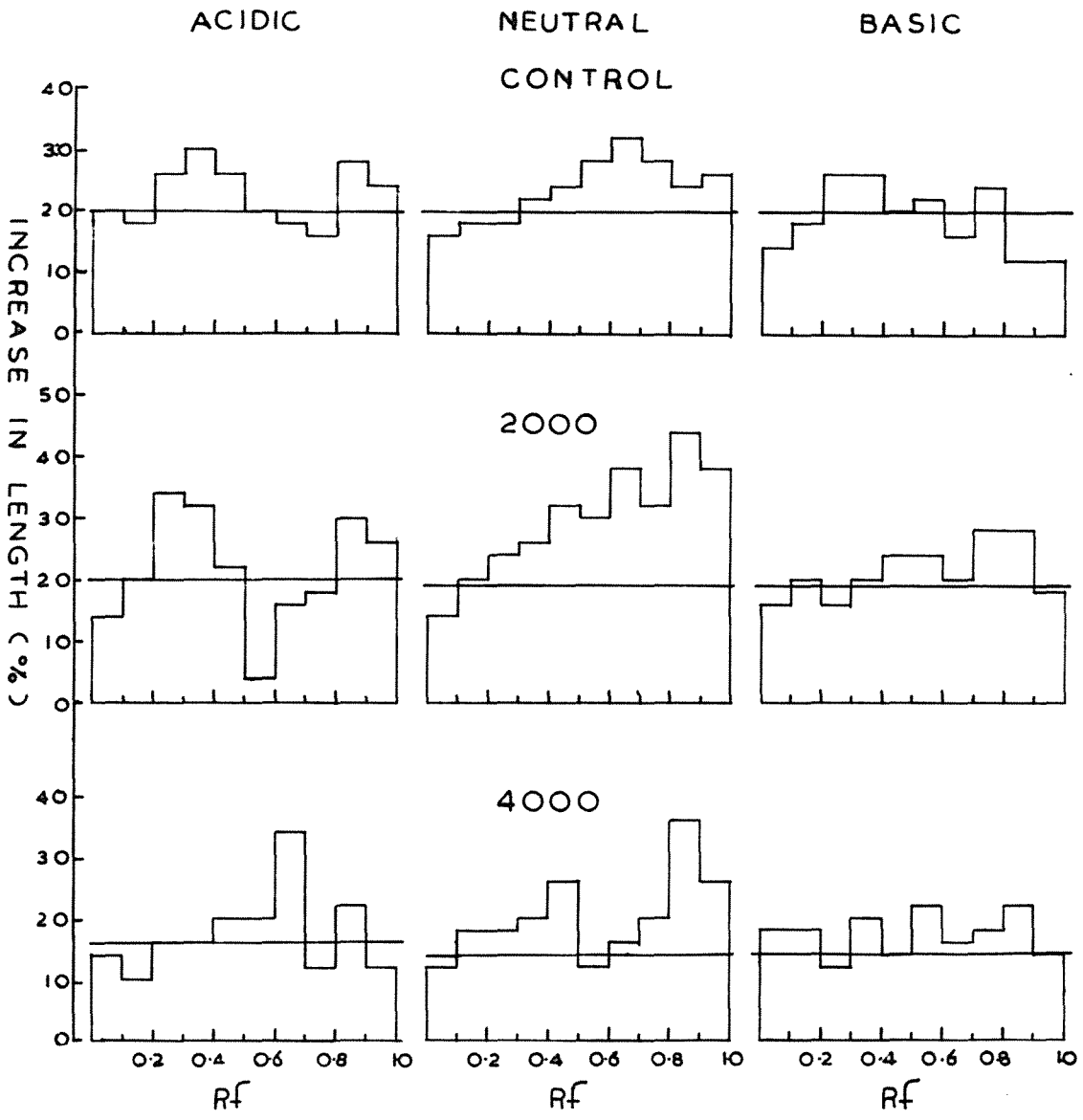


Figure 26. Response of Avena 1st internode sections to eluates from paper chromatography (Harvest date 26/12/67).



distinctive odour of skatol could be detected in the commercially prepared IAA sample.

In order to assess the degree of accuracy which could be expected from the method, histogram peaks between Rf 0.2-0.5 were converted to IAA equivalents (gm) by way of the standard curve (5 sections per 2 ml). A total of 2.35×10^{-7} gm IAA was detected as opposed to 3.5×10^{-7} gm IAA applied. The discrepancy is considered to be due to incomplete elution from chromatographic strips.

C. ENDOGENOUS AUXINS

I. General Considerations

Endogenous auxins, extracted from Alar treated shoot tissue, were assayed against the modified *Avena* 1st internode bioassay. The lengths of the 5 sections from any one bioassay were brought to a total and expressed as a percent increase in section length. Results from these calculations are presented in histogram form (Figure 24 - 26).

Immediate quantitative comparisons between treatment histograms cannot be made because of variations in the dry weight of extracted shoot tissue. Furthermore, a uniform response was not obtained with water controls from all bioassays. It was considered that this effect was due to differences between different batches of *Avena* seedlings as bioassays attempted from the same seedling batch gave similar values for control bioassays. It could be argued that inconsistant water control values were due to inhibitor presence remaining in chromatographic

TABLE 17

Endogenous auxin levels extracted from Alar treated apple shoots

Harvest Date	Alar treatment	Dry weight	Acidic auxin 10^{-10} M/gm dry weight	"Auxin units" Acidic	gm dry weight Neutral	gm dry weight Basic
7/11/67	Control	6.929	1.44	2.89	13.87	0.57
	4000 ppm	6.202	0.38	3.23	14.84	1.94
5/12/67	Control	14.30	24.90	2.87	3.78	1.12
	2000 ppm	11.09	37.30	5.51	5.51	2.98
	4000 ppm	11.43	105.90	6.29	8.31	2.54
26/12/67	Control	9.62	4.52	2.91	4.58	1.87
	2000 ppm	8.73	16.19	5.04	12.97	3.55
	4000 ppm	10.10	6.06	3.17	6.74	3.56

paper after pre-running in solvent. Had differences been due to inhibitor presence, water control values could be expected to differ between bioassays attempted from the same seedling line.

II. Acidic Auxins

In order to determine quantitative changes, if any, between treatments of any one harvest date, histogram peaks were converted to IAA equivalents (gm), divided by sample dry weight, and expressed as IAA equivalents (10^{-10} gm/gm dry weight) (Table 17). However, the response obtained with bioassays 7/11/67 (Control and 4000 ppm Alar) and 26/12/67 (4000 ppm Alar) was less than that obtained with water controls from the standard bioassay. To compensate for this effect, the magnitude of each peak was calculated; each percent increase in total section length above that of the water control being taken as one "auxin unit", (Table 17).

Comparison between treatments from individual harvest dates shows that "auxin units" per gm dry weight increase with increasing Alar concentration although at 4000 ppm Alar (26/12/67) the increase in "auxin units" was not as great as that obtained with 2000 ppm Alar. This finding precludes the hypothesis that Alar promotes enzyme activity associated with auxin degredation.

Qualitative comparisons between control and Alar treatments shows that anomalous peaks occur at Rf 0.2, 0.9-1.0 (4000 ppm, Figure 24); at Rf 0.6-0.7 (2000 and 4000 ppm, Figure 25); and at Rf 0.7 (4000 ppm, Figure 26). Some peaks which occur in control treatments are absent from 4000 ppm Alar treatments e.g. peaks at Rf 0.5 (Figure 24), at Rf 0.1 (Figure 25) and at Rf 0.3-0.4 (Figure 26).

The development of anomalous peaks with Alar treatment with concomitant reductions in neighbouring peak intensity (Figures 24 - 25) suggests that Alar inhibits one or more steps in the acidic auxin biosynthesis. However, it is also apparent that some acidic auxins increase with increasing Alar concentration (Figure 25).

III. Neutral Auxins

Histogram peaks were converted to "auxin units" per gm dry weight (as previously described) and results presented in Table 17. Quantitative analysis of results from individual harvest dates shows that "auxin units" increase with Alar treatment. Again, there is no evidence to suggest that Alar enhances neutral auxin degredation. However, assessment of neutral auxin histograms in the qualitative, rather than quantitative sense shows that Alar has a considerable influence on neutral auxin levels.

Qualitative changes occur between control and Alar treatments at Rf 0.4-0.5 and at Rf 0.8 (Figure 24), although the major peaks at Rf 0.2-0.3, 0.6-0.7 and 0.9-1.0 remain virtually unchanged. However, comparisons between control and Alar treatments (Figure 25) shows that the peak at Rf 0.9-1.0 (control) decreases with increasing Alar concentration and this decrease is accompanied by an increase in anomalous fractions at Rf 0.7-0.8 (2000 ppm) and Rf 0.6-0.7 (4000 ppm). Neutral auxins which occur at Rf 0.3 (control) appear to accumulate at high Alar concentration (4000 ppm). The effect of Alar on neutral auxin metabolism is clearly shown in Figure 26. Neutral auxin levels in control treatments (peaks at Rf 0.4-1.0) appear to increase at 2000 ppm Alar with concomitant development of anomalous peaks at Rf 0.5 and 0.9.

Anomalous peak development (at Rf 0.5 and 0.9) is more prominant with 4000 ppm Alar treatment.

Alar induces both quantitative and qualitative changes in neutral auxin fractions. Although peaks at Rf 0.2, 0.6, and 1.0 (Figure 24) and at Rf 0.3 (Figure 25) increase in magnitude with increasing Alar concentration, the development of anomalous peaks in all histograms appears to be the more prominant Alar effect.

IV. Basic Auxin

Histogram peaks were converted to "auxin units" per gm dry weight as previously described (Table 17). Quantitative comparison between treatments shows that basic auxin levels increase with increasing Alar concentration. In comparison to acidic and neutral auxins, basic auxin levels from control treatments show an increase with harvest time. However, basic auxin levels from control treatments are considerably lower than those shown for acidic and neutral auxins.

Qualitative assessment of individual histograms shows that anomalous peaks occur with Alar treatment (Figures 24 - 25) although anomalous peaks do not develop to the same extent as comparable peaks in acidic and neutral auxin histograms. Histogram peaks at Rf 0.6 (Figures 24 and 26) and at Rf 1.0 (Figure 25) appear to increase in magnitude with increasing Alar concentration while peaks at Rf 0.4 (2000 and 4000 ppm Alar, Figure 25) decrease in comparison to the control.

D. DISCUSSION

An increase in the slope of the standard graph was obtained by decreasing the number of Avena sections per ml of auxin solution. Nitsch and Nitsch 1956 stated that relative accuracy of the bioassay depended on the type of proportionality between auxin concentration and the growth response (a linear relationship gives more accurate results than a semi-logrithmic response). With a semi-logrithmic response a more accurate bioassay can be achieved when small changes in auxin concentration result in large differences in growth (Figure 23). Results from this study show that increased slope, achieved with increasing auxin concentration per section, tended to decrease the effective range of the bioassay. Between 5×10^{-7} - 5×10^{-5} M (5 sections per 2 ml) the bioassay shows an increasing degree of auxin saturation.

Expression of endogenous auxin levels in terms of "auxin units" shows that acidic, neutral, and basic auxin levels increase with increasing Alar concentration. These results are in direct contrast with those obtained by Halevy (1963) and Kuraishi and Muir (1963) - with CCC. However, qualitative assessment of histograms (Figures 24 - 26) shows that Alar treatment influences endogenous auxin to the extent that:

(i) Some acidic and neutral auxin fractions which occur in control treatments are absent from Alar treatments.

(ii) The inhibition of the above fractions is accompanied by an increase in anomalous fractions in all Alar treatments but for the exception of neutral auxins isolated from the 4000 ppm treatment (Figure 24).

(iii) Some fractions are either unaltered or increase in magnitude with Alar treatment.

Exogenous auxin has been shown to overcome the inhibitory effect of CCC on Avena coleoptile growth and 'Alaska' pea stem segments (Kuraishi and Muir 1963), and CCC induced cucumber hypocotyl retardation (Moore 1967). However, exogenous auxin failed to reverse CCC suppressed Avena coleoptile elongation (Wittwer and Tolbert 1960b), Alar and AMO 1618 induced retardation of cucumber hypocotyl (Moore 1967), although gibberellin showed a stimulatory effect in all the latter examples. This conflicting evidence is not really clarified by evidence presented in this study.

Alar treatment decreases levels of some auxin fractions found in control treatments. Had auxin extractions been attempted prior to 7/11/67, an initial decrease in auxin levels may have been detected (as shown for gibberellin-like substances). Evidence presented in this study in no way supports the claim that Alar promotes auxin degredation (Halevy 1963). Furthermore, insufficient evidence is available to distinguish between possible causes of the observed increase (or absence of change) in some auxin fractions. Alar could either interact with the site of auxin action or auxin may accumulate as a result of decreased gibberellin levels (utilization in growth).

The fact that anomalous fractions occur with Alar treatment would suggest that they represent the production of 'abnormal' auxins which are inactive in apple shoot elongation. Development of anomalous fractions is more pronounced with time from Alar treatment and this may be taken as evidence of an Alar induced block in the biosynthetic pathway leading to 'normal' auxin in apple (Reed 1965, Reed et al. 1965).

Chapter 6

INTERACTION BETWEEN ALAR, GIBBERELLIN, AND AUXIN ON *Avena* 1st INTERNODE SECTIONS

Chapter 6

INTERACTION BETWEEN ALAR, GIBBERELLIN, AND AUXIN ON *Avena* 1st INTERNODE SECTIONS

A. GROWTH RETARDATION OF *Avena* SEEDLINGS

Avena sativa cv Garton seedlings were grown in the dark for 5 days after Alar treatment. Thirty seedlings were selected at random, seedling length measured, and the degree of retardation calculated (Table 18).

TABLE 18

	Alar Treatment (ppm)		
	Control	2000	4000
Total seedling length (cm)	125.5	122.2	70.9
Growth retardation (%)	-	2.6	43.5

Little difference in seedling length was detected between control and 2000 ppm Alar. However, appreciable growth retardation was detected at 4000 ppm Alar.

TABLE 19a

Interaction between GA_3, IAA, and buffer on *Avena* 1st internode sections (mean values calculated from 2 replicates)

Seedling Media	Water			Alar (2000 ppm)			Alar (4000 ppm)		
	Gibberellin (gm/ml)								
Auxin (M)	0	10^{-7}	10^{-5}	0	10^{-7}	10^{-5}	0	10^{-7}	10^{-5}
0	23	49	49	25	40	34	27	42	42
5×10^{-7}	69	92	80	43	49	53	63	66	68
5×10^{-5}	88	105	88	40	51	53	67	67	73

TABLE 19b

Interaction between GA_3, IAA and Alar on *Avena* 1st internode sections (mean values calculated from 2 replicates

Seedling Media	Water			Alar (2000 ppm)			Alar (4000 ppm)		
Alar addition (ppm)	3000			2000			4000		
	Gibberellin (gm/ml)								
Auxin (M)	0	10^{-7}	10^{-5}	0	10^{-7}	10^{-5}	0	10^{-7}	10^{-5}
0	35	41	41	32	36	29	38	42	41
5×10^{-7}	91	91	91	60	63	60	65	73	62
5×10^{-5}	82	92	94	52	59	64	57	73	65

B. HORMONE-ALAR INTERACTIONS

In order to investigate possible interactions between Alar and plant hormones, Avena 1st internode sections were cut from seedlings grown in Alar treated media (0, 2000 and 4000 ppm) and incubated with factorial combinations of Alar, auxin (IAA), and gibberellin (GA_3). All experiments were duplicated.

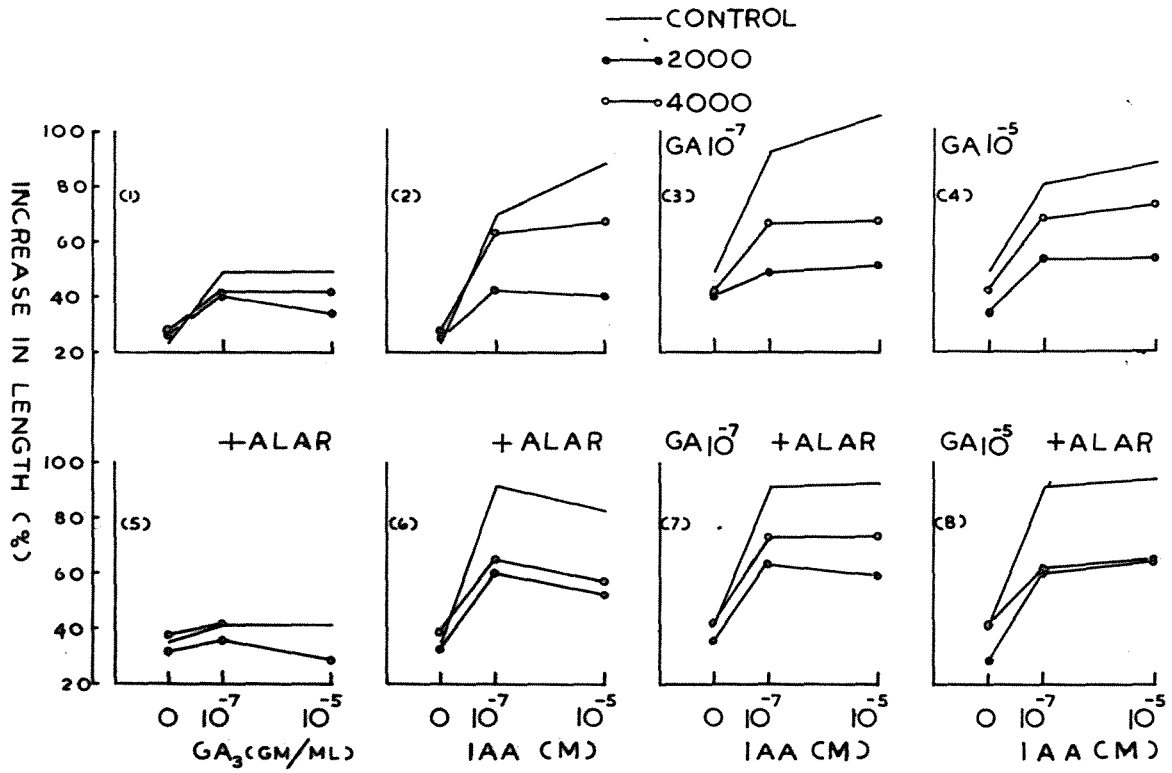
At the completion of incubation, total section length was assessed for each bioassay and expressed as a percentage increase in length. Results from replicate bioassays were combined, the mean value calculated, and presented in Table 19a, 19b. Differences between treatments (Table 19a, 19b) can be more readily demonstrated when results are presented graphically (Figure 27).

Comparison between graphs (1)-(2) (Figure 27) shows that GA_3 has some effect on the growth of Avena sections although GA_3 induced growth is about half that induced by IAA.

Section growth increases with increasing auxin concentration (Graph (2)). In the presence of auxin, GA_3 (10^{-5} gm/ml) has a small stimulatory effect on section growth (Graph (4)) although the response is not as pronounced as found with 10^{-7} gm/ml (Graph (3)). Various concentrations of IAA or GA_3, either alone or in combination, did not completely overcome the growth retardation induced by Alar treatment (Graph (1)-(4). Avena 1st internode sections from the 4000 ppm Alar treatment, however, showed a greater response to auxin and gibberellin than those from the 2000 ppm treatment.

Similar comparisons between Avena sections incubated in the

Figure 27. Response of Avena 1st internode sections to factorial combinations of IAA, GA_3, and buffer or Alar.



presence of various Alar concentrations (Graphs (5)-(8) shows that Alar (3000 ppm) lowers the response of sections from the control treatment to GA_3 (Graph (5)) and not to IAA (Graph (6)). Gibberellin (10^{-7}, 10^{-5} gm/ml) (Graphs (7)-(8)) in combination with 5×10^{-5} M IAA increases section growth over that of 5×10^{-5} M IAA alone. Again, gibberellin and auxin in combination did not completely overcome Alar induced retardation.

In order to further evaluate treatment differences, comparison was made between bioassays which had been carried out in the presence, or absence, of Alar. It can be seen from Graphs (1) and (5) that Alar increases the growth response of sections in the absence of GA_3 (Graph (5)) and decreases the response (controls) in the presence of GA_3 (Graph (5)). A similar promotion in section growth occurs with Alar treatment in the absence of auxin (Graph (6)) with a concomitant increase in section growth (in the presence of auxin) from the 2000 ppm Alar treatment. No such response was shown for the 4000 ppm Alar treatment.

Combination of Alar, IAA, and GA_3 (Graphs (7)-(8)) shows that section growth (control) was decreased at 5×10^{-5} M IAA (Graph (7)) when compared with (Graph (3)) while section growth was enhanced at 5×10^{-7} and 5×10^{-5} M IAA (Graph (8)). The increased response obtained with the 2000 ppm (Alar treatment) sections (Graphs (7)-(8)) to Alar x IAA x GA_3 was not shown by sections from the 4000 ppm Alar treatment.

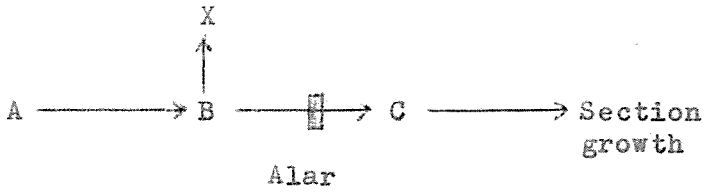
C. DISCUSSION

Wittwer and Tolbert (1960b) incubated Avena coleoptile sections with various combinations of CCC, IAA, and GA_3. CCC reduced the typical IAA response (increased growth in length) while GA_3 was only slightly active in increasing the IAA response. Kuraishi and Muir (1963) carried out a similar series of incubations. CCC inhibited coleoptile growth. Gibberellin had little effect on the inhibitory action of CCC although at 3×10^{-7} M, gibberellin was more active than at 3×10^{-5} M. In contrast, IAA overcame CCC induced growth inhibition. More recently Moore (1967) studied the kinetics of growth retardant - hormone interaction in cucumber hypocotyl elongation. GA_3 overcame Alar induced retardation of cucumber hypocotyl while IAA was without effect. This effect on a whole plant system is opposite to that found with Avena coleoptile sections. Moore concluded that Alar may affect an essential growth process different from those influenced by AMO 1618 and CCC.

Results obtained from this study show that the growth retardation of Avena 1st internode sections cut from Alar treated seedlings was not completely counteracted by auxin or gibberellin, or a combination of both. This suggests that a further growth process was affected by Alar treatment. It is evident from Graphs (6)-(8) (Figure 27) that Alar does not cause appreciable growth retardation of 1st internode sections cut from control seedlings. This is in agreement with Harada (1966) who showed that normal elongation of Avena 1st internode sections in the presence of auxin and gibberellin was not significantly inhibited by Alar, AMO 1618 or CCC.

The lack of a response in control sections, however, to simultaneous addition of Alar and gibberellin (Graph (5) Figure 27) would suggest that a slight Alar-gibberellin interaction occurred. The significance of this interaction is confounded by the interaction between Alar and the buffer (K_2HPO_4 : citric acid) (Graphs (5), (6) Figure 27). 1st internode sections cut from Avena seedlings which had been treated with 4000 ppm Alar were more responsive to auxin and gibberellin (in combination) than analogous sections cut from the 2000 ppm treatment. This effect is contrary to results obtained by Kuraishi and Muir (1963) with CCC treated Avena coleoptiles. These workers showed that growth retardation induced by 1×10^{-2} M CCC was more readily counteracted by auxin and gibberellin than the growth retardation imposed by 3×10^{-2} M CCC.

In order to suggest an explanation for the effects which were obtained with differing Alar treatments on Avena 1st internode sections, a hypothetical reaction scheme was devised such that factors A, B, and C represent reactions leading to normal section elongation.



It is suggested that factor A represents the precursor pool necessary for the production of growth factor C, via the intermediary, factor B. Let Alar inhibit the reaction;

Then levels of factor B would accumulate; the accumulation of B would trigger the formation of product X. Such a diversion is representative of feed-back control.

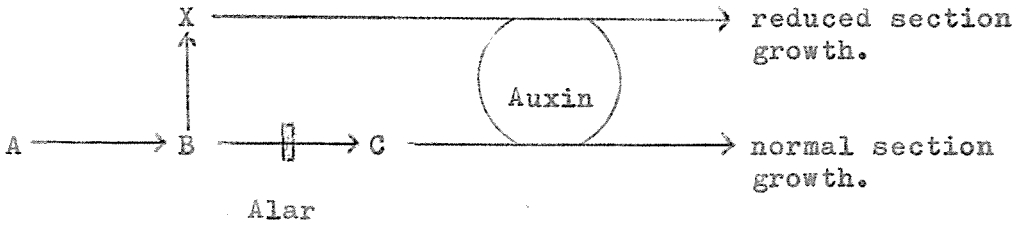
If the degree of Alar inhibition is related to the concentration applied (as shown by the degree of retardation) then the diversion of factor B to the product X would be greater at 4000 ppm than at 2000 ppm. Hence, sections cut from the 2000 ppm Alar treated seedlings would contain low levels of both factor C and product X while sections from the analogous 4000 ppm treatment would contain predominantly higher levels of product X than factor C.

Sections from Alar treated seedlings respond to auxin and to auxin and gibberellin in combination. This would argue that the product X is active in the growth promotion. This is a reasonable assumption in the light of the fact that sections from Alar treated seedlings incubated in the presence of buffer show increased growth over those of controls (Table 19a). Furthermore, this effect is more pronounced when sections are incubated in the presence of Alar (Table 19b). In all cases the stimulation in growth increases with Alar concentration. Alternatively, exogenous auxin and gibberellin could substitute for the factor C. If this was the case the increase in growth in the absence of auxin and gibberellin would not be expected.

Sections cut from Alar treated seedlings (2000 ppm) and incubated in the presence of Alar (2000 ppm) and auxin, alone and in combination with gibberellin, show a similar growth response to those from the 4000 ppm treatments. This effect can be explained in terms of the hypothetical model. Continued inhibition of the reaction B$\longrightarrow$C

during incubation may result in accumulation of factor B, and hence product X, to the level found with the 4000 ppm Alar treatment.

Even in the presence of auxin and gibberellin, sections from Alar treated seedlings do not respond to the same extent as analogous sections from control treatments. Although this effect suggests that a further process may be affected by Alar it is also possible that the product X and factor C both compete for exogenous auxin or gibberellin, or both to the extent that the overall growth response is less than that experienced where only factor C is present (control treatments). As a corollary to this proposed competition the formation of the product X - growth promoter complex must result in a reduced growth response compared to that achieved by the factor C - growth promoter interaction. The argument would be considerably strengthened if the product X is the more successful in terms of the proposed competition. In the case of Avena 1st internode sections, exogenous auxin promotes section growth to a far greater extent than exogenous gibberellin. Further, exogenous gibberellin did not appreciably promote the growth of sections incubated in the presence of Alar. This could be taken as evidence that product X and factor C compete for auxin rather than gibberellin. The overall model can be shown as:



Chapter 7

F I N A L D I S C U S S I O N

Chapter 7

FINAL DISCUSSION

Analysis of endogenous auxins and gibberellins from Alar treated apple shoots has not been previously attempted. However, evidence of a possible interaction between Alar and gibberellin in apple has been presented by Edgerton and Hoffman (1965), Greenhalgh and Edgerton (1966) and Luckwill (1966). In all the above reports shoot growth has been used as the criterion for the measurement of the interaction. Similar studies have been attempted with CO11 on Phaseolus vulgaris (Bukovac 1964) and Alar on cucumber (Moore 1966). Bukovac concluded that CO11 could interfere with the biosynthesis of endogenous gibberellin although Moore proposed that Alar may affect some other process which would be essential for shoot growth.

The weight of evidence presented in the literature concerning the mode of action of other growth retardants favours a direct interaction with the biosynthetic pathway of endogenous gibberellins. In order to clarify the observed interaction in whole plant systems, several workers have made detailed studies on Fusarium moniliforme (Kende et al. 1963, Ninnemann et al. 1964, Harada et al. 1965) and on higher plants (Ruddat 1966, Jones et al. 1967, Reid et al. 1967, Dale et al. 1968). With the exception of Alar and phosphon D, all growth retardants tested inhibited gibberellin biosynthesis in F. moniliforme. AMO 1618 and CCC were shown to inhibit gibberellin

biosynthesis in higher plants. No reports have been made of similar investigations with Alar although Dennis et al. (1964) showed that Alar caused a partial inhibition of the cyclization of trans geranyl geranyl pyrophosphate to (-)-kaurene.

It was found in this study that endogenous gibberellin-like substances extracted from Alar treated apple shoots decreased with early stages of shoot elongation. This is in agreement with results obtained by Jones et al. (1967) and Reid and Carr (1967) with CCC. However, gibberellin-like substances from apical sections of well developed extension growth increased with respect to controls. This increase was shown to be due to the presence of anomalous fractions in Alar treatments which exhibited gibberellin-like activity in the barley endosperm bioassay. The increase in anomalous fractions was accompanied by a concomitant decrease in fractions isolated in control treatments. If Alar induced a biosynthetic block sufficiently near to the gibberellin end product it is conceivable that precursor diversion would result in the production of abnormal gibberellins (Reid and Carr 1967). The mechanism of this proposed blockage is unknown.

In comparison to the research done on the gibberellin-retardant interaction, the possible interaction between growth retardants and endogenous auxins has received little attention. Kuraishi and Muir (1963) showed that diffusable auxin levels from pea 5th internode decreased with CCC treatment. Reduced auxin levels in retardant treated cucumber hypocotyls were thought to be due to increased IAA oxidase and peroxidase activity (Halevy 1963). However, an alternative mode of action was suggested by Reed (1965) Reed et al. (1965). These workers

showed that the hydrazines could inhibit tryptamine oxidation and hence decrease levels of endogenous IAA.

No evidence could be found in the present study to support the claim advanced by Halevy (1963); namely that Alar decreased auxin levels via increased IAA oxidase and peroxidase activity. Furthermore, the evidence brought forward by Reed (1965) Reed et al. (1965) has little bearing on the present study as Luckwill (1956) showed that endogenous auxins located from apple fruit and leaf tissue in no way resembled IAA. The fact that endogenous auxin (on a dry weight basis) increased with Alar treatment would argue that Alar blocks the auxin site of action. However, reappraisal of histograms in the qualitative sense showed that auxin fractions present in control treatments are generally absent from 4000 ppm Alar treatments. This evidence suggests that Alar blocks auxin biosynthesis in apple shoots. Concomitant development of anomalous fractions (acidic and neutral auxins) adds further support to the above claim.

The possibility that changes in endogenous auxins could be due indirectly to changes in endogenous gibberellins cannot be discounted. Anomalous peak development was more pronounced in harvest dates 5/12/67 and 26/12/67 than in harvest date 7/11/67 and furthermore, some auxin fractions which were present in both control and Alar treatment histograms accumulated with increasing Alar concentration.

The shoot apex has been shown to be a primary site of auxin and gibberellin synthesis. Young leaves surrounding the apical bud rather than the apical meristem itself, appear to be the principal organs of biosynthesis. Application of labelled Alar to apple seedlings and

mature trees showed that the retardant accumulated in leaves and stems (Martin et al. 1964, 1966). Alar appears to be stable over the time in which it was physiologically active (Martin et al. 1964). This evidence suggests that the succinic acid 2,2-dimethyl hydrazide (Alar) molecule is the active growth retarding compound. However, Reed et al. (1965) proposed that 0.1% hydrolysis of the administered Alar to yield 1,1-dimethyl hydrazine could produce a 50% inhibition of tryptamine oxidation. The difficulty arises when attempts are made to rationalize breakdown studies (Martin et al. 1964, 1966) with the high concentrations needed to elicit a response (2000 - 4000 ppm).

In order to clarify this point an attempt was made in the present study to assess Alar levels present in aqueous residues remaining after gibberellin extraction. These aqueous residues were passed through an ion exchange column (IR 120 H^+) and the bound retardant eluted with 7.5 N NH_4OH (Martin et al. 1964). Aliquots (1 ml) were taken from the eluate, adjusted to pH 7.0 (HCl), 0.2 ml of 0.5% $FeCl_3$: 0.5% $KFeCN_6$ added, and the volume made up to 10 ml. Colour development was read at 520 mμ. These determinations proved to be unsuccessful because colour development was influenced by compounds, apart from Alar, present in the eluate. Furthermore, analogous treatment of known Alar concentrations did not yield constant results. Never-the-less, the fact that Alar retards shoot growth in the year after application and that Alar has been detected in dormant spur and shoot tissue (Edgerton et al. 1967) would argue that the active compound is the unmetabolised molecule rather than a product of hydrolysis.

To summarize the evidence presented in this discussion the

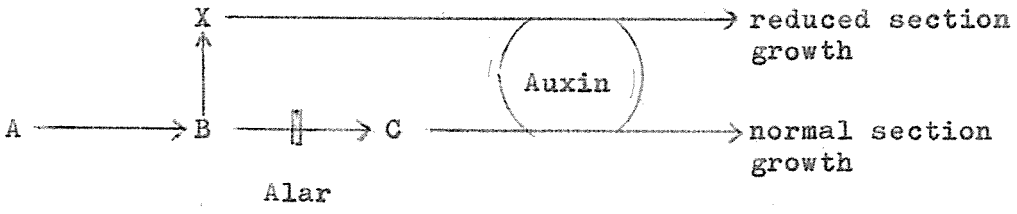
following points can be made:

(i) Alar accumulates in apple shoot apices.

(ii) Alar inhibits the biosynthesis of gibberellin-like substances in this general area.

(iii) Alar treatment alters endogenous auxin biosynthesis, this effect is either due to a direct interaction with applied Alar or to an indirect reaction with reduced gibberellin levels.

In order to explain the interaction between Alar and exogenous growth promoters (auxin and gibberellin) on Avena 1st internode sections a hypothetical model was constructed;



where A represents the precursor pool necessary for the formation of the growth factor C. Alar inhibition of the reaction B C would result in the formation of product X by means of feed back control. To complete the model, a competitive interaction was suggested to occur between product X and factor C for available auxin such that the combination of product X-auxin resulted in a much lower growth response than that obtained with factor C auxin combination.

This model can also be applied to results obtained from Alar treated Gravenstein apple:

(i) If factor C represented endogenous gibberellin, then the formation of product X may represent the diversion of the precursor pool (factor A) to the biosynthesis of 'abnormal' gibberellins. According to the model, both factor C and product X would compete for the endogenous auxin pool; the interaction between auxin and product X resulting in a reduced growth response to that achieved with auxin and factor C.

(ii) Significant differences were detected between control, 2000, 3000, and 4000 ppm Alar treatments and not between control and 1000 ppm Alar treatments. However, shoot retardation with the 2000 ppm treatment did not become significant until 77 days after Alar application as opposed to 42 days for the 3000 and 4000 ppm treatments. This differential in response can be explained; with increasing Alar concentration, product X formation would increase at the expense of factor C. With low Alar concentrations, (1000 ppm), the presence of factor C would allow shoot growth to approach that of controls. At high Alar concentrations (3000 - 4000 ppm) factor C may be absent from the reaction altogether and shoot growth would become dependent on the growth promoting effect of the auxin-product X interaction.

A further point must be considered in relation to changes in stem dry weight. Internode dry weight decreased with increasing Alar concentration and this effect became more pronounced with time from

application. Stoddart (1965) showed that CCC blocked soluble carbohydrate conversion to protein and structural polysaccharide with resultant diversion to storage polysaccharide.

If this were the case in apple and provided that storage polysaccharide was deposited in stem tissue it could be expected that stem dry weight would be approximately the same for all treatments. This, in fact, was not the case and the mechanism responsible for this weight loss remains unexplained. Some loss in storage polysaccharide could occur via glycolysis to compensate for uncoupled phosphorylation (Heatherbell et al. 1966) although it is doubtful if the loss to glycolysis would compensate for the dry weight loss.

SOME FINAL RECOMMENDATIONS

Clarification of several points raised in this study could be achieved by a similar investigation using a "soft" herbaceous plant (e.g. pea) which has one, or more dwarf cultivars.

In order to determine possible growth promoting activity of anomalous fractions detected in Alar treatment histograms the dwarf cultivar could be used as a bioassay; gibberellin promotes stem growth in dwarf cultivars.

If the Alar treatment was substituted with 1,1-dimethyl hydrazine further insight into the active form of the retardant could be obtained.

APPENDIX I

Appendix I

A. BUFFER COMPOSITION

I. Sodium Phosphate Buffer (pH 8.4)

Add 10 ml NaH_2PO_4 (0.1 M) to 990 ml Na_2HPO_4 (0.1 M).

II. Di-potassium Hydrogen Phosphate - Citric Acid Buffer (pH 5.0)

(i) Dissolve 1.794 gm K_2HPO_4 in 20 ml distilled water.

(ii) Add 1.019 gm citric acid.

(iii) Make up to 1 litre.

(iv) Add and dissolve 20 gm sucrose.

(v) Store at $5^{\circ}C$.

B. REAGENTS FOR REDUCING SUGAR ANALYSIS

I. Somogyi's Reagent (copper reagent)

(i) Dissolve 24 gm anhydrous sodium carbonate and 12 gm sodium potassium tartrate in 250 ml distilled water.

(ii) Add 40 ml copper sulphate solution (4 gm $CuSO_4$ 5. H_2O in 40 ml distilled water).

(iii) Add and dissolve 16 gm sodium bicarbonate.

(iv) Dissolve 180 gm anhydrous sodium sulphate in 500 ml distilled water, boil, and when cool add to above.

(v) Make up to 1 litre.

(vi) Store at 37° for 24 hours and filter after 3-4 days.

II. Nelson's Reagent (arsenomolybdate reagent)

(i) Dissolve 25 gm ammonium molybdate in 450 ml distilled water.

(ii) Add 21 ml concentrated H_2SO_4.

(iii) Dissolve 3 gm sodium arsenate (Na_2HAsO_4 7. H_2O) in 25 ml distilled water and add to above.

(iv) Incubate at 37° for 24 hours and store in brown bottle.

A P P E N D I X II

Appendix II

A. ANALYSIS OF VARIANCE

I. Internode Length

In order to distinguish between Alar treatment effects on internode elongation, analyses of variance (multiple classification) were carried out on internode measurements from selected harvest dates. Where treatments were shown to differ significantly by the variance ratio test, individual treatment means were compared using the t test (Snedecor 1956, Glenday 1964).

II. Leaf Area

Analyses of variance (single classification) (Snedecor 1956) were attempted between leaf area results from various Alar treatments. Reference was made to comments outlined by Glenday (1964).

TABLE A

Harvest Date 24/10/67

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.10	0.11	0.11	0.10	0.11
2	0.10	0.11	0.11	0.10	0.11
3	0.13	0.15	0.15	0.13	0.15
4	0.17	0.19	0.21	0.16	0.16
5	0.22	0.26	0.24	0.21	0.23
6	0.34	0.35	0.30	0.31	0.32
7	0.49	0.47	0.57	0.39	0.41
8	0.92	0.81	0.87	0.66	0.69
9	1.57	1.29	1.31	0.99	1.03
Total	4.04	3.74	3.87	3.05	3.21

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	0.082	4	0.0205	3.01	2.7 (4.0)	*
Varieties	5.906	8	0.7383	108.57	2.3 (3.2)	**
Residual	0.217	32	0.0068			
Total	6.206	44				

TABLE B

Harvest Date 31/10/67

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.11	0.13	0.11	0.15	0.13
2	0.13	0.15	0.15	0.15	0.14
3	0.15	0.22	0.19	0.20	0.20
4	0.23	0.27	0.23	0.25	0.25
5	0.27	0.27	0.31	0.32	0.32
6	0.37	0.35	0.43	0.42	0.39
7	0.55	0.61	0.69	0.63	0.62
8	0.92	0.91	1.16	0.99	0.91
9	1.43	1.53	1.44	1.55	1.47
Total	4.16	4.44	4.71	4.66	4.43

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	0.020	4	0.005	2.67	2.7 (4.0)	NS
Varieties	8.447	8	1.055	564.6	2.3 (3.2)	**
Residual	0.060	32	0.0018			
Total	8.527	44				

TABLE C

Harvest Date 7/11/67

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.15	0.15	0.18	0.16	0.21
2	0.19	0.13	0.15	0.15	0.12
3	0.21	0.18	0.19	0.18	0.17
4	0.27	0.27	0.27	0.24	0.23
5	0.33	0.29	0.29	0.25	0.27
6	0.39	0.34	0.35	0.35	0.35
7	0.64	0.52	0.50	0.49	0.63
8	1.04	0.77	0.73	0.77	1.04
9	1.67	1.30	1.25	1.51	1.57
10	2.57	2.00	1.82	1.99	2.11
11	2.98	2.47	2.52	2.21	2.31
Total	10.44	8.42	8.25	8.30	9.01

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	0.306	4	0.076	4.91	2.6 (3.8)	**
Varieties	35.117	10	3.511	225.1	2.1 (2.8)	**
Residual	0.627	40	0.015			
Total	36.051	54				

TABLE D

Harvest Date 28/11/67

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.17	0.13	0.23	0.19	0.19
2	0.11	0.10	0.11	0.10	0.11
3	0.16	0.11	0.17	0.13	0.12
4	0.21	0.15	0.21	0.15	0.15
5	0.23	0.17	0.27	0.17	0.15
6	0.32	0.24	0.39	0.28	0.27
7	0.49	0.47	0.67	0.54	0.40
8	0.83	0.73	1.15	0.97	0.74
9	1.47	1.30	1.71	1.37	1.28
10	2.17	1.83	2.25	2.03	1.78
11	2.97	2.41	2.73	2.41	2.12
12	3.78	2.95	3.07	2.75	2.60
13	3.83	3.25	3.07	2.73	2.71
14	3.58	2.90	2.73	2.52	2.34
Total	20.32	16.74	18.76	16.34	14.96

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	1.274	4	0.318	7.39	2.6 (3.7)	**
Varieties	94.384	13	7.26	168.56	1.95(2.56)	**
Residual	2.239	52	0.043			
Total	97.898	69				

TABLE E

Harvest Date 19/12/67

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.13	0.12	0.11	0.11	0.11
2	0.10	0.10	0.10	0.10	0.10
3	0.11	0.10	0.10	0.10	0.10
4	0.15	0.12	0.11	0.11	0.13
5	0.19	0.13	0.13	0.13	0.15
6	0.22	0.19	0.18	0.17	0.20
7	0.35	0.29	0.24	0.23	0.32
8	0.65	0.48	0.44	0.42	0.53
9	1.00	0.96	0.78	0.74	0.87
10	1.57	1.42	1.35	1.13	1.33
11	2.43	2.04	1.91	1.69	1.68
12	3.17	2.81	2.50	2.19	1.95
13	3.73	3.11	2.73	2.41	2.27
14	3.76	2.78	2.63	2.27	1.87
15	3.38	2.85	2.44	1.93	1.63
16	3.11	2.45	2.10	1.77	1.73
17	2.84	2.53	1.97	1.59	1.49
18	2.89	2.57	2.00	1.39	1.51
Total	29.78	25.05	21.82	18.48	17.97

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	5.364	4	1.091	11.06	2.5 (3.7)	**
Varieties	97.232	17	5.719	58.00	1.8 (2.3)	**
Residual	6.709	68	0.0986			
Total	109.307	89				

TABLE F

Harvest Date 2/1/68

Mean internode length (cm) from fifteen samples

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.18	0.10	0.10	0.10	0.10
2	0.10	0.10	0.10	0.10	0.10
3	0.11	0.10	0.10	0.10	0.10
4	0.15	0.10	0.10	0.10	0.10
5	0.17	0.12	0.19	0.15	0.10
6	0.17	0.21	0.22	0.23	0.12
7	0.22	0.25	0.29	0.25	0.24
8	0.37	0.41	0.52	0.49	0.41
9	0.80	0.85	0.87	0.91	0.71
10	1.24	1.24	1.44	1.05	1.17
11	2.21	1.75	1.87	1.71	1.60
12	2.85	2.33	2.57	2.10	1.90
13	3.77	2.99	2.92	2.52	2.18
14	3.58	2.89	2.64	2.42	2.22
15	3.14	2.75	2.68	2.35	2.01
16	3.00	2.71	2.40	2.11	2.07
17	2.82	2.23	2.23	1.67	1.89
18	2.62	2.25	2.03	1.75	1.78
19	2.57	2.47	2.03	1.78	1.71
20	2.53	2.20	1.94	1.63	1.68
Total	32.60	26.55	27.24	23.52	22.19

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	1.78	4	0.445	6.27	2.5 (3.7)	**
Varieties	109.15	19	5.74	80.95	1.8 (2.3)	**
Residual	5.395	76	0.070			
Total	116.333	99				

TABLE G

Harvest Date 12/2/68

Mean internode length (cm) from one sample

Internode Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	0.1	0.2	0.1	0.1	0.1
2	0.2	0.1	0.1	0.1	0.1
3	0.2	0.1	0.2	0.1	0.2
4	0.3	0.1	0.1	0.1	0.1
5	0.5	0.3	0.2	0.1	0.1
6	1.2	0.4	0.3	0.1	0.2
7	2.5	0.9	0.5	0.2	0.3
8	3.9	1.6	1.1	0.3	0.7
9	4.0	2.0	1.9	0.5	1.3
10	3.5	2.6	1.9	1.2	1.5
11	3.6	3.6	2.8	1.6	1.5
12	3.5	2.6	2.3	1.8	1.6
13	2.4	2.5	2.3	2.3	1.5
14	3.1	2.7	2.6	2.4	1.0
15	3.6	2.1	2.1	1.8	1.1
16	3.3	2.3	2.4	1.8	0.9
17	3.6	2.2	2.4	1.4	0.7
18	3.1	2.6	2.4	2.1	1.2
19	3.2	2.4	1.8	1.8	1.3
20	3.2	2.0	2.5	2.0	0.9
21	3.1	2.4	2.3	1.8	1.2
22	2.9	2.1	2.9	2.1	1.2
23	3.0	3.2	2.2	1.9	1.4
24	2.3	2.3	1.7	1.9	1.2
25	2.1	2.0	2.0	2.1	1.2
26	2.5	1.8	2.7	1.1	1.0
27	1.6	2.0	1.5	1.3	0.8
28	1.5	1.4	1.5	1.6	1.2
29	1.4	1.1	1.7	1.3	1.0
30	0.7	0.8	1.1	1.1	1.3
31	0.1	0.3	0.4	0.3	0.1
32	0.1	0.2	0.1	0.1	0.1
Total	70.3	52.9	50.1	38.4	28.0

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	31.808	4	7.95	32.59	2.5 (3.5)	**
Varieties	113.658	31	3.66	15.00	1.6 (1.9)	**
Residual	30.344	124	0.24			
Total	175.81	159				

TABLE H

Average leaf area (x 10 cm^2) of each shoot sample

Harvest Date	Sample Number	Alar Treatment (ppm)				
		Control	1000	2000	3000	4000
24/10/67	1	3.67	2.96	1.73	2.93	3.90
	2	3.76	3.81	4.70	2.88	3.90
	3	3.22	3.85	2.28	3.29	2.48
	4	3.52	4.37	2.34	3.74	2.78
	5	2.86	3.03	2.99	3.33	2.88
	Total	17.12	18.02	15.04	16.17	15.94
31/10/67	1	3.85	4.57	3.73	3.40	4.58
	2	4.75	4.56	3.30	4.30	3.88
	3	4.42	4.00	3.90	3.82	5.24
	4	4.09	3.16	3.99	3.88	3.91
	5	4.58	3.70	2.92	3.98	4.09
	Total	21.69	19.99	17.84	19.38	21.70
7/11/67	1	5.14	3.87	3.89	3.43	4.98
	2	4.06	3.87	4.31	3.50	4.38
	3	4.49	4.25	3.15	4.63	3.98
	4	3.34	4.41	4.78	4.52	4.27
	5	3.55	4.39	4.52	3.12	4.28
	Total	20.58	20.79	20.65	19.20	21.89
14/11/67	1	5.09	3.42	4.32	4.01	5.57
	2	3.56	4.05	4.60	2.76	3.59
	3	5.01	4.84	3.78	4.29	4.74
	4	4.41	3.19	3.81	3.47	2.87
	5	5.88	3.37	4.12	3.80	5.28
	Total	23.95	18.87	20.63	18.33	22.05
21/11/67	1	3.99	3.66	4.16	4.01	2.94
	2	3.44	3.99	4.21	3.40	3.80
	3	3.66	4.50	4.40	3.78	3.08
	4	3.93	3.56	3.34	4.29	3.45
	5	3.67	3.66	3.64	4.37	4.05
	Total	18.69	19.37	19.75	19.85	17.32
28/11/67	1	3.44	3.34	4.51	3.58	4.45
	2	3.11	4.37	4.37	3.64	4.18
	3	4.56	4.17	3.37	3.95	4.04
	4	5.01	3.61	4.44	3.86	3.61
	5	4.32	4.40	4.09	4.66	3.93
	Total	20.44	19.89	20.78	19.69	20.21
12/12/67	1	2.68	3.76	3.86	3.68	3.45
	2	4.03	4.61	4.61	3.64	3.97
	3	4.72	4.35	4.16	4.62	3.93
	4	4.00	3.36	3.37	4.09	4.22
	5	3.94	3.71	4.62	3.33	4.09
	Total	19.37	19.79	20.62	19.36	19.66

TABLE I

Harvest Date 14/11/67

Average leaf area (x 10 cm^2) of each shoot sample

Sample Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	5.09	3.42	4.32	4.01	5.57
2	3.56	4.05	4.60	2.76	3.59
3	5.01	4.84	3.78	4.29	4.74
4	4.41	3.19	3.81	3.47	2.87
5	5.88	3.37	4.12	3.80	5.28
Total	23.95	18.87	20.63	18.33	22.05

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	4.266	4	1.066	1.78	2.9 (4.4)	N.S.
Residual	11.949	20	0.597			
Total	16.215	24				

TABLE J

Harvest Date 5/12/67

Average leaf area (x 10 cm^2) of each shoot sample

Sample Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	3.70	4.33	3.79	3.85	4.43
2	3.65	4.85	3.69	3.56	3.70
3	3.54	3.84	3.78	4.03	3.85
4	4.32	4.63	4.28	2.92	4.68
5	5.08	3.49	4.27	3.93	4.14
Total	20.29	21.14	19.81	18.29	20.80

Analysis of variance

Source of variation	Sums of squares	d.f.	Mean squares	F.	5% (1%) points of F.	
Blocks	0.992	4	0.248	1.05	2.9 (4.4)	N.S.
Residual	4.718	20	0.235			
Total	5.710	24				

TABLE K

Harvest Date 19/12/67

Average leaf area (x 10 cm^2) of each shoot sample

Sample Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	4.44	4.08	3.54	3.80	4.02
2	3.14	3.72	3.98	3.17	4.51
3	4.21	3.48	3.23	3.67	3.82
4	4.02	3.07	3.81	4.56	4.41
5	4.79	4.11	3.39	3.30	3.61
Total	20.60	18.46	17.95	18.50	20.37

Analysis of variance

Source of variation	Sums of squares	d.f.	squares	F.	5% (1%) points of F.	
Blocks	1.185	4	0.296	1.33	2.9 (4.4)	N.S.
Residual	4.437	20	0.221			
Total	5.622	24				

TABLE L

Harvest Date 2/1/68

Average leaf area (x 10 cm^2) of each shoot sample

Sample Number	Alar Treatment (ppm)				
	Control	1000	2000	3000	4000
1	2.59	3.04	2.44	3.84	3.53
2	2.96	2.95	4.19	3.50	2.00
3	3.72	3.53	3.51	2.91	3.28
4	3.80	2.81	3.86	2.89	3.21
5	2.24	3.13	2.85	2.80	3.00
Total	15.31	15.46	16.85	15.94	15.02

Analysis of variance

Source of variation	Sums of squares	d.f.	squares	F.	5% (1%) points of F.	
Blocks	0.410	4	0.102	0.314	5.8 (14.2)	N.S.
Residual	6.492	20	0.324			
Total	6.902	24				

A P P E N D I X III

Figure I. Optical density response to increasing cyanidin chloride concentration.

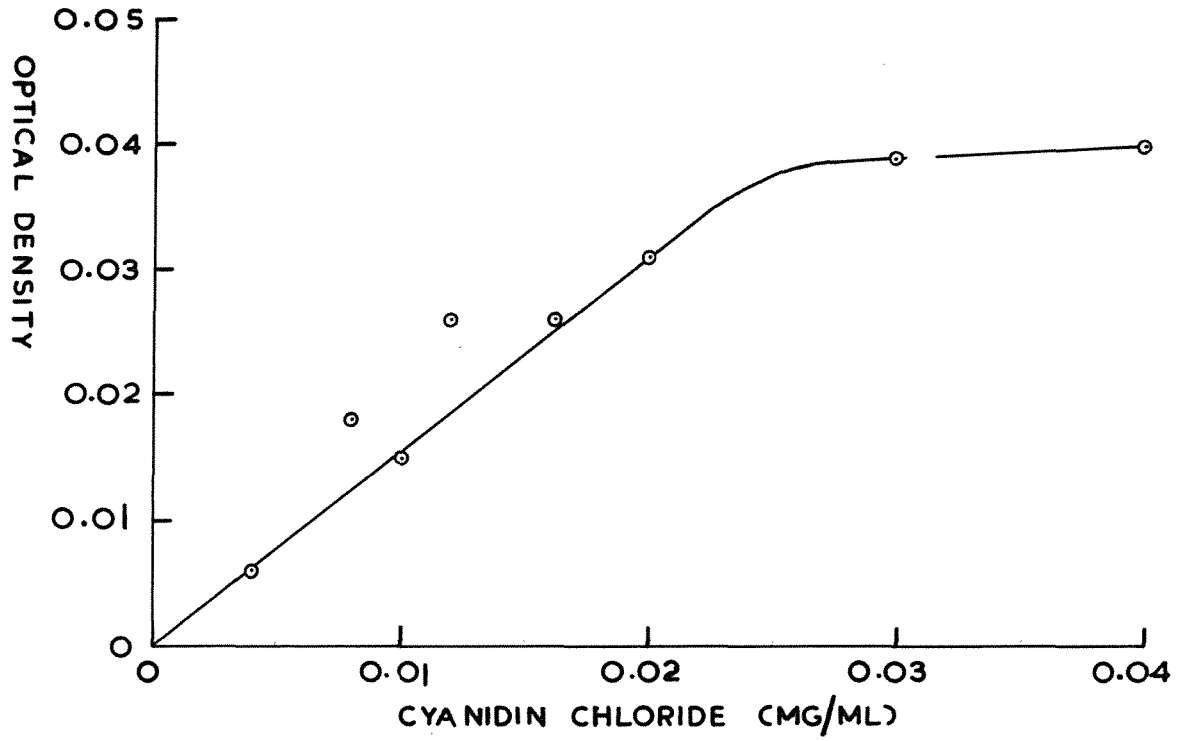
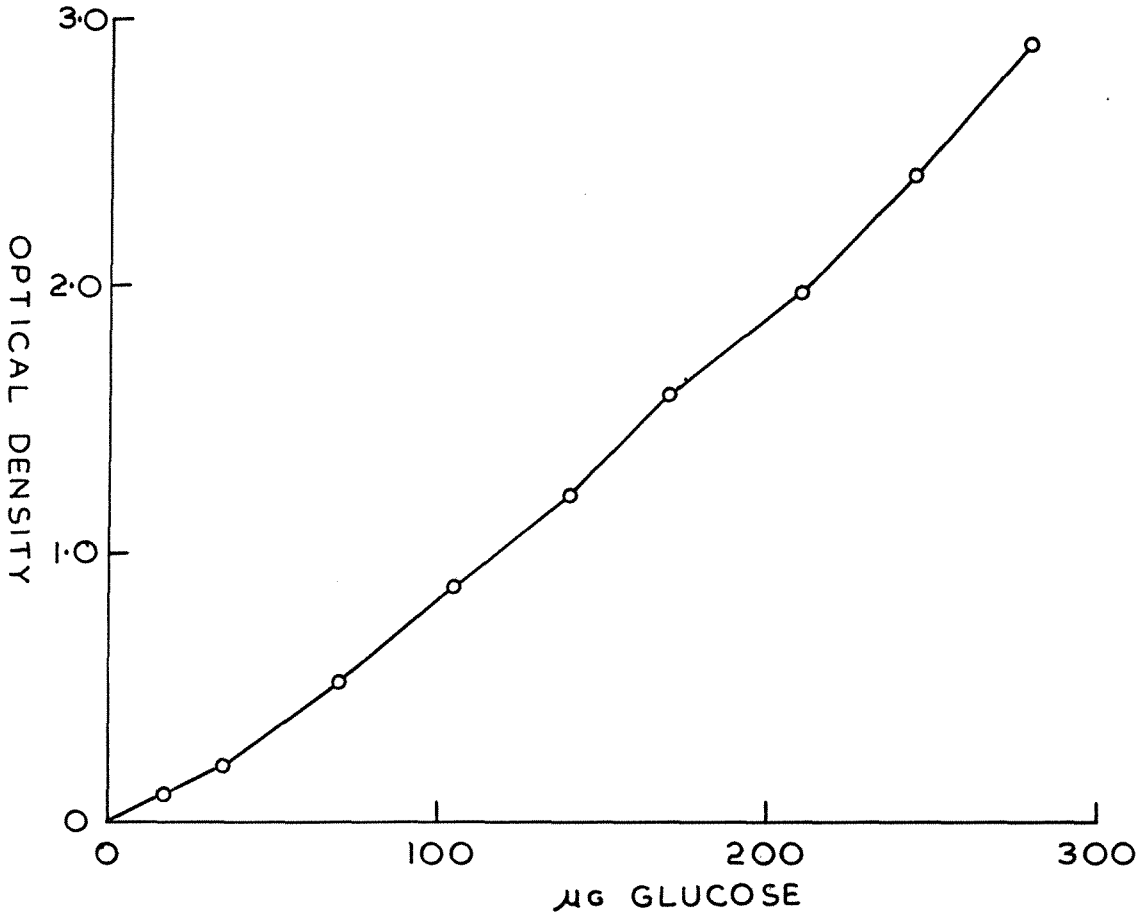


Figure II. Optical density response to increasing glucose concentration.



REFERENCES

ARNEY, S.E., and MANCINELLI, P. (1966)
The basic action of gibberellic acid in elongation of meteor pea stems.
New Physiol. 65 : 161.

AUDUS, L.J. (1965)
Plant growth substances.
(Polunin, L. Ed), London, Leonard Hill, 2nd Edn. pp.126-128.

BATJER, L.P., WILLIAMS, M.W., and MARTIN, G.C. (1964a)
Dwarfing fruit trees.
Ag. Res. Wash. 13 : (5).

BATJER, L.P. (1965)
Progress report on the use of B-nine on fruit trees.
Proc. Wash. St. Hort. Ass. p.27.

BATJER, L.P., WILLIAMS, M.W., and MARTIN, G.C. (1964b)
Effects of B-nine on vegetative and fruit characteristics of apples, pears, and sweet cherries.
Proc. Am. Soc. Hort. Sci. 85 : 11.

BATJER, L.P., and WILLIAMS, M.W. (1966)
Effects of N-dimethyl amino succinamic acid (Alar) on watercore and harvest drop of apples.
Proc. Am. Soc. Hort. Sci. 88 : 77.

BALDEV, B., LANG, A., and AGATEP, A.O. (1965)
Gibberellin production in pea seeds developing in excised pods. Effect of growth retardant AMO 1618.
Science 147 : 155.

BRIAN, P.W. (1959)
Effects of gibberellins on plant growth and development.
Biological Review 34 : 37.

BROOK, J., WEST, S.H., and ANTHONY, D.S. (1967)
Effects of Phosphon S on nucleic acid metabolism in Psium sativum 'Alaska'.
Plant Physiol. 42 (6) : 785.

BROOKS, H.J. (1964)
Responses of pear seedlings to B_{995}, a growth retardant.
Nature 203 : 1303.

BUKOVAC, M.J. (1964)
Modification of the vegetative development of Phaseolus vulgaris with N,N-dimethyl amino maleamic acid.
Amer. J. Bot. 51 (5) : 480.

BURNETT, D., AUDUS, L.J., and ZINSMEISTER, H.D. (1965)
Growth substances in the roots of Vicia faba. II.
Phytochem. 4 : 891.

CATHEY, H.M., and MARTH, P.C. (1960)
Effectiveness of a quaternary ammonium carbamate and a phosphonium in controlling growth of Chrysanthemum morifolium (Ramat.).
Proc. Amer. Soc. Hort. Sci. 76 : 609.

CATHEY, H.M. (1964)
Physiology of growth retarding chemicals.
Ann. Rev. Plant Physiol. 15 : 271.

COOMBE, B.G., COHEN, D., and PALEG, L.G. (1967)
Barley endosperm bioassay for gibberellins. I. Parameters of the response system.
Plant Physiol. 42 : 105.

DAHLGREW, G., and SIMMERMAN, N.L. (1963)
Intramolecular catalysis of the hydrolysis of N-dimethyl amino maleamic acid.
Science 140 : 485.

DALE, J.E., FELIPPE, G.M. (1968)
The gibberellin content and early seedling growth of plants of Phaseolus vulgaris treated with the growth retardant CCC.
Planta (Berl.) 80 : 288.

DENNIS, D.T., UPPER, C.D., and WEST, C.A. (1965)
An enzymic site of inhibition of gibberellin biosynthesis by AMO 1618 and other plant growth retardants.
Plant Physiol. 40 : 948.

DOWNS, R.J., and CATHEY, H.M. (1960)
Effects of light, gibberellin, and a quaternary ammonium compound on the growth of dark-grown red kidney beans.
Botanical Gazette 121 : 233.

EDGERTON, L.J. (1964)
New growth retardant for fruit trees.
Farm Res. XXX No.1 : 11.

EDGERTON, L.J., and HOFFMAN, M.B. (1965)
Some physiological responses of apple to N-dimethylamino succinamic acid and other growth regulators.
Proc. Amer. Soc. Hort. Sci. 86 : 28.

EDGERTON, L.J., and GREENHALGH, W.J. (1967)
Absorption, translocation and accumulation of labeled N-dimethylamino succinamic acid in apple tissue.
Proc. Amer. Soc. Hort. Sci. 91 : 25.

EMERSON, F.H., and DOSTAL, H.C. (1966)
The differential response of apple cultivars to foliar applications of N-dimethylamino succinamic acid.
Proc. XVII International Hort. Cong. 1.

GALSTON, A.W., and WARBURG, Hava. (1959)
An analysis of auxin-gibberellin interaction in pea stem tissue.
Plant Physiol. 34 : 16.

GALSTON, A.W., and PURVES, W.K. (1960)
The mechanism of action of auxin.
Ann. Rev. Plant Physiol. 11 : 239.

GLENDAY, A.C. (1964)
Biometrics lecture course.
Massey University.

GREENHALGH, W.J., and EDGERTON, L.J. (1966)
Interactions of growth retardant (Alar) and gibberellin sprays on growth and flowering of apple.
Proc. XVII International Hort. Cong. 1.

GREENHALGH, W.J., and EDGERTON, L.J. (1967)
Interaction of Alar and gibberellin on growth and flowering of apple.
Proc. Amer. Soc. Hort. Sci. 91 : 9.

GUTTRIDGE, C.G. (1966)
The interaction of (2-chloroethyl) trimethyl ammonium chloride and gibberellic acid in strawberry.
Physiol. Plant 19 : 397.

HALEVY, A.H. (1962a)
Interaction between gibberellin and quaternary ammonium carbamates in growth of cucumber Cucumis sativus (L) seedlings.
Bull. Res. Council Israel. 11D (2).

HALEVY, A.H. (1962b)
Effect of hardening and chemical treatments on drought resistance of gladiolus plants.
Proc. XVI International Hort. Cong. 4.

HALEVY, A.H., and MONSELISE, S.P. (1963)
Interaction of gibberellin and CCC on changes in dry weight in bean leaves during the night.
PHYTON 20 (2) : 79.

HALEVY, A.H. (1963)
Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings.
Plant Physiol. 38 (6) : 731.

HALEVY, A.H., and WITTWER, S.H. (1965)
CCC, B-nine overnight treatment prolongs life of cut flowers.
F. and N. Exchange, April 24.

HALEVY, A.H., DILLEY, D.R., and WITTWER, S.H. (1966)
Senescence inhibition and respiration induced by growth retardants and N^6-benzyladenine.
Plant Physiol. 41 : 1085.

HARADA, H., and LANG, A. (1965)
Effect of some (2-chloroethyl) trimethyl ammonium chloride analogs and other growth retardants on gibberellin biosynthesis in Fusarium moniliforme.
Plant Physiol. 40 : 176.

HARADA, Hiroshi. (1966)
Interactions between plant growth retardants and gibberellic acid (GA_3) or indoleacetic acid (IAA) as they effect the elongation of Avena first internode sections : Especially the synergistic effect of Phosphon D and GA_3.
Abstract Bot. Mag. 73 (935) : 236.

HARE, R.C. (1964)
Indoleacetic acid oxidase.
Botanical Rev. 30 : 129.

HEATHERBELL, D.A., HOWARD, B.H., and WICKEN, A.J. (1966)
The effect of growth retardants on the respiration and coupled phosphorylation of preparations from etiolated pea seedlings.
Phytochem. 5 : 635.

HUMPHRIES, E.C., and FRENCH, S.A.W. (1965a)
A growth study of sugar beet treated with gibberellic acid and (2-chloroethyl) trimethyl ammonium chloride (CCC).
Ann. Appl. Biol. 55 : 159.

HUMPHRIES, E.C., WELBANK, P.J., and WITTS, K.J. (1965b)
Effect of CCC (chlorocholine chloride) on growth and yield of spring wheat in the field.
Ann. Appl. Biol. 56 : 351.

JACKSON, D.I. (1967)
Personal communication.

JONES, R.L., and PHILLIPS, I.D.J. (1966)
Organs of gibberellin synthesis in light-grown sunflower plants.
Plant Physiol. 41 : 1381.

JONES, R.L., and PHILLIPS, I.D.J. (1967)
Effect of CCC on the gibberellin content of excised sunflower organs.
Planta (Berl.) 72 : 53.

KEFFORD, N.P. (1959)
Some growth regulators of tobacco in relation to the symptoms of the physiological disease 'Frenching'.
J. Exper. Bot. 10 : 462.

KEFFORD, N.P., and GOLDACRE, P.L. (1961)
The changing concept of auxin.
Amer. J. Bot. 48 : 643.

KENDE, H., NINNEMANN, H., and LANG, A. (1963)
Inhibition of gibberellic acid biosynthesis in Fusarium moniliforme by AMO 1618 and CCC.
Die Naturwissenschaften 50 : 599.

KENDE, H., and LANG, A. (1964)
Gibberellins and light inhibition of stem growth in peas.
Plant Physiol. 39 : 435.

KURAISHI, S., and MUIR, R.M. (1963)
Mode of action of growth retarding chemicals.
Plant Physiol. 38 : 19.

LARSEN, F.F., and CROMARTY, R.W. (1966)
Effect of N-dimethylamino succinamic acid (Alar) on micro-organism growth in relation to cut flower senescence.
Proc. Amer. Soc. Hort. Sci. 89 723.

LOCKHART, J.A. (1959)
Studies on the mechanism of stem growth inhibition by visible radiation.
Plant Physiol. 34 : 457.

LOCKHART, J.A. (1961a)
Photo-inhibition of stem elongation by full solar radiation.
Amer. J. Bot. 48 : 387.

LOCKHART, J.A. (1961b)
Interaction between gibberellins and various environmental factors on stem growth.
Amer. J. Bot. 48 : 516.

LOCKHART, J.A. (1962)
Kinetic studies of certain antigibberellins.
Plant Physiol. 37 : 759.

LOCKHART, J.A. (1964)
Physiological studies on light sensitive stem growth.
Planta (Berl.) 62 : 97.

LOONEY, N.E., FISHER, D.V., and PARSONS, J.E.W. (1967)
Some effects of annual applications of N-dimethylamino succinamic acid (Alar) to apples.
Proc. Amer. Soc. Hort. Sci. 91 : 19.

LUCKWILL, L.C. (1956)
Absence of indoleacetic acid in the apple.
Science 123 : 225.

LUCKWILL, L.C., and WEAVER, P.S. (1965)
Field trials with the growth inhibitor B-nine on apples.
Long Ash. Agric. Hort. Res. Stat. p.73.

LUCKWILL, L.C. (1966)
The effect of growth regulators on growth and apical dominance of young apple trees.
Proc. XVII International Hort. Cong. 1.

MacMILLAN, J., and SUTER, P.J. (1963)
Thin layer chromatography of the gibberellins.
Nature 197 : 790.

McCUNE, D.C., and GALSTON, A.W. (1959)
Inverse effects of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn.
Plant Physiol. 34 : 416.

MARTH, P.C. (1963)
Effect of growth retardants on flowering, fruiting, and vegetative growth of holly (Ilex).
Proc. Amer. Soc. Hort. Sci. 83 : 777.

MARTIN, G.C., BATJER, L.P., and WILLIAMS, M.W. (1964)
Movement and fate of labeled B_{995}, a size controlling compound, in apple seedlings.
Proc. Amer. Soc. Hort. Sci. 84 : 7.

MARTIN, G.C., and WILLIAMS, M.W. (1966)
Breakdown products of C^{14} labeled N-dimethyl amino succinamic acid (Alar) in the apple tree.
Proc. Amer. Soc. Hort. Sci. 89 : 1.

MISHRA, D., and PAUL, S.C. (1967)
M.H. and B-nine dependant reversal of CCC induced retardation of early shoot growth in rice.
Planta (Berl.) 74 : 368.

MITCHELL, J.W., WIRWILLE, J.W., and WEIL, L. (1949)
Plant growth regulating properties of some nicotinium compounds.
Science 110 : 252.

MONSELISE, S.P., and HALEVY, A.H. (1964)
Chemical inhibition and promotion of citrus flower bud induction.
Proc. Amer. Soc. Hort. Sci. 84 : 141.

MONSELISE, S.P., GOREN, R., and HALEVY, A.H. (1966)
Effects of B-nine, Cycocel, and benzothiazole oxyacetate on flower bud induction of lemon trees.
Proc. Amer. Soc. Hort. Sci. 89 : 195.

MOORE, T.C. (1967)
Kinetics of growth retardant and hormone interaction in affecting cucumber hypocotyl elongation.
Plant Physiol. 42 : 677.

NICHOLLS, P.B.N., and PALEG, L.G. (1963)
A barley endosperm bioassay for gibberellins.
Nature 199 : 823.

NINNEMANN, H., ZEEVART, J.A.D., KENDE, H., and LANG, A. (1964)
The plant growth retardant CCC as inhibitor of gibberellin biosynthesis in Fusarium moniliforme.
Planta (Berl.) 61 : 229.

NITSCH, J.P. and NITSCH, C. (1956)
Studies on the growth of coleoptile and first internode sections : A new sensitive straight growth test for auxin.
Plant Physiol. 31 : 94.

OSBORNE, D. (1966)
Protecting plants from cold.
New Scientist 29 : 773.

PALEG, L.G. (1960a)
Physiological effects of gibberellic acid. I. On carbohydrate metabolism and amylase activity of barley endosperm.
Plant Physiol. 35 : 293.

PALEG, L.G. (1960b)
Physiological effects of gibberellic acid. II. On starch hydrolyzing enzymes of barley endosperm.
Plant Physiol. 35 : 902.

PALEG, L.G. (1961)
Physiological effects of gibberellic acid. III. Observations on its mode of action on barley endosperm.
Plant Physiol. 36 : 829.

PALEG, L.G., COOMBE, B.G., and BUTTROSE, M.S. (1962)
Physiological effects of gibberellic acid. V. Endosperm responses of barley, wheat and oats.
Plant Physiol. 37 : 798.

PALEG, L.G., KENDE, H., NINNEMANN, H., and LANG, A. (1965)
Physiological effects of gibberellic acid. VIII. Growth retardants on barley endosperm.
Plant Physiol. 40 : 165.

PALEG, L.G. (1965)
Physiological effects of gibberellins.
Ann. Rev. Plant Physiol. 16 : 291.

PHINNEY, B.O. (1961)
Dwarfing genes in Zea mays and their relation to the gibberellins.
Fourth International Conf. Plant Growth Regul.
Iowa State Univ. Press. p.489.

PLAUT, Z., HALEVY, A.H., and SHMUELI, E. (1964)
The effect of growth retarding chemicals on growth and transpiration of bean plants grown under various irrigation regimes.
Israel J. Agric. Res. 14 (4).

REED, D.J. (1965a)
Tryptamine oxidation by extracts of pea seedlings : Effect of growth retardant β-hydroxyethyl hydrazine.
Science 148 : 1097.

REED, D.J., MOORE, T.C., and ANDERSON, J.D. (1965b)
Plant growth retardant B_{995} : A possible mode of action.
Science 148 : 1468.

REID, D.M., and CARR, D.J. (1967)
Effects of a dwarfing compound, CCC, on the production and export of gibberellin-like substances by root systems.
Planta (Berl.) 73 : 1.

RIDDELL, J.A., HAGEMAN, H.A., J'ANTHONY, C.M., and HUBBARD, W.L. (1962)
Retardation of plant growth by a new group of chemicals.
Science 136 : 391.

RUDDAT, M. (1966)
Inhibition of the biosynthesis of steviol by a growth retardant.
Nature 211 : 971.

RYUGO, K. (1966)
Persistance and mobility of Alar (B_{995}) and its effects on anthocyanin metabolism in sweet cherries, Prunus avium.
Proc. Amer. Soc. Hort. Sci. 88 : 160.

SACHS, R.M., LANG, A., BRETZ, C.F., and ROACH, J. (1960)
Shoot histogenesis : Subapical meristematic activity in a caulescent plant and the action of gibberellin and AMO 1618.
Amer. J. Bot. 47 : 260.

SACHS, R.M., and KOFRANEK, A.M. (1963)
Comparative cytohistological studies on inhibition and promotion of stem growth in Chrysanthemum morifolium.
Amer. J. Bot. 50 : 772.

SACHS, R.M., and WOHLERS, M.A. (1964)
Inhibition of cell proliferation and expansion in vitro by three stem growth retardants.
Amer. J. Bot. 51 : 44.

SACHS, R.M. (1965)
Stem elongation.
Ann. Rev. Plant Physiol. 16 : 73.

SCHERFF, R.A. (1952)
The effect of an ammonium phenylcarbamate on growth and development of bean plants.
M.A. Thesis.
(Taken from Cathey (1964) p.282).

SNEDECOR, G.W. (1956)
Statistical methods applied to experiments in agriculture and biology.
Ames : Iowa State College Press, 5th Edn.

STAHLY, E.A., and WILLIAMS, M.W. (1966)
Size control of apple, pear, cherry, and plum nursery stock with Alar.
Proc. XVII International Hort. Cong. 1.

STODDART, J.L. (1965)
Chemical changes in Lolium temulentum (L) after treatment with (2-chloroethyl) trimethyl ammonium chloride (CCC).
J. Exper. Bot. 16 : 604.

STUART, N.W. (1962)
Stimulation of flowering in azaleas and camellias.
XVI International Hort. Cong. 1.

SWAIN, T., and HILLIS, W.E. (1959)
The penolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents.
J. Sci. Food Agric. 10 : 63.

TOLBERT, N.E. (1960)
(2-chloroethyl) trimethyl ammonium chloride and related compounds as plant growth substances. II. Effect on growth of wheat.
Plant Physiol. 35 : 380.

VAN OVERBEEK, J. (1966)
Plant hormones and regulators.
Science 152 : 721.

WILLIAMS, M.W., BATJER, L.P., and MARTIN, G.C. (1964)
Effects of B-nine on apple quality.
Proc. Amer. Soc. Hort. Sci. 85 : 17.

WILLIAMS, M.W. (1967)
Personal communication.

WITTWER, S.H., and TOLBERT, N.E. (1960a)
(2-chloroethyl) trimethyl ammonium chloride and related compounds as plant growth substances. III. Effect on growth and flowering of the tomato.
Amer. J. Bot. 47 : 560.

WITTWER, S.H., and TOLBERT, N.E. (1960b)
(2-chloroethyl) trimethyl ammonium chloride and related compounds as plant growth substances. Growth, flowering, and fruiting responses as related to those induced by auxin and gibberellin.
Plant Physiol. 35 : 871.

ZEEVART, J.A.D. (1966)
Reduction of the gibberellin content of Pharbitis seeds by CCC and after effects in the progeny.
Plant Physiol. 41 : 856.