# ASPECTS OF THE HORMONAL PHYSIOLOGY OF FRUIT DEVELOPMENT IN VITIS VINIFERA L.



A thesis submitted

by

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# ASPECTS OF THE HORMONAL PHYSIOLOGY OF FRUIT DEVELOPMENT IN VITIS VINIFERA.

## SUMMARY

Two complementary studies were undertaken of the hormonal physiology of set and development of the fruit of Vitis vinifera, with special reference to gibberellin promoters and inhibitors. In one approach the effects of applications of factorial combinations of GA and CCC were measured on various parameters of cluster development. Time of application of each compound was also examined. In the second approach, estimations of endogenous GA-like and ABA-like compounds were made in extracts of flowers and fruits during the critical periods of anthesis and early pericarp development.

Pre-anthesis applications of GA increased fruit-set in the stenospermocarpic Sultana, decreased set in the parthenocarpic Cape and had no effect on three other cultivars. Anthesis applications increased the set of seedless berries in Doradillo, had no effect on Muscat, Cape or Zante, and decreased set in Sultana. Post-anthesis applications increased the set of seedless berries on cultivars other than the parthenocarpic. Pre-anthesis applications of CCC increased set though not always significantly. Post-anthesis applications were without effect on set.

Applications of GA prior to anthesis were the most effective in reducing seed development; post-anthesis applications were without effect on Doradillo but reduced seed development in Muscat. Intermediate results were generally obtained from anthesis applications. With one exception (Muscat pre-anthesis 1966-7) CCC did not alter the proportion of seeded and seedless berries set.

In general both compounds exerted significant, usually opposite, effects on pericarp development. Where seed growth was unaffected by GA application, this compound had no influence on the final fresh weight of the berry. However, if seed development was imperfect then GA had a profound effect on the final size of the berry. The magnitude of this effect depended upon the cultivar and time of application: those berries in which seed development was incomplete showed greatest response to GA applied at set, but where no seed growth occurred at all then response was greatest to anthesis applications. CCC, irrespective of time of application, significantly reduced berry size in all cultivars except those which were parthenocarpic.

GA significantly affected the shape of berries, especially when applied at anthesis. The degree of the response was unrelated to seed development, although in the seeded cultivar Muscat the change in shape was unaccompanied by any change in berry weight. Cape was the most responsive, while Muscat, Sultana and Zante were less so. Doradillo was unaffected. CCC significantly reduced the length/width ratio of all berries with a normal ratio greater than about 1.1.

GA often significantly increased the length of the rachis and pedicel but only when applied pre-anthesis. There was a trend, sometimes significant, for CCC to retard the elongation of the rachis and the pedicel.

Cluster weight was affected by the treatments in a manner similar to berry weight. Pre-anthesis applications of GA to seeded cultivars resulted in decreased yield because a greater proportion of berries had inhibited seed growth and were smaller. Later applications of GA, notably in Doradillo, increased yield because of reduced inhibition of seed growth and greater set and development of seedless berries. Anthesis applications of GA were most effective in increasing yield in Zante and Cape, while post-anthesis applications were more effective in Sultana.

In the examination of endogenous growth regulators in the fruit of Doradillo, Sultana and Cape, qualitative differences in GA-like compounds were noted between the parthenocarpic cultivar, Cape, and the other two cultivars; the difference between Sultana and Doradillo appeared to be only quantitative. Significant changes in concentration of GA-like substances during early development occurred only in the seeded cultivar Doradillo. No significant response to CCC was noted.

An ABA-like compound was present in all extracts examined and significant changes occurred in its concentration. There was a trend in all three cultivars (significant in Doradillo, P= 0.05) for the level of the inhibitor, which was generally highest at anthesis, to decline more slowly after treatment with CCC.

These results and the results of others are used to formulate a general hypothesis of the control of fruit-set and pericarp development in Vitis vinifera.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my belief, no material which has previously been published or written by another person has been included, except when due reference is made in the text of this thesis.

# I. GENERAL INTRODUCTION

The control of fruit set and pericarp development is economically important to the viticultural industry. In spite of the advent of plant growth regulators and their use as a tool to modify plant growth, we still have not achieved control of such factors as set, pericarp size and shape of grape berries. In the normal situation these factors are controlled in the first instance by the genetic make-up of the plant and in the second instanc by the environment. In order to control fruit cluster development we need to know what processes are involved and how they are controlled.

## (1) Structure and development of the fruit

The fruit of Vitis vinifera L. is classified as a berry (Perold 1927) and it develops on a panicle (cluster) that may contain from a few to several thousand flowers. Each flower is separately attached to a pedicel which in turn is attached to the rachis which often has secondary or even tertiary branches. The rachis is attached to the vegetative shoot by the peduncle.

The development of the fruit cluster initials up to the stage of anthesis has been described for a number of cultivars (Barnard 1932; Barnard and Thomas 1933; Winkler and Shemsettin 1937; Manzoni 1953; Winkler 1962). Two workers (Pearson 1932; Stout 1936) have concentrated their studies on the development of the embryo as it affects seed development following pollination. These workers have shown that parthenocarpy as exemplified in Red and White Corinth (Currant) is due to a defective type of embryo formation in which the inner integument and embryo sac fail to develop. In the Black Corinth (Zante Currant), although the integuments develop normally the egg apparatus either

fails to develop or degenerates before fertilization. The stenospermocarpic cultivar Sultana also exhibits defective ovule formation in that there is excessive growth of the inner integument prior to anthesis, and the embryo sac is frequently abnormal. However, it is possible that defective formation of the embryo is not causally related to parthenocarpy; rather it may result from the same physiological cause that produces parthenocarpic development. Fruit clusters in such seeded cultivars as Muscat Gordo Blanco and Doradillo generally contain a proportion of seedless berries. Seedlessness in these cultivars is a result of one or more of the following factors: lack of pollination (facultative parthenocarpy), imperfect ovule development, or seed abortion following pollination and partial seed development in which case pericarp growth is said to be stenospermocarpic. A further type of seed development is apogamy in which complete seed development takes place in the absence of fertilization. All these forms of berry development have been demonstrated in Muscat (Stout 1936).

The type of seed development is an important factor in determining not only the final size of the berry, but also, in elongated berries, the shape. There is an obvious positive correlation between amount of seed development per berry and the final size of the berry (Winkler and Williams 1935; Stout 1936; Olmo 1946). Parthenocarpic berries generally weigh less than 1g, stenospermocarpic 1 to 2g, and seeded up to 5g or more. In cultivars which normally have elongated seeded berries but also set seedless berries, the seedless berries, apart from being smaller, are generally more spherical than the seeded (Müller-Thurgau (1908) cited by Pearson 1932).

Coombe (1960) compared the growth rates in the different seed and fruit structures of Sultana (stenospermocarpic) and Muscat (seeded) and found that the rates of cell division were very similar in the pericarp and septum; the only major differences were in seed growth and these differences, apart from the early abortion of the embryo in Sultana, were mainly quantitative. His results also indicated that both cell division and cell expansion contributed to the first growth phase of the berry, while cell expansion was the sole contributing factor in the second phase of growth. Harris et al. (1968) examined quantitatively the rate of cell division and cell expansion in the placenta and inner and outer pericarp in Sultana. They found that for this cultivar pericarp growth was basically responsible for the overall growth of the berry, and that this tissue represented 64 per cent of the berry volume at maturity.

No work has been done at the cellular level to see at what stage in berry growth the differences in berry size and shape are determined. Are these differences determined before anthesis or are they a consequence of pollination ? The above observations on berry growth as affected by degree of seed development would seem to indicate that there is little difference in the state of the pericarp in the different cultivars up to the stage of anthesis and that differences in size and shape are a consequence of pollination, fertilization and seed development. The contribution of each of these processes has yet to be fully established for the fruit of Vitis vinifera although Müller-Thurgau (1908 , cited by Pearson 1932) has established that pollination is necessary for berry elongation and Olmo (1936) found that pollination was essential for the setting of the parthenocarpic Zante Currant. Millardet (1901) found that he could induce "fausse hybridation" in grape flowers with pollen from Ampelopsis hederacea.

Massart (1902), by splitting a self-incompatible cucurbit flower in half and pollinating one half with self-incompatible pollen and the other half with compatible pollen, was able to show that the stimulation which inhibits abscission and initiates the first wave of growth in the ovary wall is derived from pollen, but that the stimulation which causes growth in both the ovary wall and the placenta comes only from the fertilized ovules. Also it has been established that set, size, and shape of the Deglet Noor date are affected by pollen source (metaxenia) (Nixon 1934; Schroeder and Nixon 1958). Similar effects have been noted in mandarins by Stafford (pers.comm.). Perhaps an analogous relationship exists in the fruit of Vitis vinifera.

(2) The relationship between plant growth regulators and fruit set and development

(i) Gibberellin

During the last decade gibberellic acid ($GA_3$) has come into widespread use as a fruit sizing agent for seedless grapes. It has assumed particular importance in the production of Sultana clusters for fresh fruit. Such has been the impact of this treatment that fruit less than two or three times its untreated size is virtually unsalable in the USA.

Much attention has been paid in the last 10 years to the gross effects of GA on fruit cluster development, and many of these effects have been quantitatively analysed, for seeded as well as seedless cultivars. The most obvious effect and the first to be documented (Weaver 1958) is the ability of GA to increase markedly the size and weight of seedless berries. Sachs and Weaver (1968) studied the histology of this effect in the cultivars of Zante Currant and Sultana and observed that the

tissue most affected was the inner pericarp. This tissue responded by an increase in cell expansion within 24 hours of the application of 40 ppm GA. This expansion was so intense in the region of the inner pericarp that the cells of this tissue became distorted and compressed the cells of the outer pericarp against the epidermis. The authors do not rule out an effect on cell division as well as cell expansion although they did not observe any increase in the rate of cell division. Seeded berries are notably unresponsive to GA (Weaver and McCune 1959 a,b; Lavee 1960). Smirnov and Perepelisyna (1965) examined 18 seedless cultivars and established that the responsiveness of a cultivar varied inversely as the weight of the seed rudiments it contained. Lavee (1960) established a similar correlation for berries within the seeded cultivar Queen of the Vineyard, and observed that two or three seeds were required to offset the effect of GA applied at 3 weeks post-anthesis. Weaver et al. (1962) examined Muscat and found that in this cultivar one seed was sufficient to offset the effect of a post-anthesis treatment with GA.

The increase in weight of seedless berries following the application of GA is a result mainly of an increase in the length of the berry (Weaver 1958), although there is also an associated but relatively smaller increase in the width of the berries. It has been observed (Sachs and Weaver loc.cit.) that time of application is important in this effect and it is due to the greater responsiveness of the distal parenchymatous tissue to GA. Weaver and McCune (1959a) observed that Muscat berries (seeded) treated with GA were elongated.

Recently work has been carried out on the influence of GA on the processes associated with fertilization and further to evaluate the importance of any such influence on fruit set and size. Weaver and McCune (1959 a, b) observed that the application of GA to seeded cultivars resulted in the formation of small seedless berries ("millerandage", "shot berries"). It was later found that application of GA at anthesis produced shot berries in Sultana and also reduced the number of berries set (Weaver and Pool 1965; Christodoulou et al. 1966, 1967, 1968; Lynn and Jensen 1966; Mosesian and Nelson 1968). Weaver and McCune (1960) postulated that these effects were due to gibberellin acting as a pollenicide and supported this with the information that 25 p.p.m. GA applied to Carignane clusters reduced the germinability of the pollen from 90% to 7%. Pollen from clusters treated with 100 p.p.m. GA failed to germinate at all. A number of authors (Watanabe 1963a, cited by Sachs and Weaver 1968, 1963b; Smirnov and Perepelisyna 1965; Nishitani et al. 1968) have studied the influence of GA on embryo development and found that pre-anthesis applications of GA caused atrophy and degeneration of embryo-sac tissue in seeded and seedless cultivars. Nishitani et.al. 1968) proposed that this effect was the probable cause of seedlessness in seeded cultivars and that in the cultivars they studied pollen germinability was not limiting. Thus it would appear that GA on one hand may prevent normal fertilization and on the other may replace seed development as a requirement for fruit set and pericarp development. However this does not explain the full situation since one of the notable effects of GA at high concentrations is the setting, by pre-anthesis treatments, of almost completely undeveloped ovaries.

In this situation GA has obviously prevented the abscission of the ovary but at the same time it has prevented its development. Furthermore Weaver and McCune (1960) and Pratt and Shaulis (1961) have demonstrated that, for fruit set and development, GA cannot fully replace pollination.

Many often conflicting reports have been made on the ability of GA to alter fruit quality, and it is apparent that time of application is an important factor. Pre-anthesis application of gibberellin resulted in earlier flowering and earlier maturity (Weaver and McCune (1959c). It is therefore possible that in this situation the earlier maturity (as measured by sugar content) is a direct result of the earlier flowering. Anthesis applications have been reported by several authors to hasten maturity (Lynn and Jensen 1966; Mosesian and Nelson 1968), but it may be that this is due to the marked degree of berry thinning induced by this treatment with a consequent reduction in vine load. There is general agreement that post-anthesis applications of GA greater than 10 mg/l will result in lower levels of sugar and a higher acid titre (Weaver and McCune 1959c; Smirnov and Perepelisyna 1965; Mosesian and Nelson 1968). Weaver and McCune (1959c) demonstrated that at a given level of crop a post-anthesis application of GA had no significant effect on the sugar content of the berries at harvest, and thus it is possible that the retardation of maturity is a consequence of the increased crop. It should be noted that the above experiments were carried out on the cultivar Sultana and the conclusions may not be true of other cultivars.

A number of authors have measured the berry number (set), berry size and juice density (Brix, Baumé) (e.g. Weaver and McCune 1959 a & c, 1962; Lynn and Jensen 1966; Mosesian and Nelson 1968), but no attempt has been made to assign a relative value to these as a component of yield. In order to do this, size would need to be measured in terms of volume rather than length or width or weight.

Pre-anthesis sprays of GA result in rachis elongation in seeded cultivars (Alleweldt 1959; Rives and Pouget 1959; Weaver and McCune 1959 a & b, 1961), but only small effects have been reported for seedless cultivars (Weaver and McCune 1959c, 1962). Associated with the increase in rachis length has been an increase in the toughness and the weight of the rachis and pedicels (Weaver and McCune 1959 a; Smirnov and Perepelisyna 1965).

(ii) Growth retardants

The effects of a number of growth retardants on fruit cluster development have been examined for Vitis vinifera. Increases in fruit set have been obtained with two chemicals, 2-(chloro ethyl) trimethyl ammonium chloride (CCC) (Coombe 1965, 1967), and tributyl-2, 4-dichlorobenzylphosphonium chloride (Phosphon D) (Coombe loc.cit.). N,N-dimethylaminomaleamic acid (C011) was without effect (Coombe loc.cit.). Increases in fruit set have only been obtained with pre- or early anthesis treatments. Phosphon D, although it increases set, is phytotoxic and leaf applications result in marked chlorosis. Alar was ineffective in the early experiments (Coombe 1967) and thus most attention has been paid to CCC.

The application of CCC as well as resulting in increased set also causes a reduction in berry size, which offsets to a significant extent the increased berry number (Coombe loc.cit.). In these respects it is the reverse of GA. However, where GA application results in increased seedlessness in seeded cultivars, CCC has no effect (Coombe 1967). Further, CCC has been shown to increase set and decrease berry size in stenospermocarpic and seeded cultivars. Although it increased set in such parthenocarpic cultivars as Zante Currant it does not cause a significant decrease in berry size; perhaps this is due to there being little scope for a reduction in size of this cultivar. In Muscat concentrations of CCC low enough to have no effect on set can still decrease berry size (Coombe 1967). Coombe (pers. comm.) has also shown that CCC application to either bunch, leaves or the whole shoot will result in decreased shoot growth and he uses this and other observations to explain the fruit setting effect of CCC in terms of an increase in availability of nutrients to the bunch. This effect is also the reverse of GA since GA applications have been shown to increase shoot growth (Weaver and McCune 1959).

(3) The relationship between endogenous growth regulators and fruit set and development

(i) Endogenous gibberellin-like compounds

Coombe (1960) assayed crude extracts from seeded and seedless cultivars of grapes and found significant activity in the stamens of seeded and stenospermocarpic cultivars but none in the stamens from the parthenocarpic Zante Currant. An examination of the ovaries of Zante Currant and Seedless Emperor (stenospermocarpic) revealed highest activity at the stage of fruit set. Sultana showed a small rise in activity during anthesis, and

the two seeded cultivars showed no activity at all. Weaver and Pool (1965b) found that by using a combination of liquid/liquid partitioning and paper chromatography they could demonstrate significant activity in seeded cultivars, although they did not look at changes in concentration with stage of development. Coombe et al. (1965) similarly demonstrated gibberellin-like activity in Doradillo ovaries. Iwahori et al. (1968) compared seeded and stenospermocarpic variants of the cultivar Tokay. They found that one acidic gibberellin was present in both variants, and that greatest activity coincided with early fruit set. In the seeded variant high gibberellin-like activity persisted for three weeks, and was followed by another smaller rise in activity four to five weeks later, whereas in the seedless variant the early high level of activity dropped very rapidly and was followed by another smaller rise in activity one or two weeks later. They ascribe these differences to the ability of the well developed seed of the perfect-seeded variant to produce more gibberellin.

(ii) Endogenous abscisic acid-like compounds

There is little published work relating to the presence of endogenous abscisic acid-like compounds in Vitis. Coombe (pers. comm.) has found that there is an acidic inhibitor of the barley endosperm bio-assay in young berries of Vitis vinifera, and Skene (1967) has detected an inhibitor which has similar chromatographic properties to abscisic acid (ABA). Lott (1968) found ABA-like activity in mature grape seeds. It is therefore likely that an ABA-like compound does exist in developing berries although there is no information on the role it plays in determining fruit cluster development.

(4) The relationship between the growth retardant CCC and endogenous gibberellin-like and abscisic acid-like compounds

Because essentially opposite effects are obtained with GA and growth retardants much attention has been paid to the possibility that growth retardants such as CCC exert their influence on plant growth by inhibiting the synthesis of gibberellin. Such a relationship was first demonstrated by Kende et al. (1963), and this approach has since been widely applied to the tissue of higher plants (Dennis et al. 1965; Zeevaart 1966; Radley 1967; Jones 1967; Reid et al. 1968; Dale and Fillipe 1968). CCC application results in reduced levels of unconjugated gibberellins and at the same time it increases the levels of the more polar gibberellins (Reid et al. loc.cit.), perhaps a gibberellin glycoside (Bristow and Simmonds 1968). Thus there is the possibility that CCC may not inhibit the production of gibberellin per se; rather it may cause it to be treated as a toxic product and consequently "detoxified" by the addition of a glycosidic group, and then stored. Such gibberellin glycosides have been described although their role in plant growth regulation has yet to be determined (Murakami 1961; Schreiber et.al. 1967; Tamura et al. 1968).

No information has been published on the relationship between CCC and endogenous inhibitors in spite of the fact that an increase in the level of endogenous inhibitors could account for such effects of CCC as the retardation of shoot elongation (Downs and Cathey 1960; Guttridge 1966; Knypl 1966; Dale and Fillipe 1968).

## II. FIELD EXPERIMENTS TO EXAMINE THE INTERACTION OF GA AND CCC ON REPRODUCTIVE FRUIT CLUSTER DEVELOPMENT

### (1) Introduction

The results of experiments in which GA and CCC were applied independently to flower clusters indicate that the relationship between GA and CCC is an antipodal one : in general, the application of GA results in increased berry size and decreased berry set, (e.g. Weaver1958; Weaver and McCune 1959a,b, c; Stewart et al. 1958; Coombe 1959), while the application of CCC results in decreased berry size and increased berry set (Coombe 1965, 1967; Claus 1965). These facts are consistent with the deduction, made from other work, that CCC may have its effect by inhibiting the production of some physiologically active gibberellins within the plant (see e.g. Dennis et al. 1965; Reid and Carr 1967; Radley 1967).

The ability of a grape berry to increase in size in response to the application of GA is influenced to a marked degree by the extent of seed development within that berry (Weaver and McCune 1959a,b; Lavee 1960; Smirnov and Perelisyna 1965). Why this should be so is a question which has yet to be answered. It is all the more intriguing because the response to CCC is independent of the presence of seed in a berry (Coombe 1965, 1967). In view of these observations it was decided to examine how concurrent applications of GA and CCC interacted in terms of reproductive cluster development and how this interaction was altered by the type of ovule development in the berry. Accordingly the tests were done on five cultivars:

(a) Zante Currant (syn. Black Corinth) - parthenocarpic;
(b) Cape Currant (known locally as Tunn Currant) - parthenocarpic (see Appendix E);
(c) Sultana (syn. Sultanina, Thompson Seedless) - stenospermocarpic;
(d) Muscat Gordo Blanco (syn. Muscat of Alexandria, hereafter referred to as Muscat) - seeded;
(e) Doradillo - seeded.

The morphogenesis of Zante Currant and Sultanina berries has been described by Pearson (1932).

Coombe's (1960) analyses for gibberellin-like activity in Vitis vinifera berries demonstrated that, in the cultivars Seedless Emperor and Zante Currant, there was a marked peak of gibberellin-like activity a week to ten days after flowering, i.e., at fruit set. With this result in mind the second series of experiments were designed to examine how the compounds interacted with respect to time of application, and in particular to examine how the interaction between GA and CCC altered with progressively later applications of GA.

## (2) Materials and Methods

### Cultivars

Three cultivars were chosen for the experiments in 1966-7, Muscat, Zante Currant, and Cape Currant. A further two cultivars, Sultana and Doradillo, were included in the 1967-8 series.

Treatments and Design

In the 1966-7 experiments the experimental design was a 3 x 4 factorial, replicated 10 times. CCC was applied at rates of 0, 100, or 1000 ppm (w/v), in factorial combination with GA at 0, 0.2, 2.0 or 20.0 ppm (w/v). All treatments included 0.075% Agral 60 as a wetting agent, and were applied as a cluster dip at one time two or three weeks before anthesis. The vines, which were trained to four arms on a "T" trellis system, were divided into quarters, one per cordon. The twelve treatments were randomised between three adjacent vines which were selected for minimal differences due to inequalities caused by soil type, irrigation, drainage and other between - vine variations.

In the 1967-8 series the basic design was a 3 x 3 factorial, replicated 10 times. The concentrations were CCC at 0, 50 or 2000 ppm (w/v), applied in factorial combination with GA at 0, 0.5 or 20.0 ppm (w/v). Tween 20 0.05% was included as a wetting agent. The nine treatments were applied as a dip to single clusters and were randomly distributed over a single vine. The treatments were applied over five combinations of application times:

A. CCC early, GA early;
B. CCC early, GA at anthesis;
C. CCC early, GA late;
D. CCC late, GA early;
E. CCC late, GA late.

"Early" and "late" here mean approximately two weeks before and after anthesis respectively.

Measurements

The clusters were harvested at, or a little before, maturity and were placed in a cool store until they were measured, generally within the next ten days.

Fresh weight and length of each cluster were measured, and then the berries were separated into two groups, seeded and seedless, and counted. The seededness or otherwise of the berries was determined by holding the berries up to the light, and checked occasionally by opening some berries. The presence of any hardened structures was used as the criterion for seededness, and thus the term as used here does not necessarily imply the presence of viable seeds. Pedicel length, when measured, was obtained from the cumulative length of five or ten randomly chosen pedicels. Berry length and width was obtained from a random sample of ten berries, also measured cumulatively. Refraction of the juice expressed from these ten berries was measured with a hand refractometer.

Statistical Analysis

The statistical analysis of the measured and derived parameters was carried out by the Biometry Section of the Waite Agricultural Research Institute.

The 1966-7 results were analysed as a two-way factorial with a total of 119 degrees of freedom. The analysis of the 1967-8 results was carried out using an extended version of the Wellesbourne-Waite 'Genstat' programme. The trial was treated as a split plot analysis, with the differences between the major

plots (vines) being confounded with the differences between the time of application combinations. A factorial analysis was carried out within the major plots. The only interaction derived was the first order interaction between GA and CCC. Thus no "F" values were derived for either the first order interaction of each compound with the time of application, or for the second order interaction of the two compounds together with time. However it is held that the LSD for the comparison of means within each major plot is valid for comparisons across major plots (times of application).

(3) Results

In the following discussion both years results are considered together where they are in agreement; differences are then stated and discussed though emphasis is placed in the 1967-8 results because these were more extensive.

(i) Doradillo

Set:

There was a small but highly significant positive response to GA in terms of total number of berries set when the GA was applied either at anthesis or two weeks later. CCC had no significant effect on the total number of berries set (Fig. 2.7).

GA applied at all three times caused a highly significant increase in the percentage of seedless berries set (Fig. 2.8). At the early application time this change was due to the replacement of seeded berries with seedless berries (Fig. 2.5A & 2.6A) the total number of berries remaining unchanged (Fig.2.7A). However at the later times of application, and particularly the last, there was no decrease in the number of seeded berries set (Fig. 2.5B,C & E).

Berry Growth:

Analysis of the fresh weight of seeded berries revealed little response to GA, however CCC applied either early or late caused a highly significant decrease in the fresh weight of the berries (Fig. 2.9). It would appear from fig. 2.9C that late application of GA offsets the effect of an early application of CCC (based on LSD) although the overall analysis for the interaction between GA and CCC did not reveal a significant "F" value.

A small but highly significant change in berry shape (an increase in length/width ratio) was brought about by GA, especially by early application. CCC had no effect on its own and did not offset the effect of GA (Fig. 2.10).

Rachis Growth:

The application of 20ppm GA either at anthesis or at set resulted in a small increase in rachis length (Fig. 2.11), but CCC had no significant effect.

Yield:

The response of cluster weight to GA interacted markedly with time of application. The response shifted from markedly negative with increasing concentrations of GA at the pre-anthesis application (Fig 2.12A) to markedly positive at the post-anthesis application (Fig 2.12C). The former effect was due to the replacement of seeded berries with smaller, seedless berries. The latter effect was due to the setting of more seedless berries without reduction in the number of seeded berries and to the larger size of these seedless berries.

CCC had no significant effect on cluster weight.

(ii) Muscat

Set:

The application of CCC two weeks prior to anthesis resulted in an increase in fruit set. This effect was more pronounced in the 1966-7 experiment in which the number of seeded berries was increased by 47% (significance P = 0.001), and the number of seedless berries was increased by 24% (n.s.). GA at 20ppm increased slightly the total number of berries set at the late time of application in the 1967-8 experiment, and at 0.5ppm it decreased set in most experiments (Fig 2.15).

CCC had no significant effect on the ratio of seeded to seedless berries set in either year. GA produced highly significant effects in both years, although the effects were completely reversed each year. In 1966-7 pre-anthesis treatments with GA decreased the number of seedless berries per cluster and had no effect on the number of seeded berries that set; as a consequence of this GA decreased the relative number of seedless berries per cluster (see Fig. 2.1). However in the 1967-8 series the application of GA at all three times resulted in an increase in the relative number of seedless berries set per cluster (Fig. 2.16).

Berry Growth:

The application of CCC at all times resulted in a large significant reduction in fresh weight of seeded berries (Fig. 2.17). There was also a slight but not significant reduction in the weight of seedless berries (Fig. 2.18). A highly significant reduction in the fresh weight of seeded berries was obtained from the application of GA in 1966-7 (Fig. 2.1 ), though this effect was not as marked in the 1967-8 experiments. Late applications of GA did however produce a large increase in the size of seedless berries (Fig. 2.18, 2.21 & 2.22).

Rachis and Pedicel Growth:

Cluster length and pedicel length showed similar responses: both increased in length relative to untreated after treatment with increasing concentrations of GA, and decreased with increasing concentrations of CCC.

These effects were more marked in 1966-7 (Fig. 2.1). The interactions, as indicated in the Table 2.1 for 1966-7, are probably a consequence of the domination of the response by high concentrations of GA (See Fig. 2.1).

An interesting point in relation to the physiological differences between a cluster and a tendril (which have the same morphological origin) is that at high levels of GA the rachis behaved like a tendril in that it toughened, coiled, and became thigmotropic. This effect was noted especially in the first year and mainly in the cultivars Muscat and Cape Currant.

Yield:

GA when applied two weeks before anthesis caused a marked reduction in cluster weight (Fig. 2.26) (highly significant in 1967-8, not significant in 1966-7). But, when applied at later stages, cluster weight was unaffected. Like Doradillo the reduction in cluster weight was due to a reduction in the number of seeded berries set, (2.13) and their replacement with smaller seedless berries (Fig. 2.14 & 2.18). In both years CCC applied before anthesis caused a significantly larger set (not significant in the overall analysis for 1968-9). However yield was not increased because the set increase was offset by a reduction in berry size. When CCC was applied after anthesis, it still resulted in the production of smaller berries (Fig. 2.17), but had no effect on set (2.15); the combination of these two effects resulted in a reduction in cluster weight as illustrated in Fig. 2.26. The reduction in cluster weight brought about by an early application of GA followed by a post-anthesis

application of CCC appears to be independent and additive, as was noted in Doradillo.

(iii) Sultana

Set:

GA had large effects on set which varied markedly with its time of application; when applied during anthesis it decreased set but before or after anthesis it increased set (Fig. 2.27). CCC had little effect on set except in one time combination (CCC early, GA late Fig. 2.27C) where it increased set; here there was no interaction between GA and CCC.

Berry Growth:

GA when applied during or after flowering brought about significant increases in fruit size: the later the treatment the greater the response (Fig. 2.28). CCC when applied early brought about a significant decrease in berry weight. An interesting aspect shown in Fig. 2.28C is that berries treated with CCC before flowering are less responsive to GA applied after flowering.

Maturity, as measured by the refractive index of the berries at harvest was unaffected by all treatments, except that the highest level of GA at the late application resulted in a significantly lower refractive index (Fig. 2.32).

GA increased berry length and the length/width ratio when applied during or after anthesis, especially the former; pre-anthesis GA had no significant effect on berry shape (Fig. 2.31).

CCC applied before anthesis brought about a decrease in the length/width ratio of the berries due to a significant decrease in berry length (Fig. 2.31). This effect was independent of the increase which resulted from the application of GA.

Rachis Growth:

Early application of GA resulted in a very marked increase in cluster length. Later applications had lesser effects (Fig. 2.33). No consistent effects were obtained from the application of CCC.

Yield:

A large positive response to 20ppm GA was obtained when it was applied post-anthesis (Fig. 2.34C & E), because more (Fig. 2.27) and much larger berries were set (Fig. 2.28). The application of 0.5ppm GA at anthesis reduced cluster weight when compared with either no GA or 20ppm GA, because the increase in berry weight was in proportion to increasing concentrations of GA (Fig. 2.28) while the number of berries set was equally reduced by both 0.5 and 20ppm GA (Fig. 2.27).

(iv) Cape Currant

Set:

Cape Currant sets poorly under normal circumstances. In these experiments it responded well to pre-anthesis CCC showing, in 1966-7, a 60% increase in the

number of berries set in response to the application of 1000 ppm before anthesis (Fig.2.2). The response in 1967-8, though not quite as marked, was still highly significant (Fig. 2.35). Late application of CCC had no clear effects. The early application of GA in both years caused a significant reduction in fruit set. In 1966-7 GA dominated the CCC increase, but in 1967-8 the responses were additive. The comparison of later application of GA in 1967-8 showed no effect on fruit set when applied at or after flowering. However, a late application of GA apparently had the ability to offset the effect of an early application of CCC (See Fig. 2.35C).

Berry Growth:

The fresh weight of the seedless berries was increased by GA in both years and at all times of application (Fig. 2.2 & 2.37). In 1967-8 the greatest response was obtained from the anthesis application. In neither year did CCC have any effect on berry weight.

The shape of the berries as measured by the length/width ratio was significantly affected only by the application of GA. The effect was most prominent when the GA was applied during anthesis (Fig. 2.38), as with the other cultivars.

Despite the scarcity of seeded berries and thus the great variability in this parameter, there were, in the year they were measured (1966-7), suggestions that they responded similarly to seeded Muscat berries: CCC 100ppm reduced berry weight, and tended to reduce berry length and width; GA in the presence of CCC significantly increased the length/width ratio (Fig. 2.3).

Rachis Growth:

GA again had a dominant effect and, as with Sultana, exerted its greatest influence when applied before anthesis. CCC application had inconsistent effects though there was a trend for early application to decrease rachis elongation (Fig. 2.39).

Yield:

Twenty parts per million of GA applied prior to anthesis in 1966-7 resulted in a yield increase of 167% (Fig. 2.2). In 1967-8 anthesis treatment gave a similar response (Fig. 2.40). Treatment either prior to or after anthesis in 1967-8 resulted in smaller but still highly significant increases in yield. The lower increase in 1967-8 was due to counteraction of the increase in berry size by a decrease in berry number (Fig. 2.35); this decrease in berry number was not significant in 1966-7. As discussed above, no effect on berry number as a result of GA application was recorded at any other time, and thus the effect of GA is primarily one of increased berry size. There was a trend for an increase in yield following an early application of CCC. In 1966-7 this increase amounted to 90%, comparing 1000ppm CCC with nil (Fig. 2.2). Though most of these increases were not significant, there was a significant interaction of CCC with GA (See Table 2.1).

(v) Zante

Set:

In 1966-7 GA significantly decreased the number of berries set, and CCC significantly increased the number of berries set (Fig. 2.4). However in 1967-8 no significant response was obtained to either compound, at any time of application.

Berry Growth:

The response of berry weight to GA was similar to that obtained with Cape Currant except that the berries were less responsive at the earliest time of application. Twenty parts per million of GA nearly tripled the berry weight when applied during anthesis, and nearly doubled it when applied post-anthesis; the early application resulted in only 50% increase in berry weight. The results in Fig. 2.42 suggest that a late application of CCC may offset the effect of an early application of GA though the interaction was not significant.

GA when applied early caused a small but significant hastening of berry maturity as indicated by juice refraction (Fig. 2.44); this is interesting because it occurred despite an increase in cluster weight. When GA was applied either during or after flowering it produced a significant retardation of maturity rate, especially with the latest application. CCC when applied early showed a trend towards hastening maturity, but, when applied late in association with a late application of GA, the rate of maturity appeared to

be additively reduced (Fig. 2.44E). When the GA was applied early it apparently dominated and overcame the retarding effect of a late application of CCC (Fig. 2.44D).

The only consistent effect on berry shape was an increase in the length/width ratio produced by GA. This effect, as in Sultana and Cape Currant, was most marked when the GA was applied at anthesis (Fig. 2.43).

Rachis and Pedicel Growth:

GA increased cluster length (Fig. 2.46), early treatments being the most effective. The only effect of CCC appeared to be in the ability of a late application to offset to a marked degree the response to an early application of GA (Fig. 2.46D).

The response of pedicel elongation was closely similar to that of rachis elongation, with the exception of the interaction just mentioned (Table 2.1 Fig. 2.45).

Yield:

The cluster weight responses were very similar to that of Cape Currant with GA again being the dominant factor. The maximum response was obtained when GA was applied at anthesis, although nearly as good a result was obtained by late application. The main component of the yield response was berry fresh weight.

The overall analysis for CCC in both 1966-7 and 1967-8 revealed no significant yield response.

Fig. 2.1 Muscat 1966-7

GA and CCC applied in factorial combination two (2) weeks pre-anthesis

Cluster parameter: as per legend

GA concentrations: as per legend

CCC concentrations: 0ppm .____.

100ppm o____o

1000ppm Δ____Δ

Vertical lines represent LSD at P= 0.05 for each compound where significant. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

# MUSCAT 1966-7

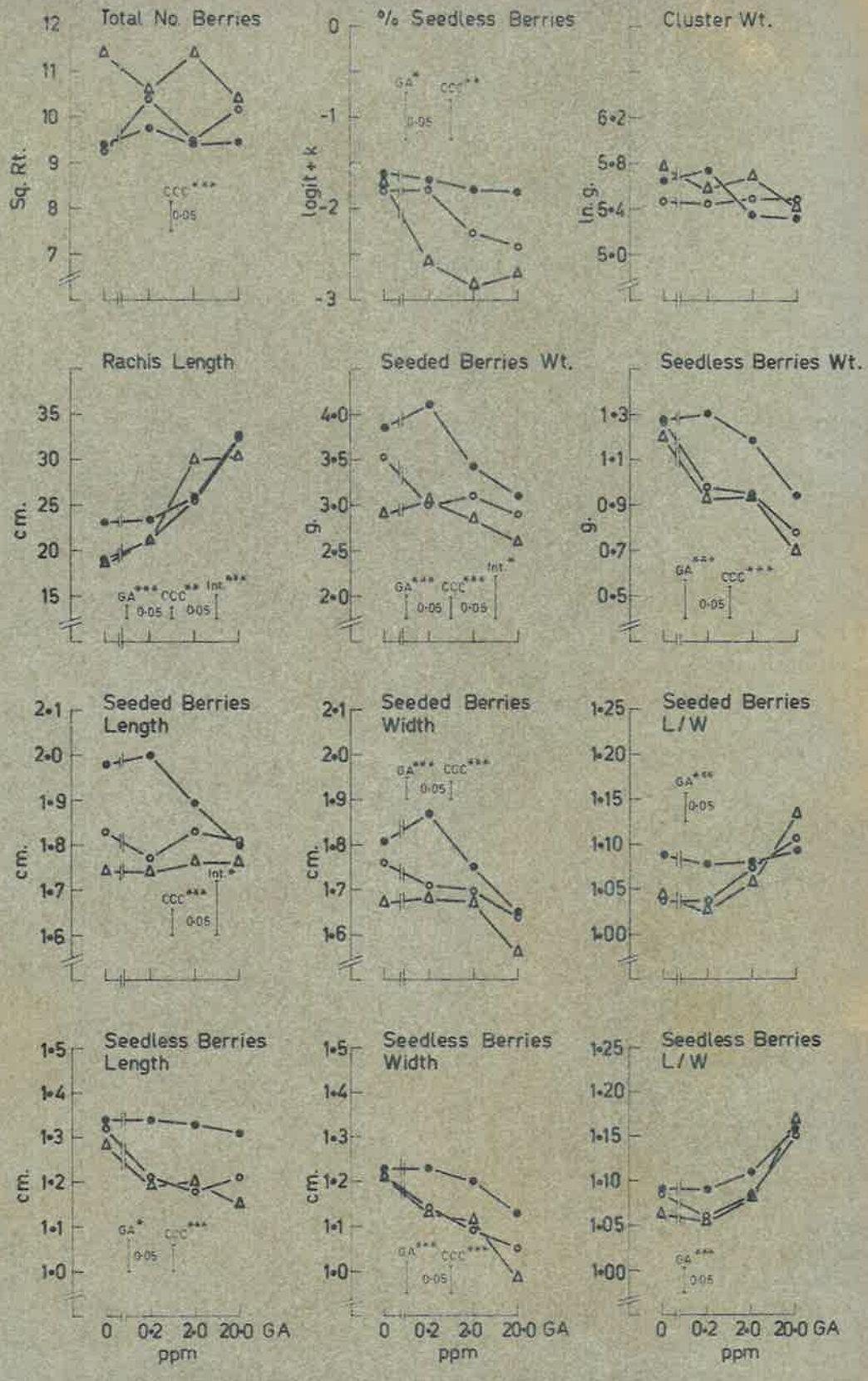


Fig. 2.2 Cape 1966-7

GA and CCC applied in factorial combination two (2) weeks pre-anthesis

Cluster parameter: as per legend

GA concentration: as per legend

CCC concentration:

| | |
|---|---|
| 0ppm | ._____. |
| 100ppm | o_____o |
| 1000ppm | Δ____Δ |

Vertical lines represent LSD at P=0.05 for each compound where significant. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

# CAPE 1966-7

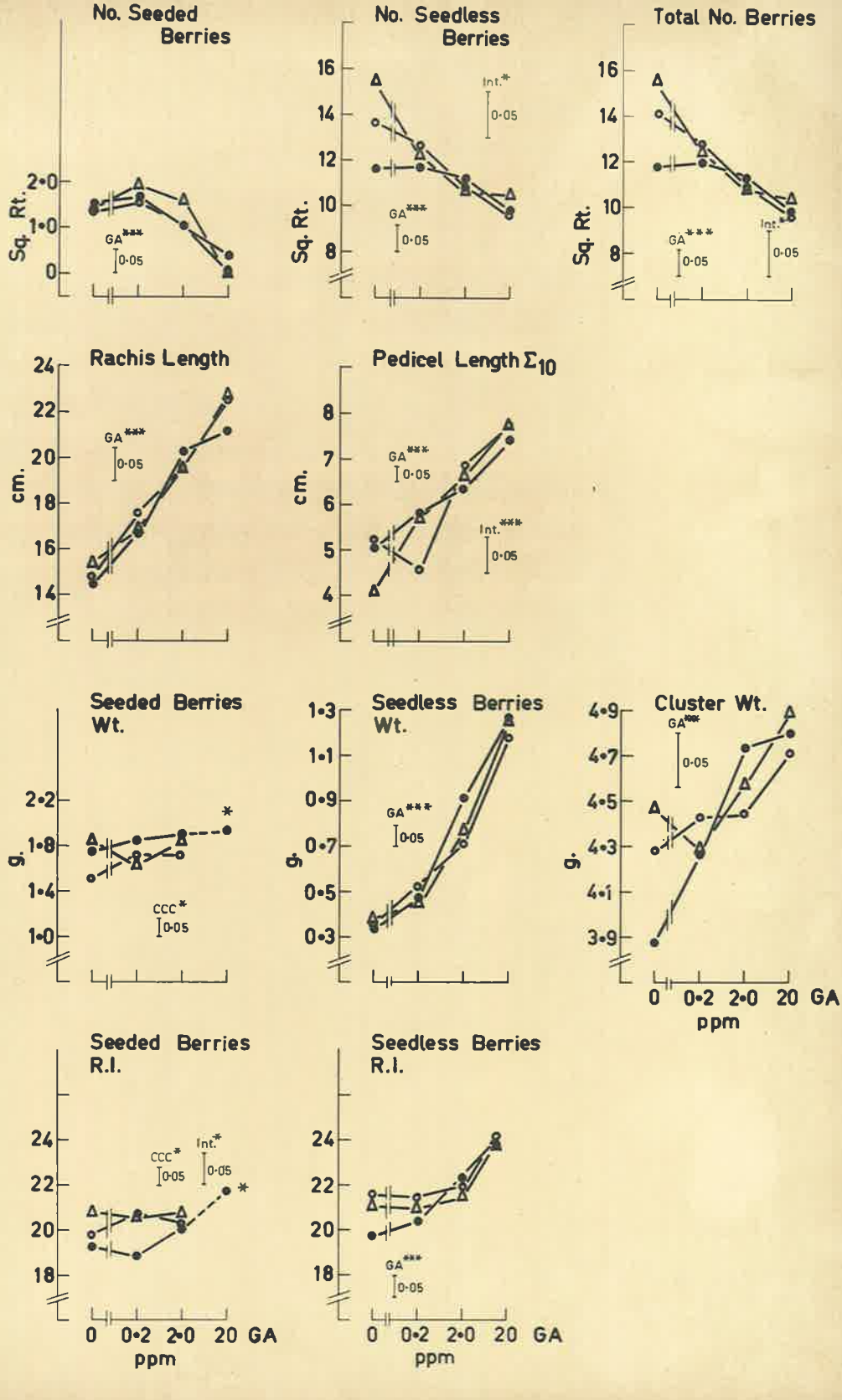

No. Seeded Berries
Sq. Rt.
2·0
1·0
0
GA*** 0·05
No. Seedless Berries
Sq. Rt.
16
14
12
10
8
Int.* 0·05
GA*** 0·05
Total No. Berries
Sq. Rt.
16
14
12
10
8
GA*** 0·05
Int.* 0·05
Rachis Length
cm.
24
22
20
18
16
14
GA*** 0·05
Pedicel Length Σ10
cm.
8
7
6
5
4
GA*** 0·05
Int.*** 0·05
Seeded Berries Wt.
g.
2·2
1·8
1·4
1·0
CCC* 0·05
Seedless Berries Wt.
g.
1·3
1·1
0·9
0·7
0·5
0·3
GA*** 0·05
Cluster Wt.
g.
4·9
4·7
4·5
4·3
4·1
3·9
GA*** 0·05
0 0·2 2·0 20 GA
ppm
Seeded Berries R.I.
24
22
20
18
CCC* 0·05
Int.* 0·05
0 0·2 2·0 20 GA
ppm
Seedless Berries R.I.
24
22
20
18
GA*** 0·05
0 0·2 2·0 20 GA
ppm

Fig. 2.3 Cape 1966-7 continued

Fig. 2.4 Zante 1966-7

GA and CCC applied in factorial combination two (2) weeks pre-anthesis

Cluster parameter: as per legend

GA concentration: as per legend

CCC concentration:

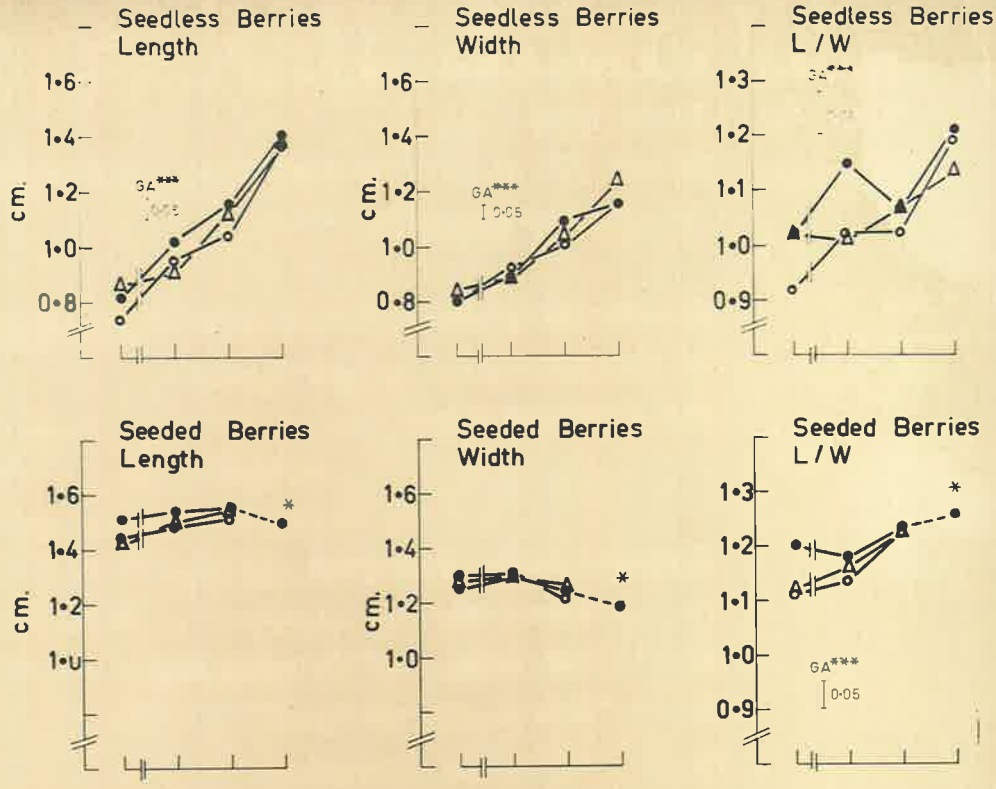
0ppm .——.

100ppm o——o

1000ppm △——△

Vertical lines represent LSD at P=0.05 for each compound where significant. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

## CAPE 1966-7




## ZANTE 1966-7

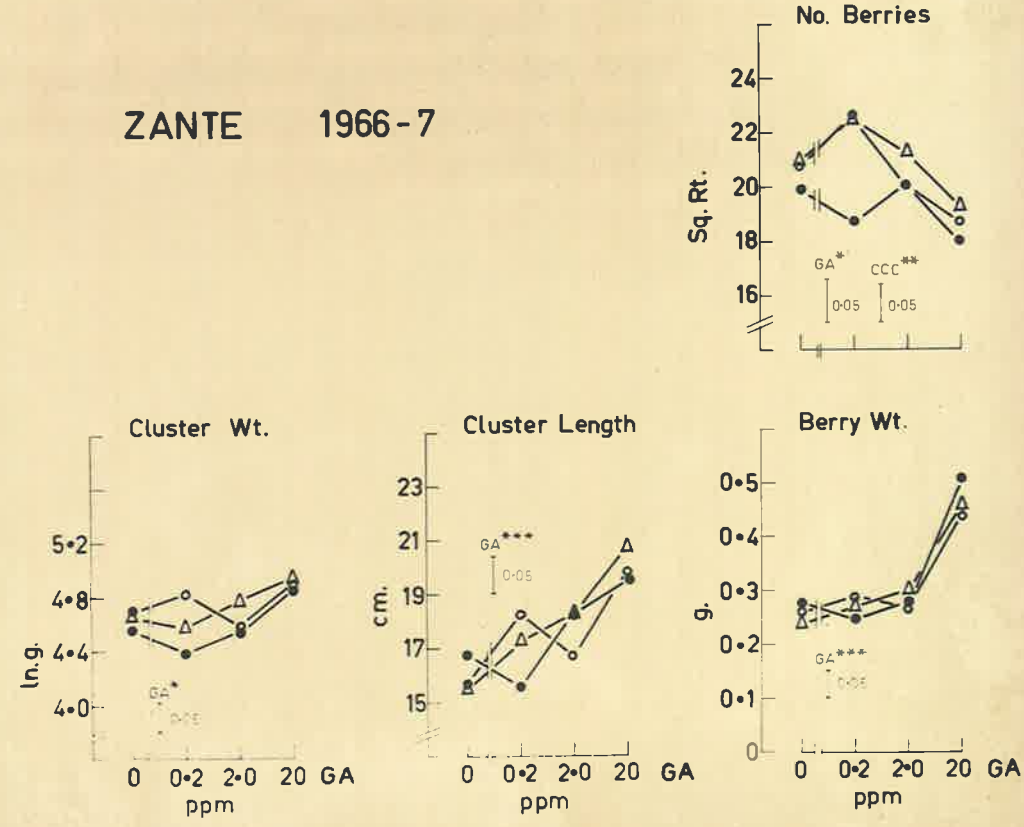


Fig. 2.5 Doradillo 1967-8 - Number of seeded berries per cluster (sq.rt.)

Fig. 2.6 Doradillo 1967-8 - Number of seedless berries per cluster (sq.rt.)

GA and CCC applied in factorial combination

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o____o

1000ppm △____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

# DORADILLO

Figure 2.5

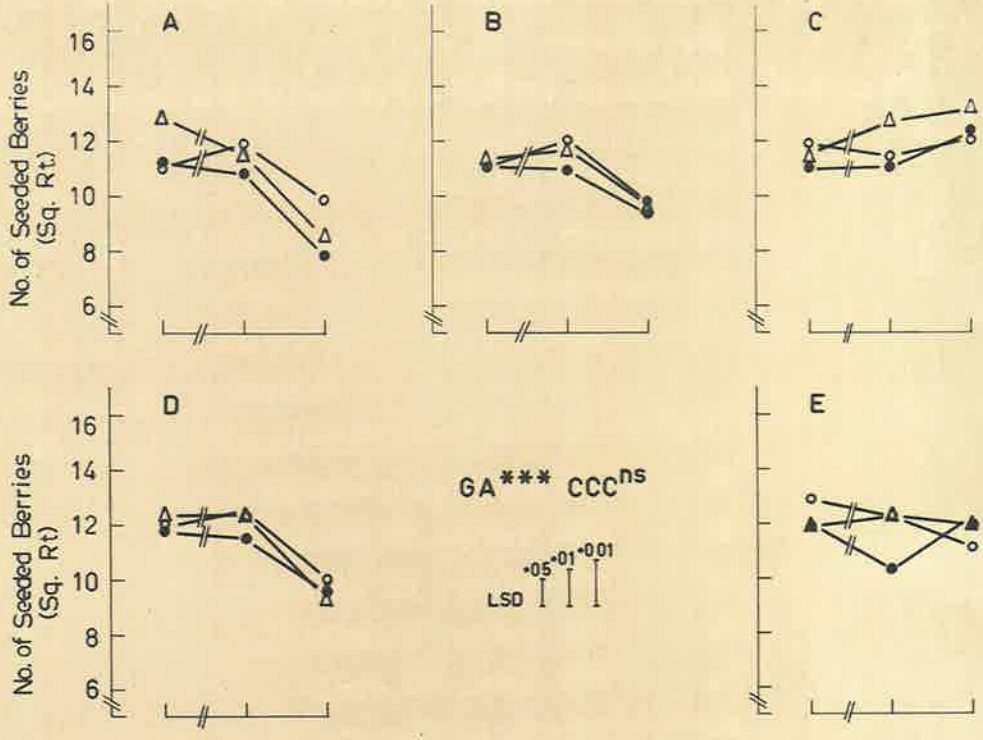



Figure 2.6

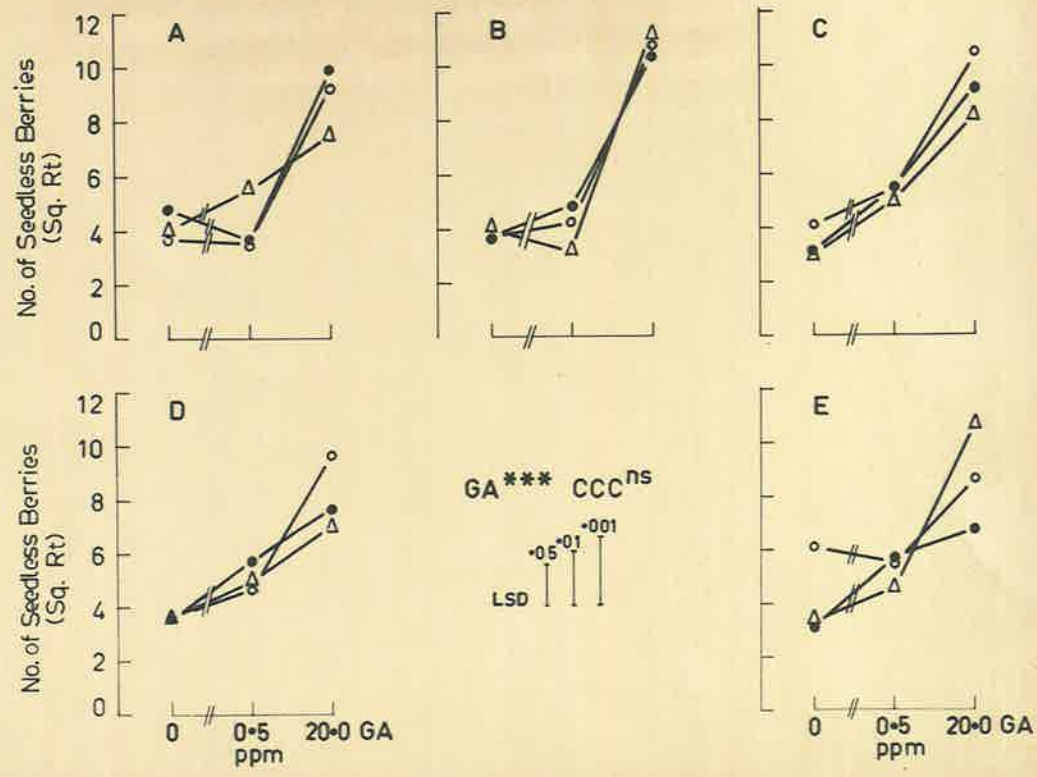


Fig. 2.7 Doradillo 1967-8 - Total number of berries per cluster

Fig. 2.8 Doradillo 1967-8 - Percent seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △_____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance, thus:

*P=0.05, **P=0.01, ***P=0.001.

## DORADILLO

Figure 2.7

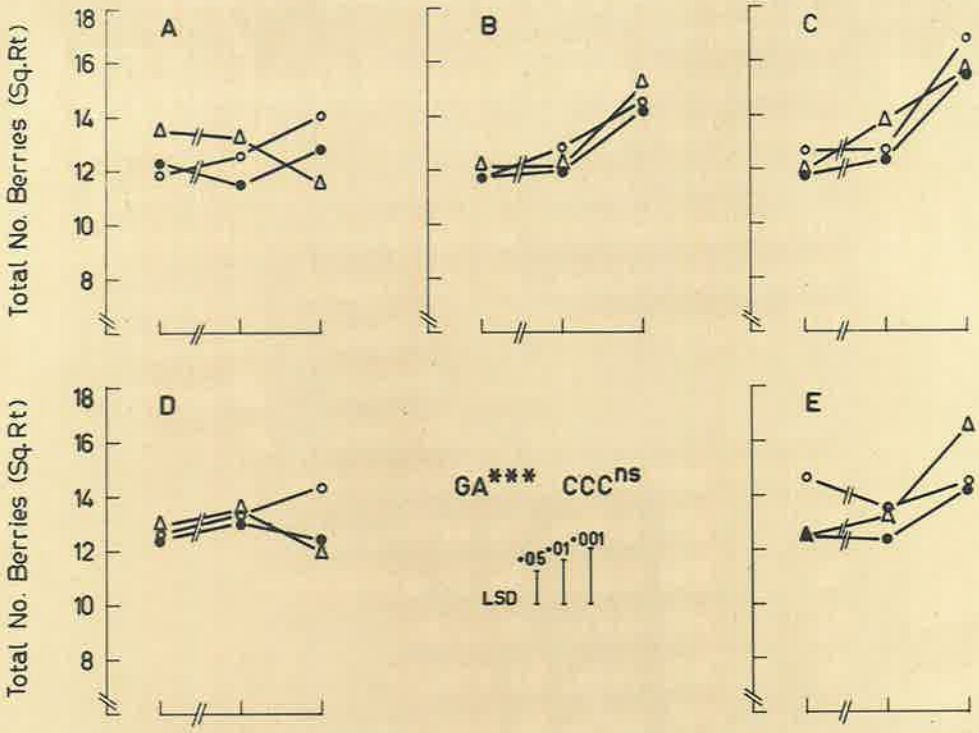



Figure 2.8

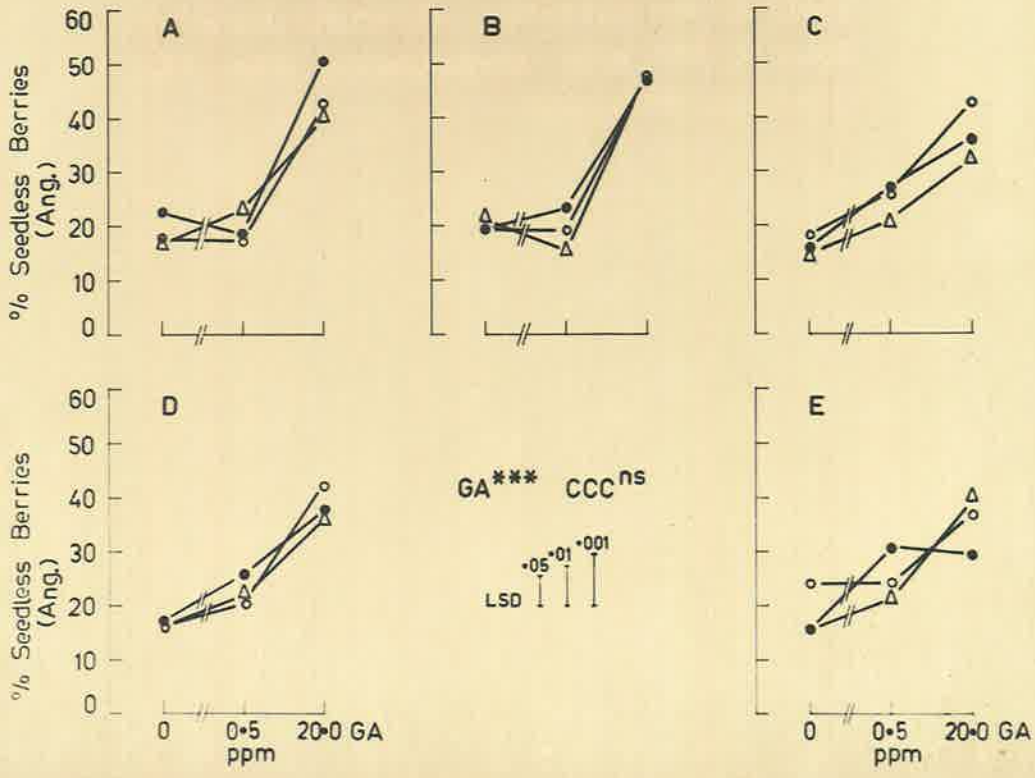


Fig. 2.9 Doradillo 1967-8 - Fresh weight of seeded berries.

Fig. 2.10 Doradillo 1967-8 - Length/width ratio of seeded berries.

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △_____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

# DORADILLO

Figure 2.9

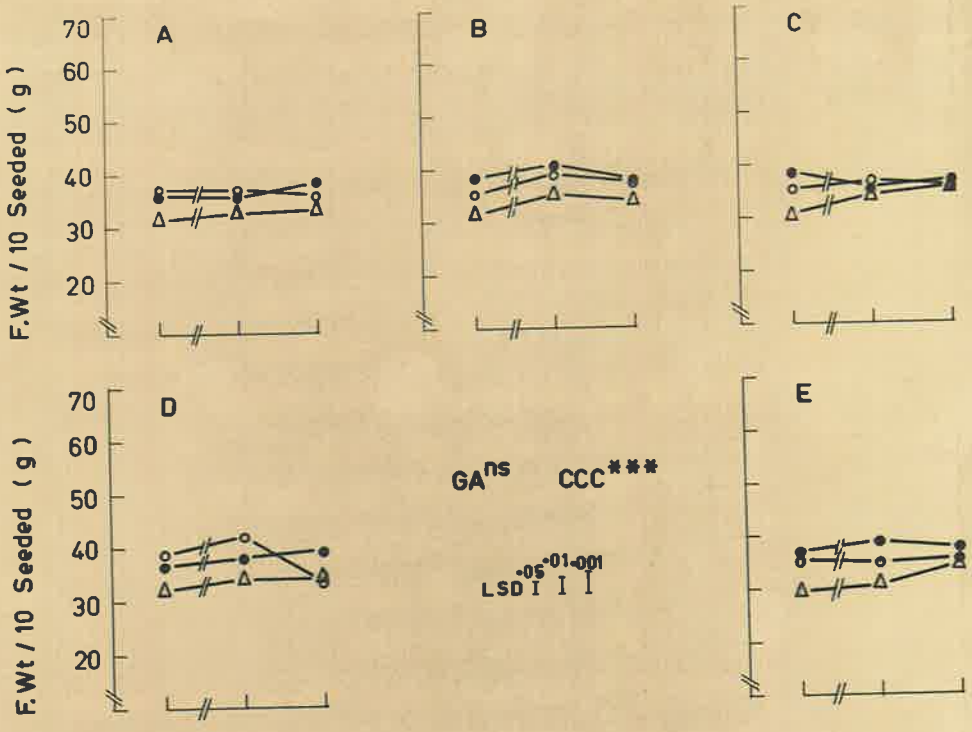



Figure 2.10

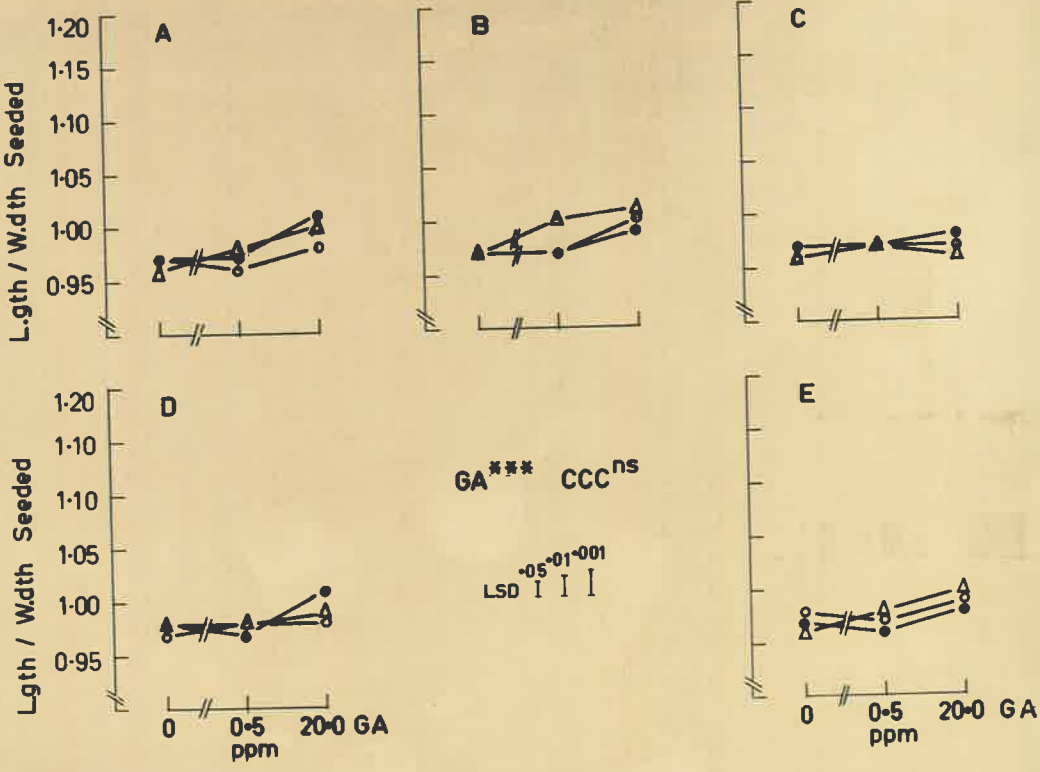


Fig. 2.11 Doradillo 1967-8 - Rachis length

Fig. 2.12 Doradillo 1967-8 - Cluster weight

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △_____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance, thus: *P=0.05, **P=0.01, ***P=0.001.

# DORADILLO

Figure 2.11

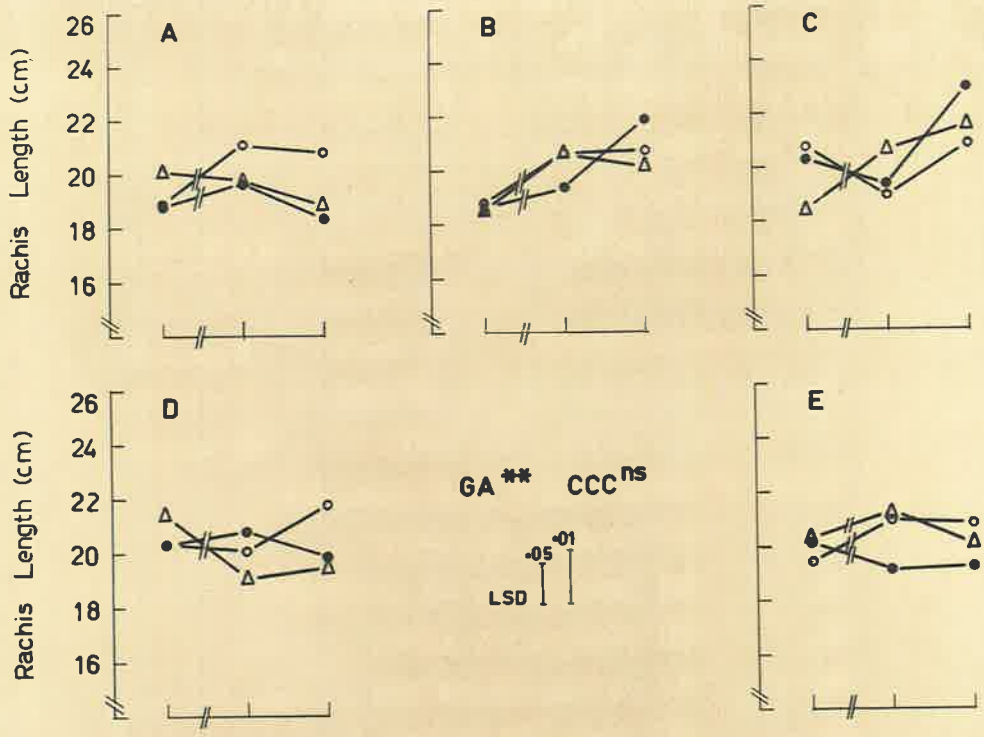



Figure 2.12

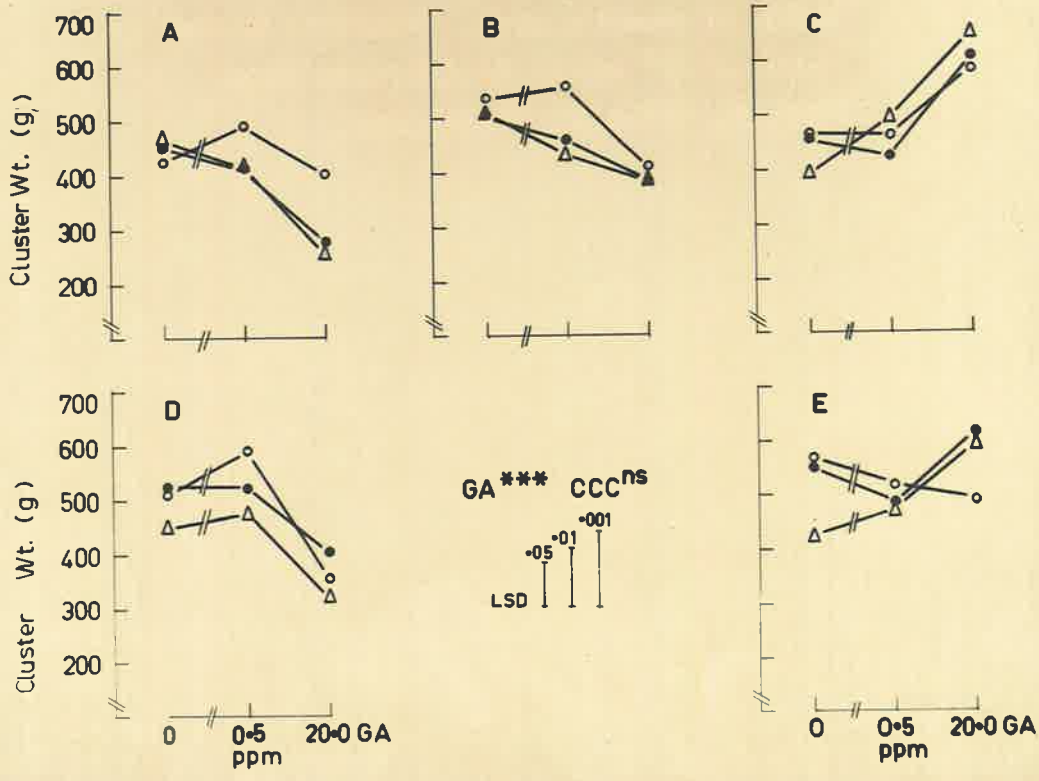


Fig. 2.13 Muscat 1967-8 - Number of seeded berries per cluster

Fig. 2.14 Muscat 1967-8 - Number of seedless berries per cluster

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .——.

100ppm o——o

1000ppm △——△

Times of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

## MUSCAT

Figure 2.13

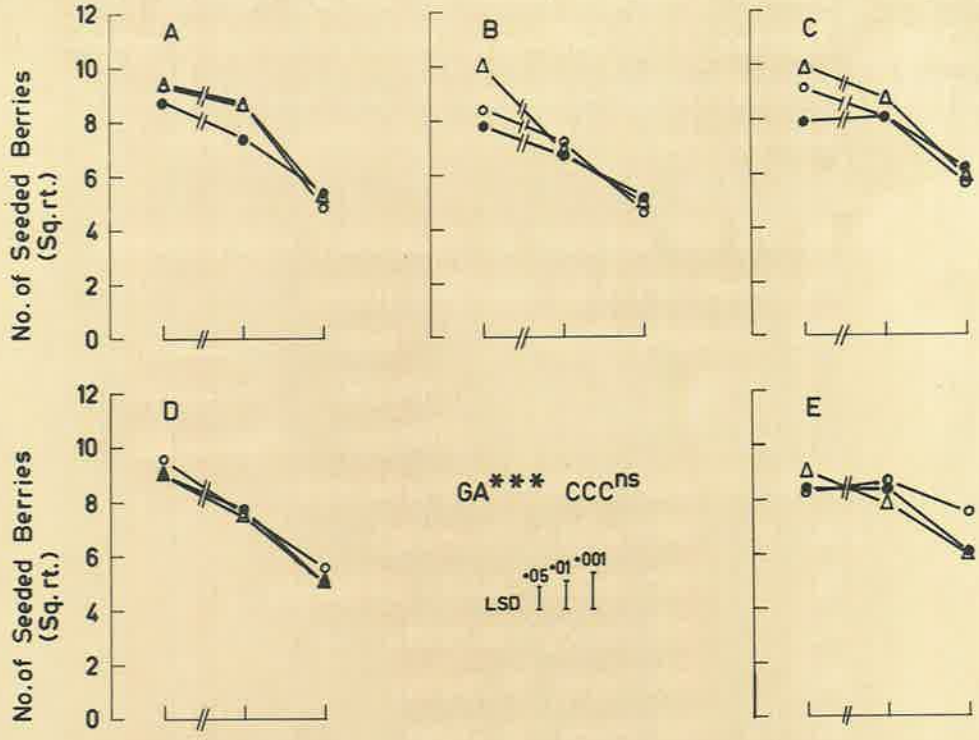



Figure 2.14

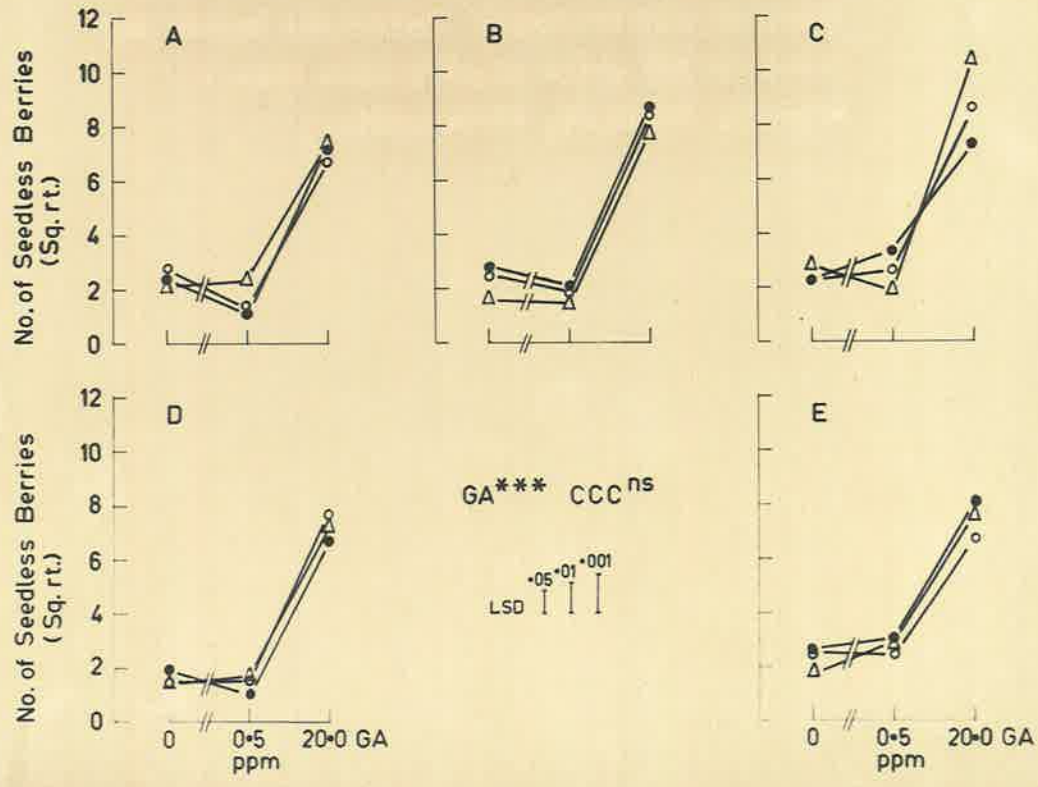


Fig. 2.15 Muscat 1967-8 - Total number of berries per cluster

Fig. 2.16 Muscat 1967-8 - Percent seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .____.

100ppm o____o

1000ppm Δ____Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus :

*P=0.05, **P=0.01, ***P=0.001.

MUSCAT

Figure 2.15

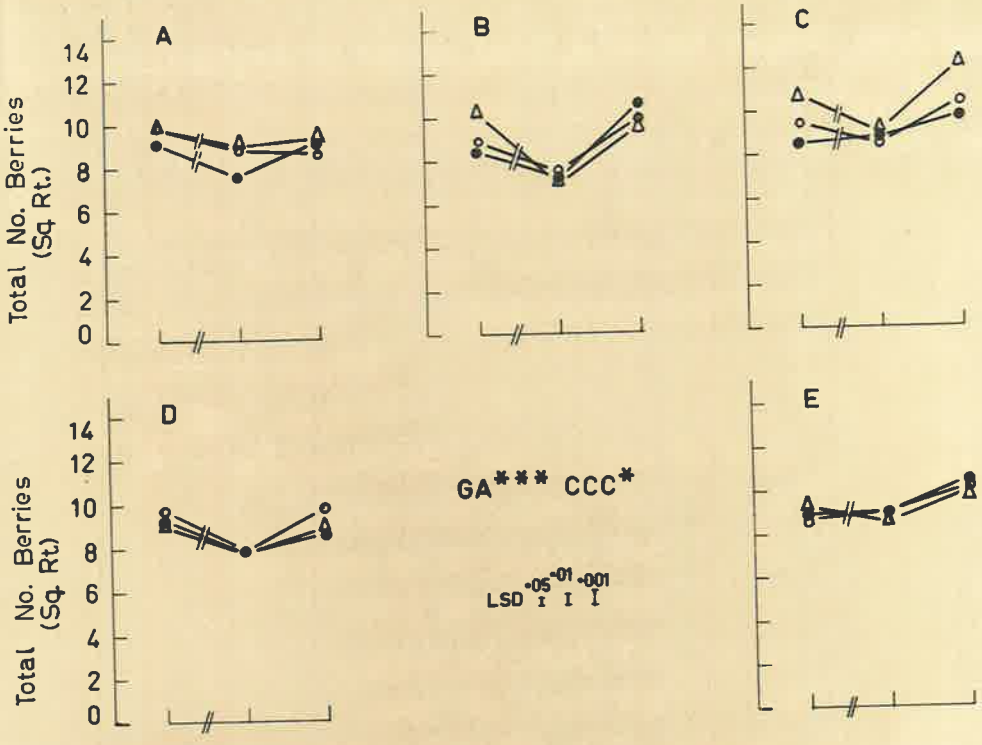



Figure 2.16

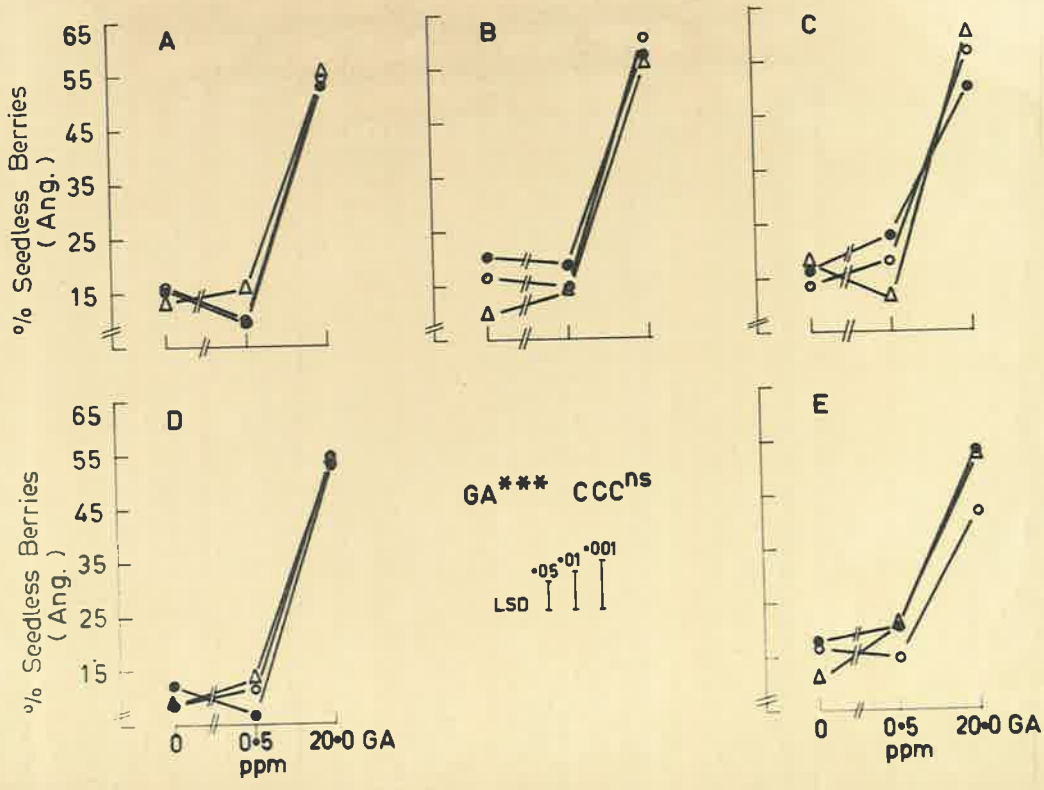


Fig. 2.17 Muscat 1967-8 - Fresh weight of seeded berries

Fig. 2.18 Muscat 1967-8 - Fresh weight of seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentrations: 0ppm ._____.

100ppm o_____o

1000ppm △____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# MUSCAT

Figure 2.17

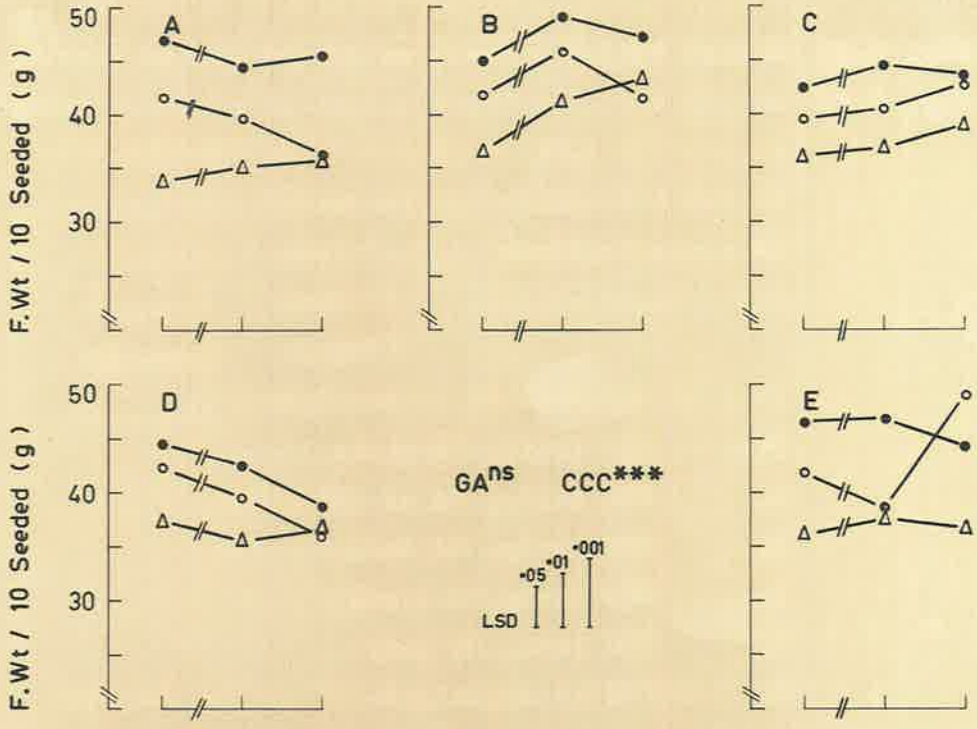



Figure 2.18

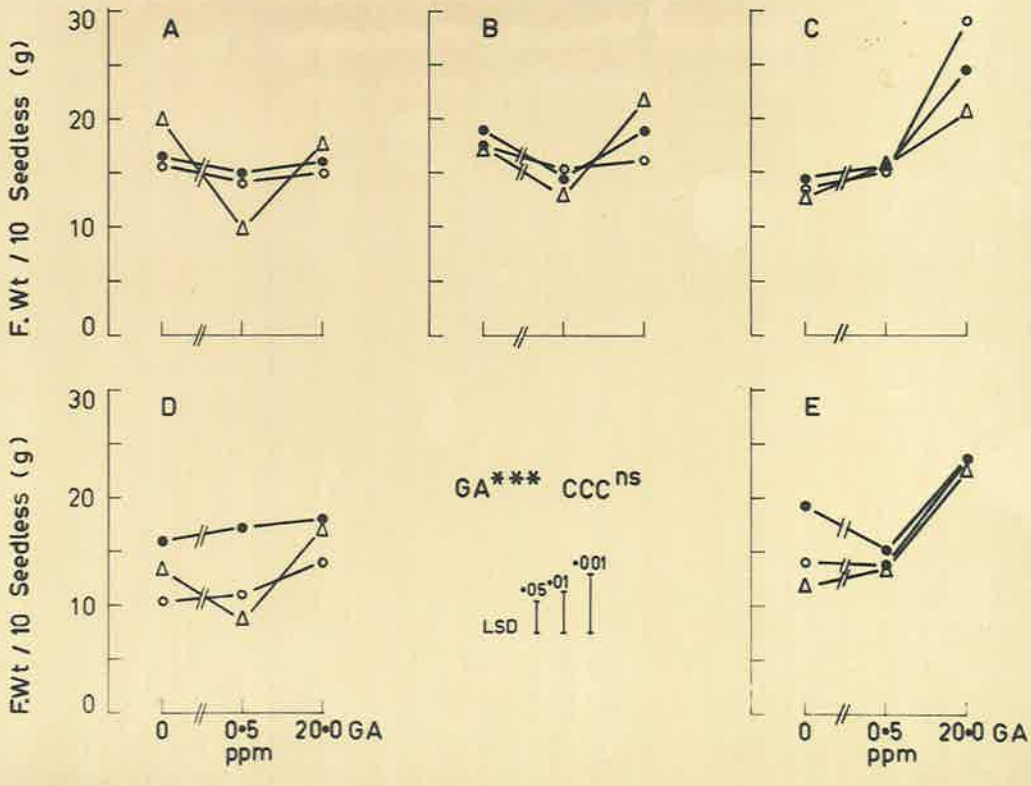


Fig. 2.19 Muscat 1967-8 - Length of seeded berries

Fig. 2.20 Muscat 1967-8 - Width of seeded berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

GA concentration: 0ppm ._____.

100ppm o_____o

1000ppm Δ_____Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# MUSCAT

Figure 2.19

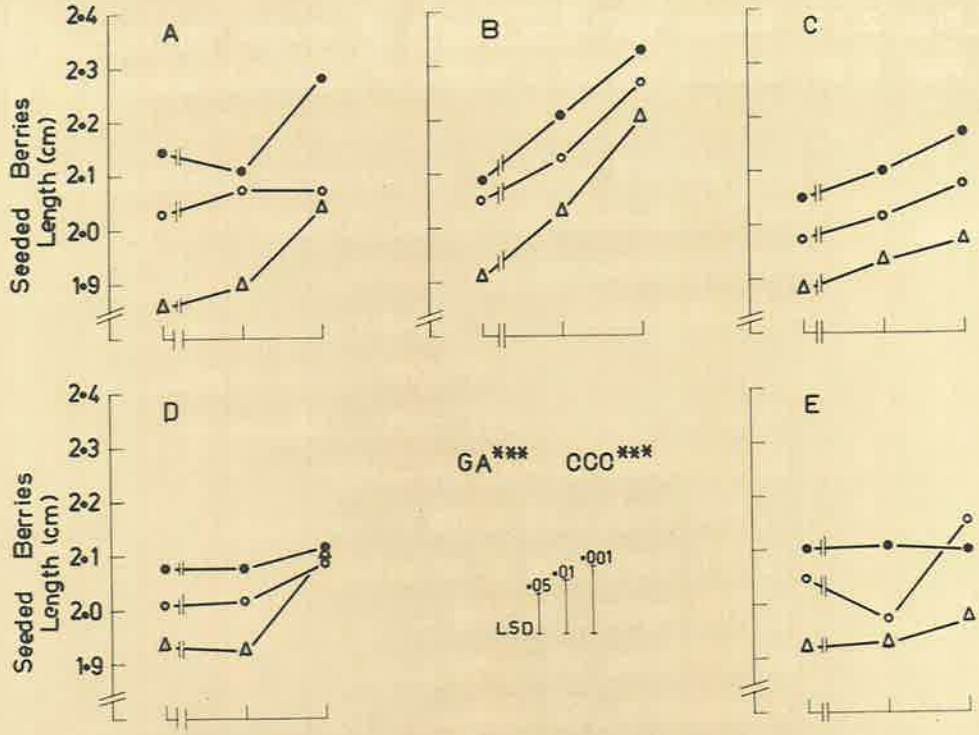



Figure 2.20

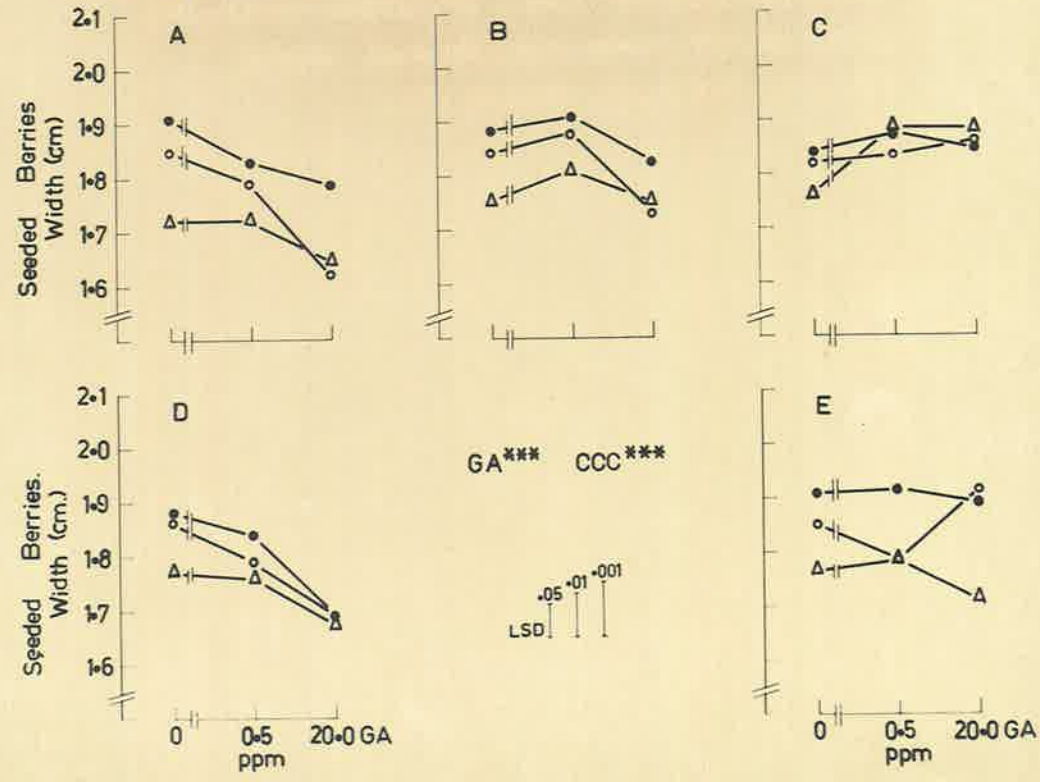


Fig. 2.21 Muscat 1967-8 - Length of seedless berries

Fig. 2.22 Muscat 1967-8 - Width of seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# MUSCAT

Figure 2.21

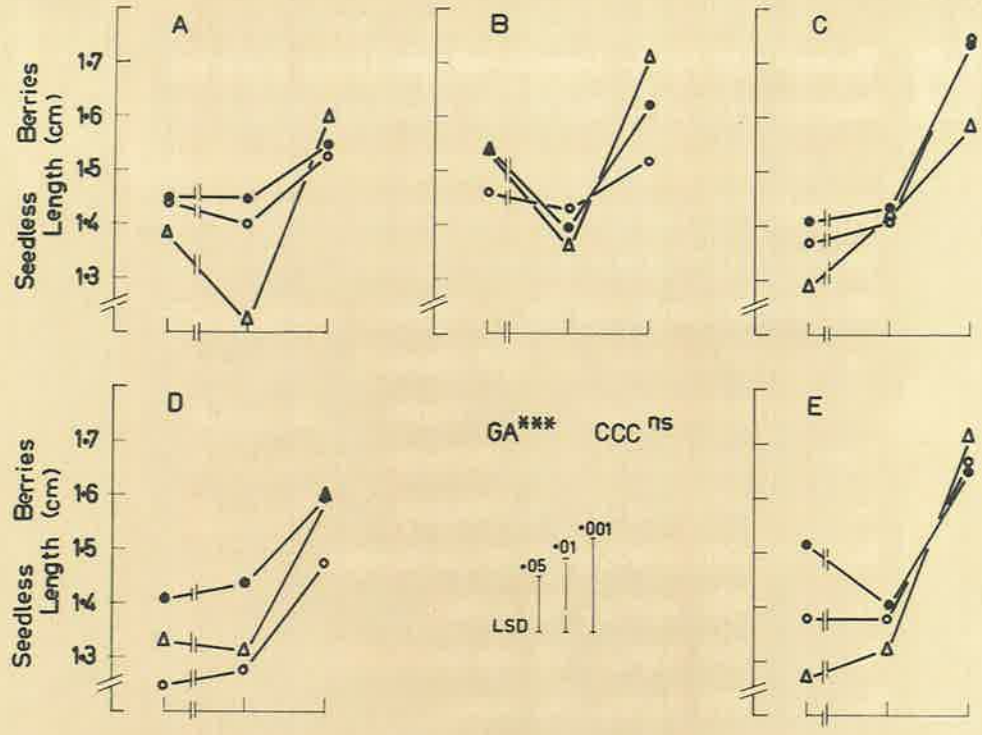



Figure 2.22

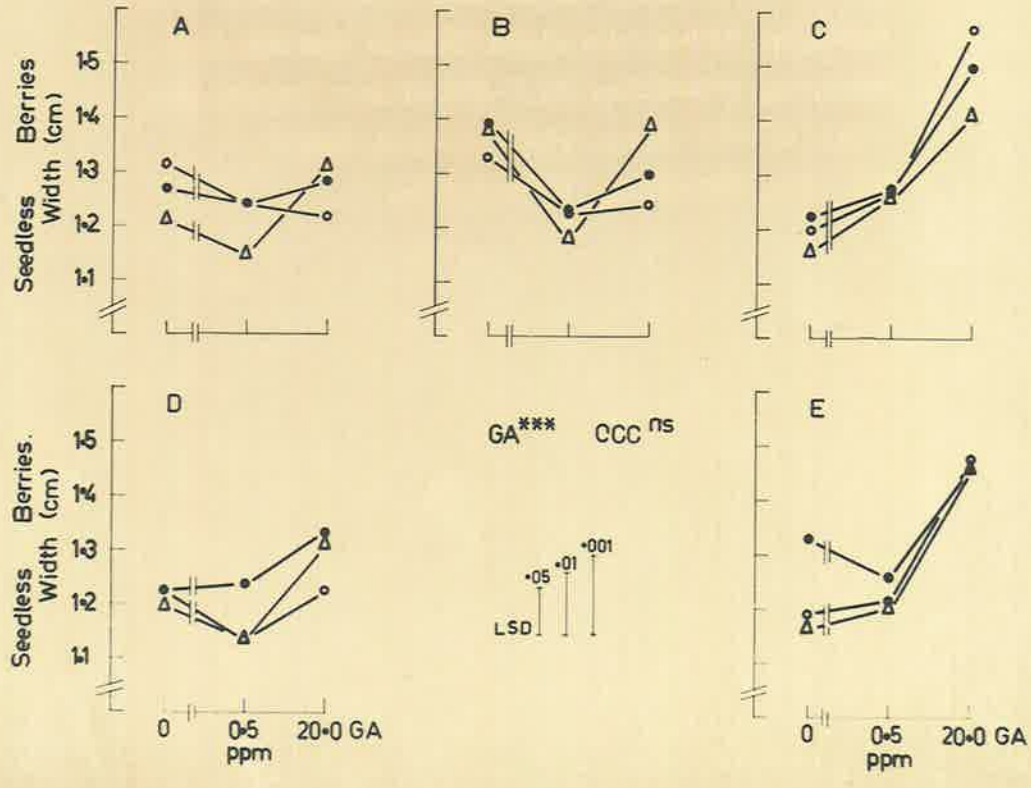


Fig. 2.23 Muscat 1967-8 - Length/width ratio seeded berries

Fig. 2.24 Muscat 1967-8 - Length/width ratio seedless berries

GA and CCC applied in factorial combination

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm Δ_____Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

MUSCAT

Figure 2.23

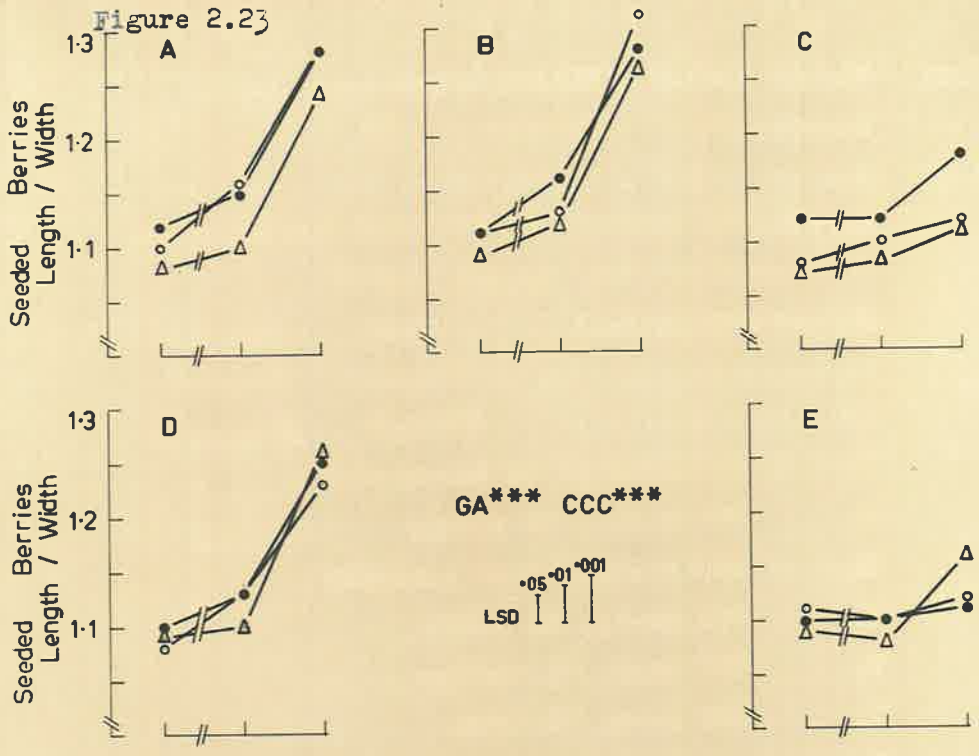



Figure 2.24

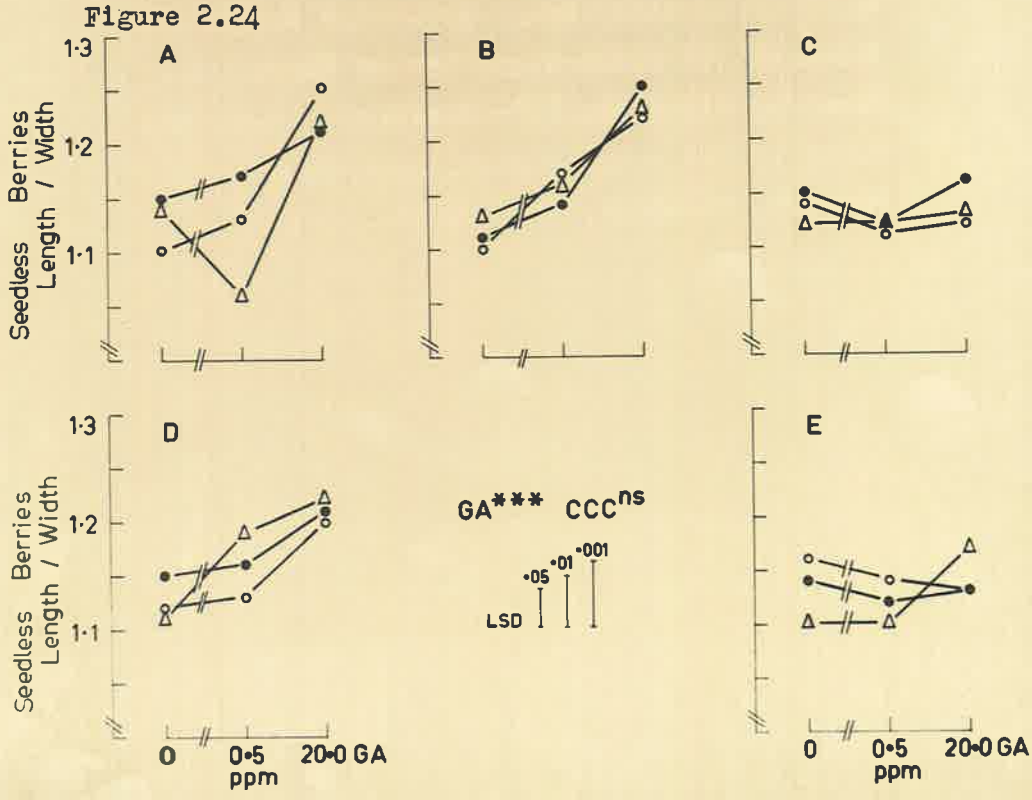


Fig. 2.25 Muscat 1967-8 - Rachis length

Fig. 2.26 Muscat 1967-8 - Cluster weight

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △____△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# MUSCAT

Figure 2.25

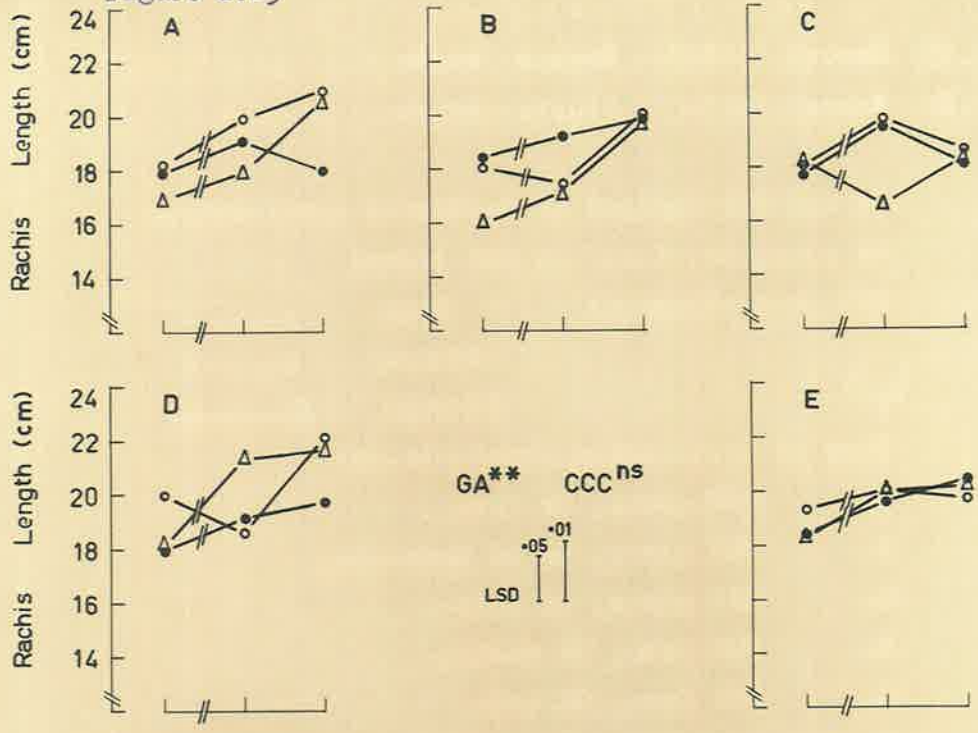



Figure 2.26

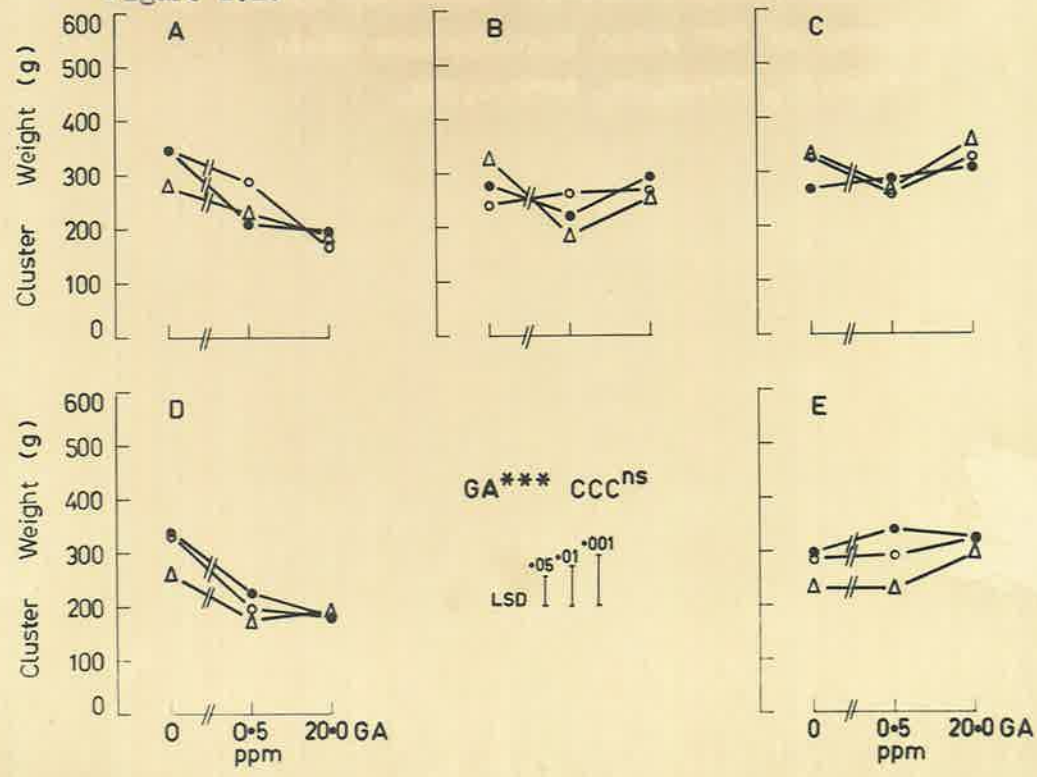


Fig. 2.27 Sultana 1967-8 - Number of berries per cluster

Fig. 2.28 Sultana 1967-8 - Fresh weight of berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .____. 100ppm o____o 1000ppm Δ____Δ

Time of application combinations:

A. CCC early, GA late;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

## SULTANA

Figure 2.27

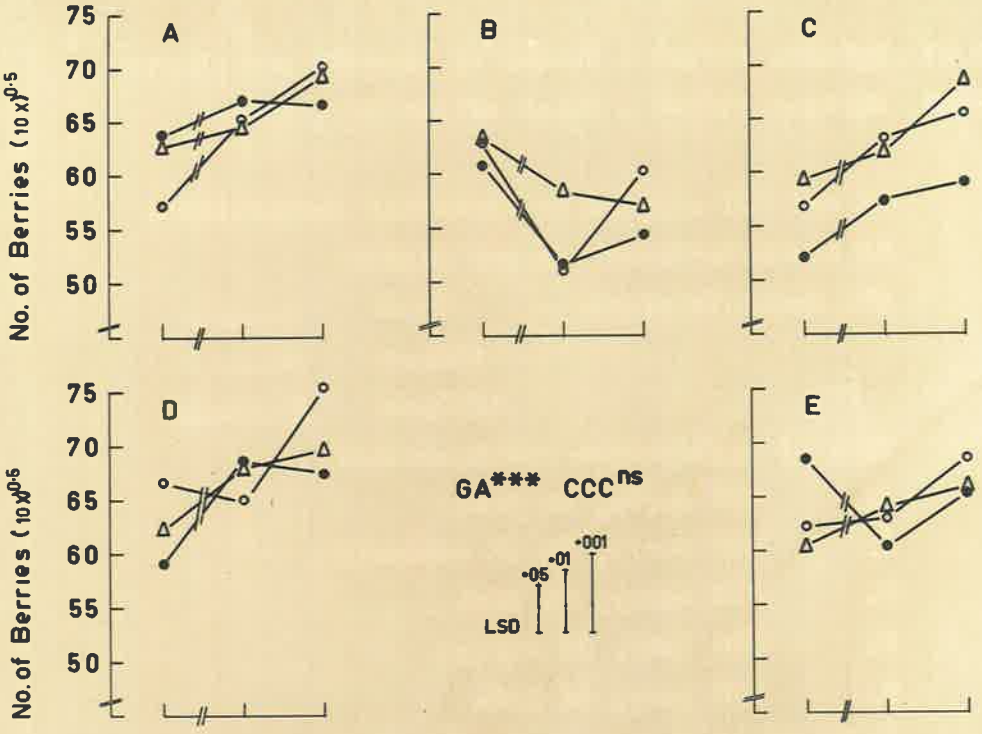



Figure 2.28

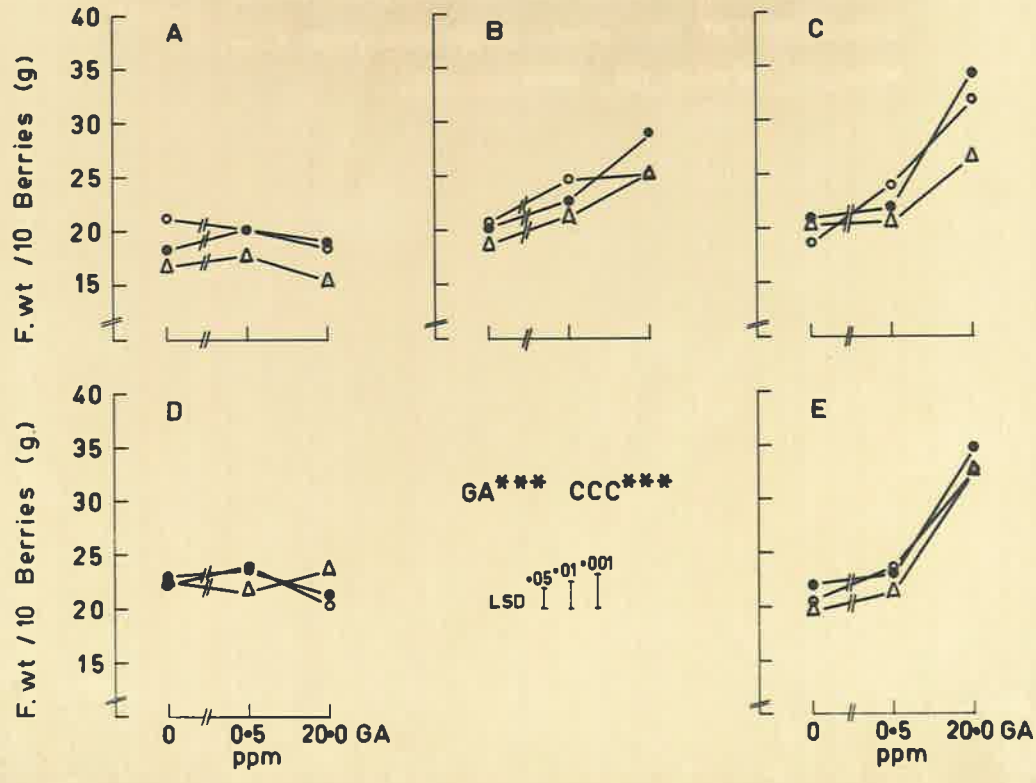


Fig. 2.29 Sultana 1967-8 - Length of berries

Fig. 2.30 Sultana 1967-8 - Width of berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .____.

100ppm o____o

1000ppm Δ——Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus : *P=0.05, **P=0.01, ***P=0.001.

# SULTANA

Figure 2.29

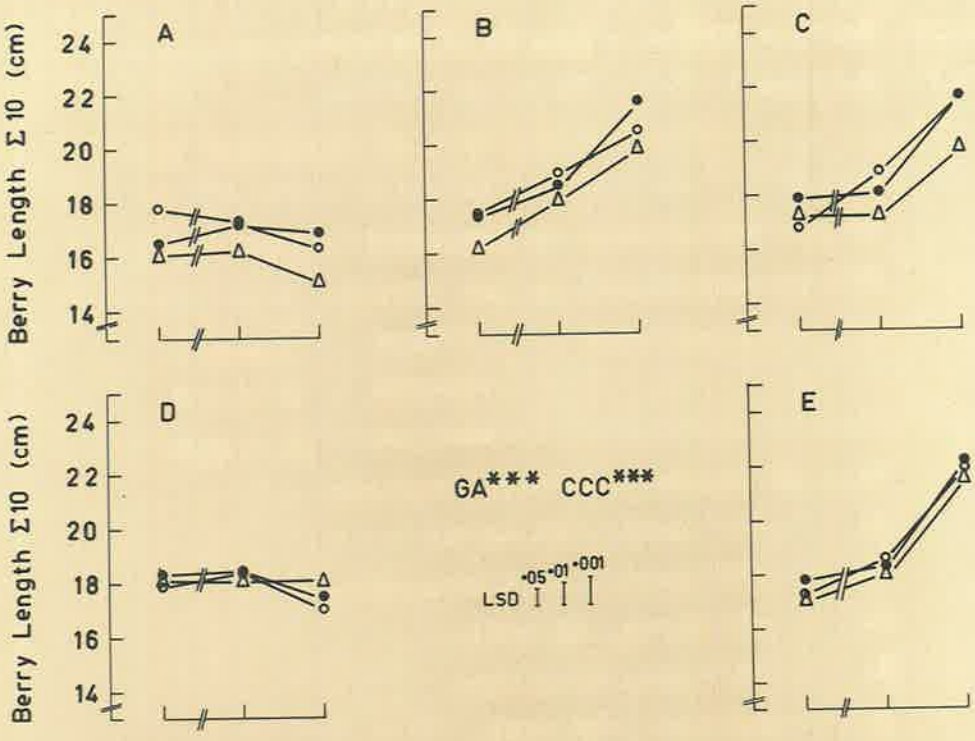



Figure 2.30

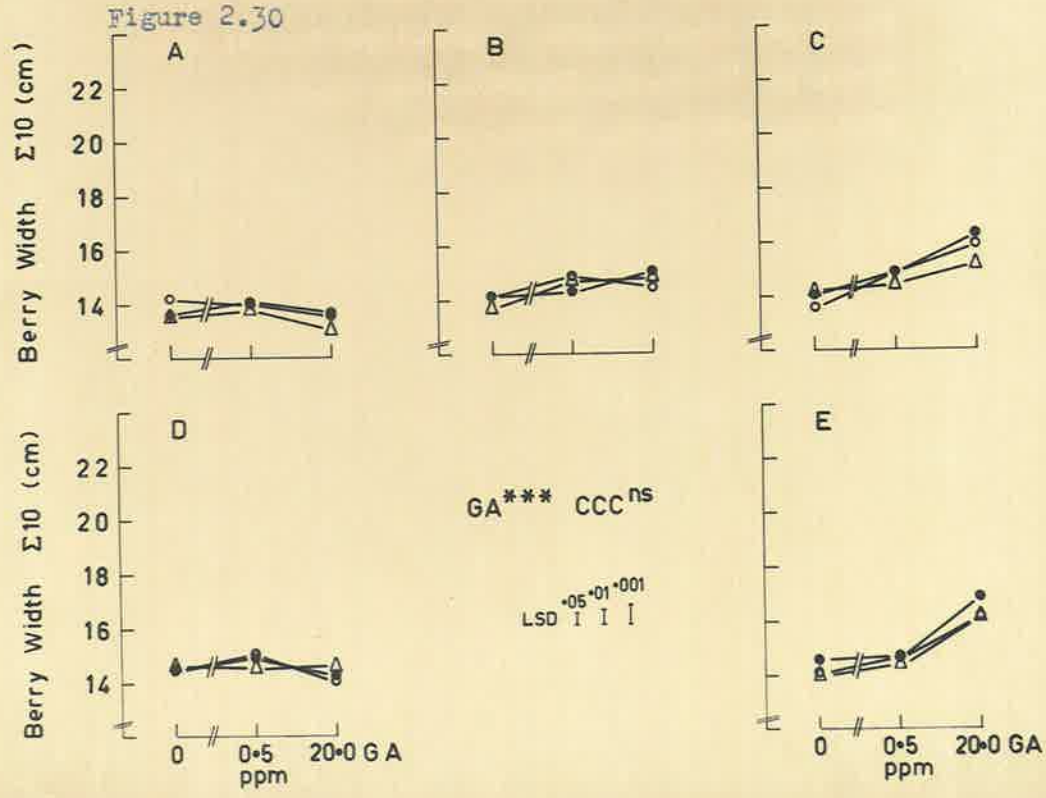


Fig. 2.31 Sultana 1967-8 - Length/width ratio of berries

Fig. 2.32 Sultana 1967-8 - Refraction of expressed juice

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .——. 100ppm o——o 1000ppm △——△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# SULTANA

Figure 2.31

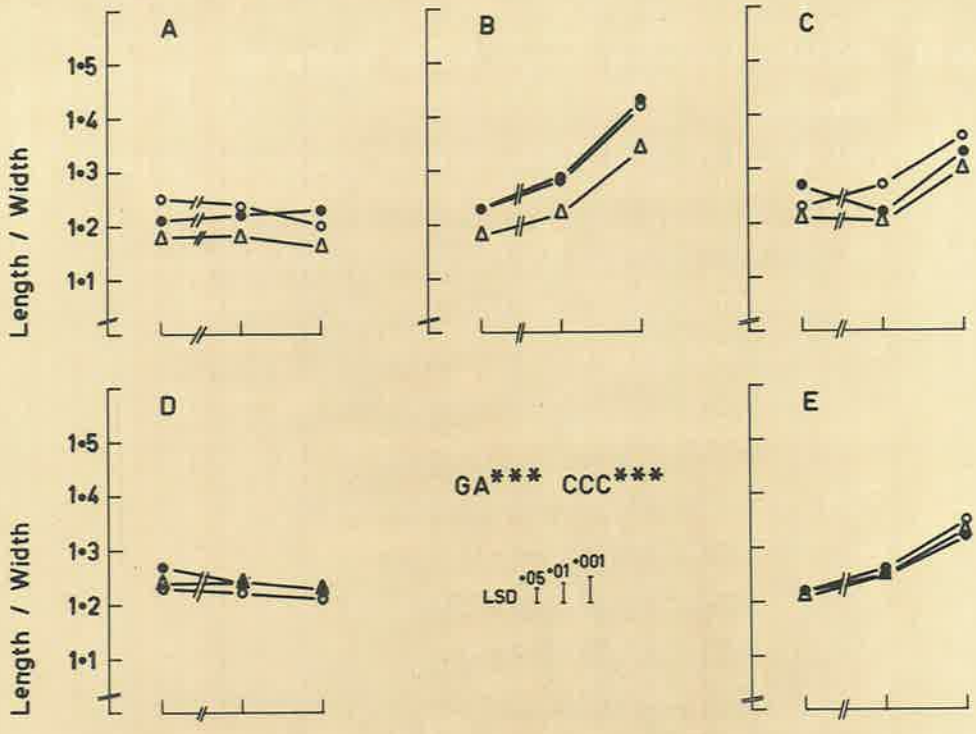



Figure 2.32

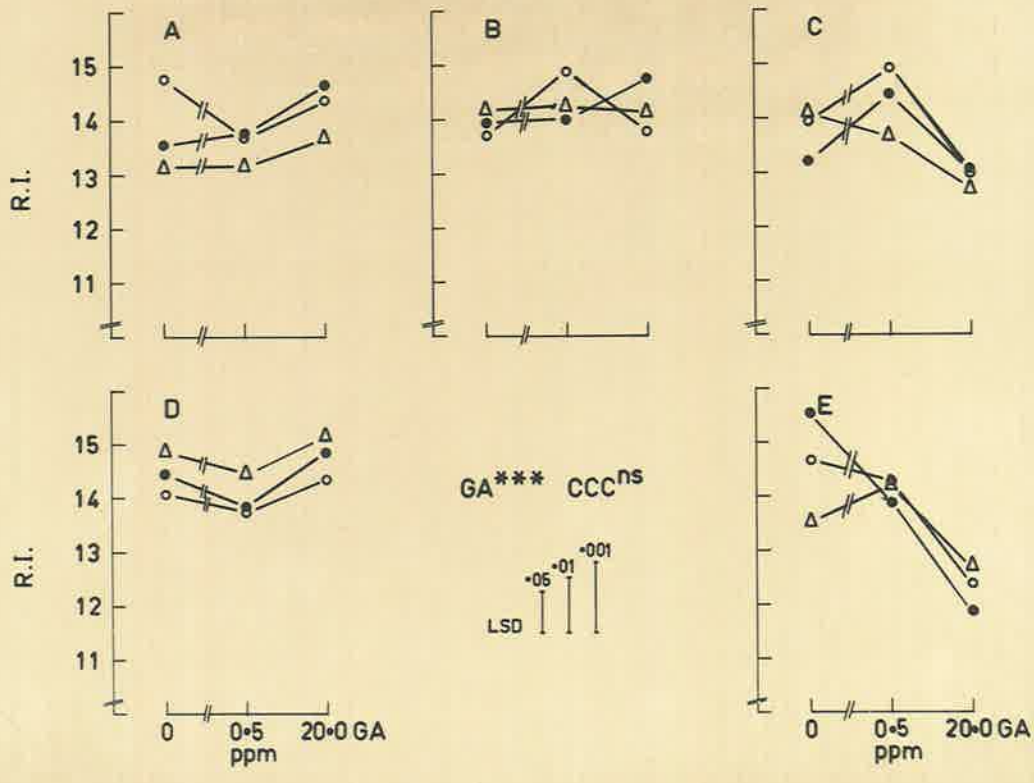


Fig. 2.33 Sultana 1967-8 - Rachis length

Fig. 2.34 Sultana 1967-8 - Cluster weight

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .——.

100ppm o—— o

1000ppm △—— △

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# SULTANA

Figure 2.33

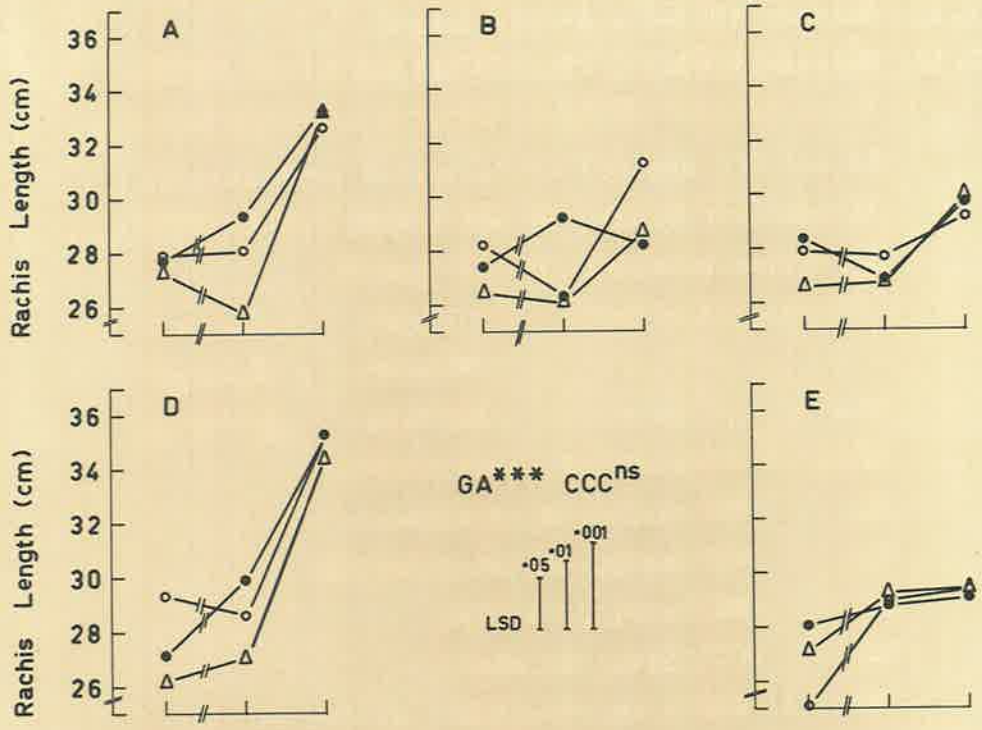



Figure 2.34

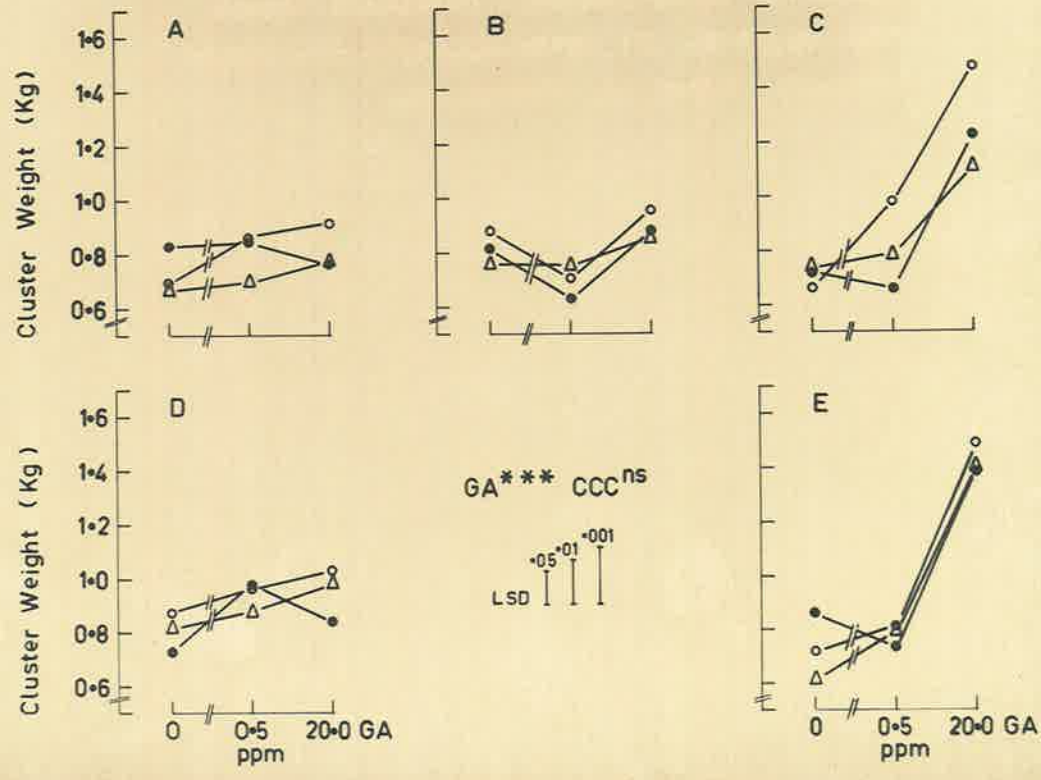


Fig. 2.35 Cape Currant 1967-8 - Total number of berries

Fig. 2.36 Cape Currant 1967-8 - Percent seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .——.

100ppm o——o

1000ppm Δ——Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

# CAPE

Figure 2.35

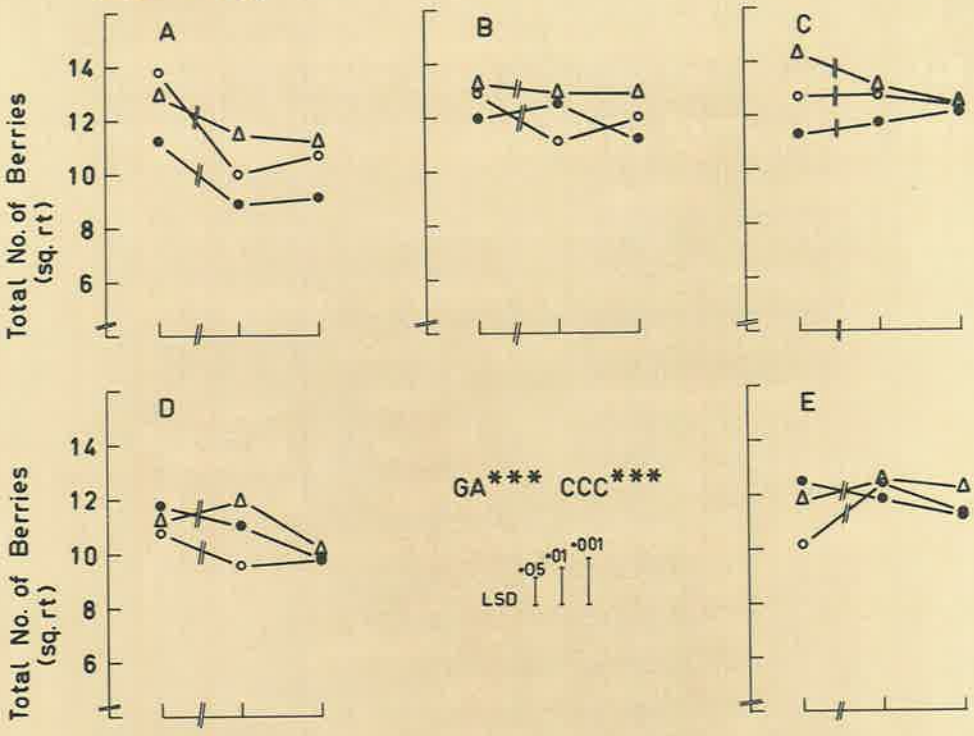



Figure 2.36

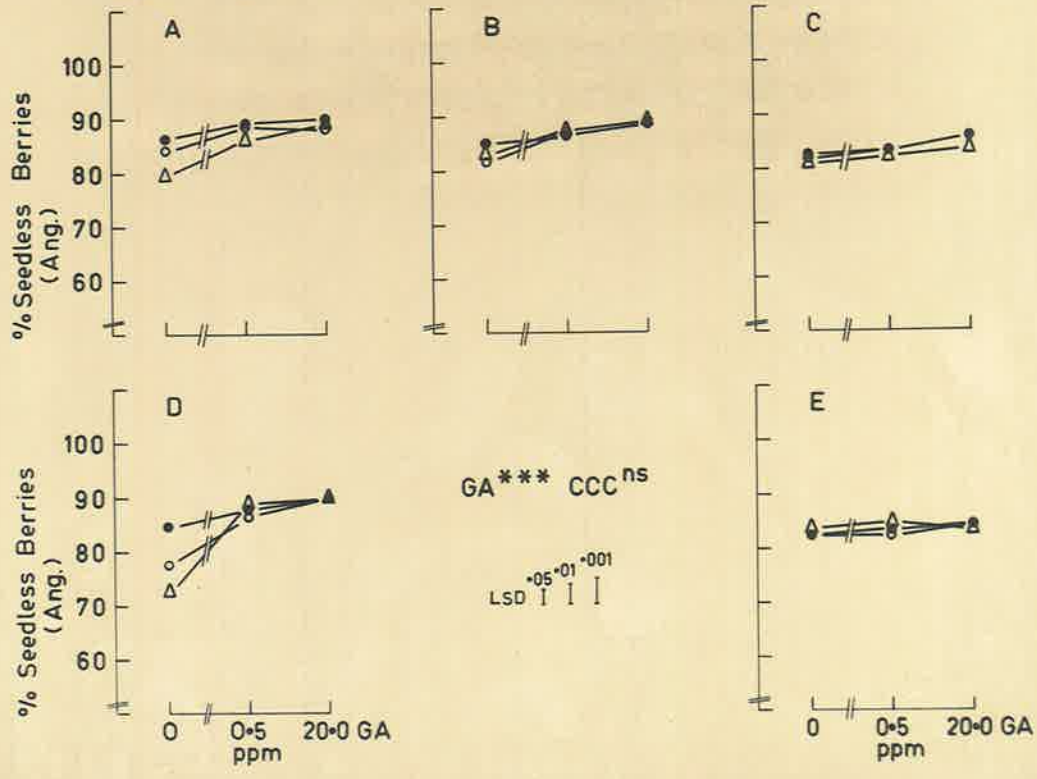


Fig. 2.37 Cape Currant 1967-8 - Fresh weight of seedless berries

Fig. 2.38 Cape Currant 1967-8 - Length/width ratio of seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration:

0ppm .——.

100ppm o——o

1000ppm Δ——Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

# CAPE

Figure 2.37

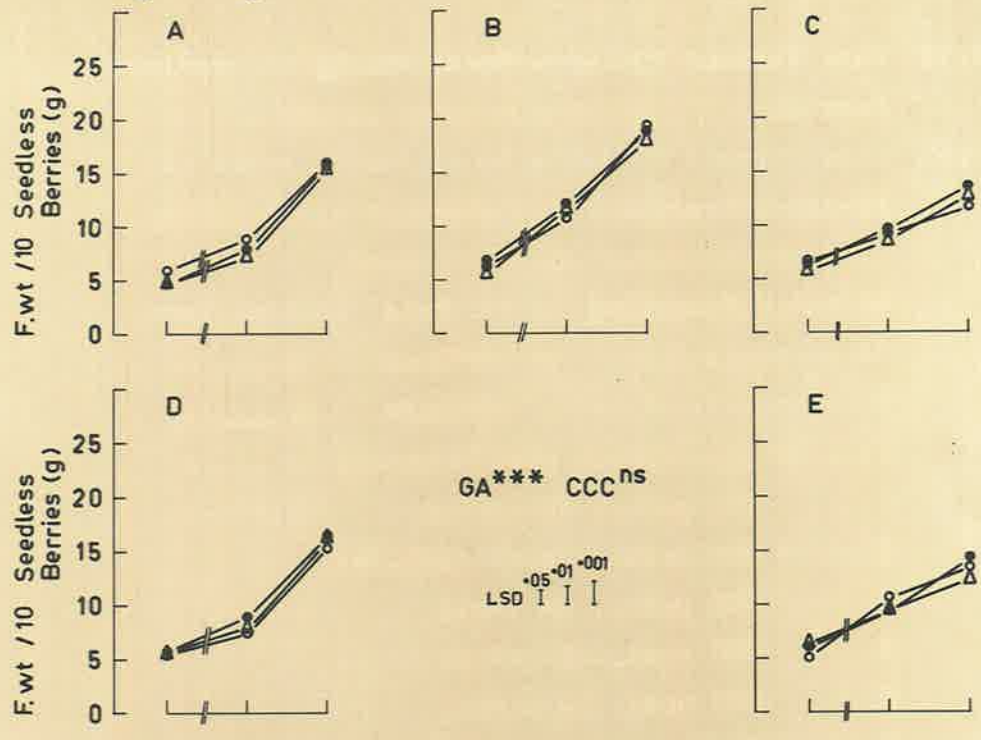



Figure 2.38

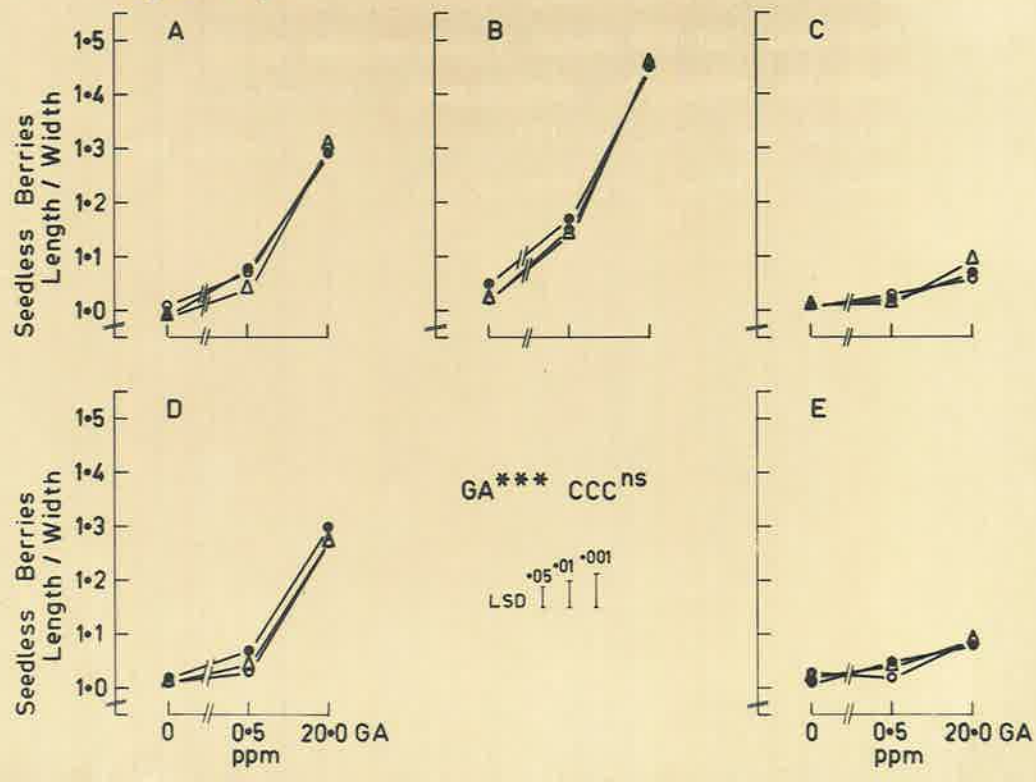


Fig. 2.39 Cape Currant 1967-8 - Rachis length

Fig. 2.40 Cape Currant 1967-8 - Cluster weight

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm Δ_____Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

# CAPE

Figure 2.39

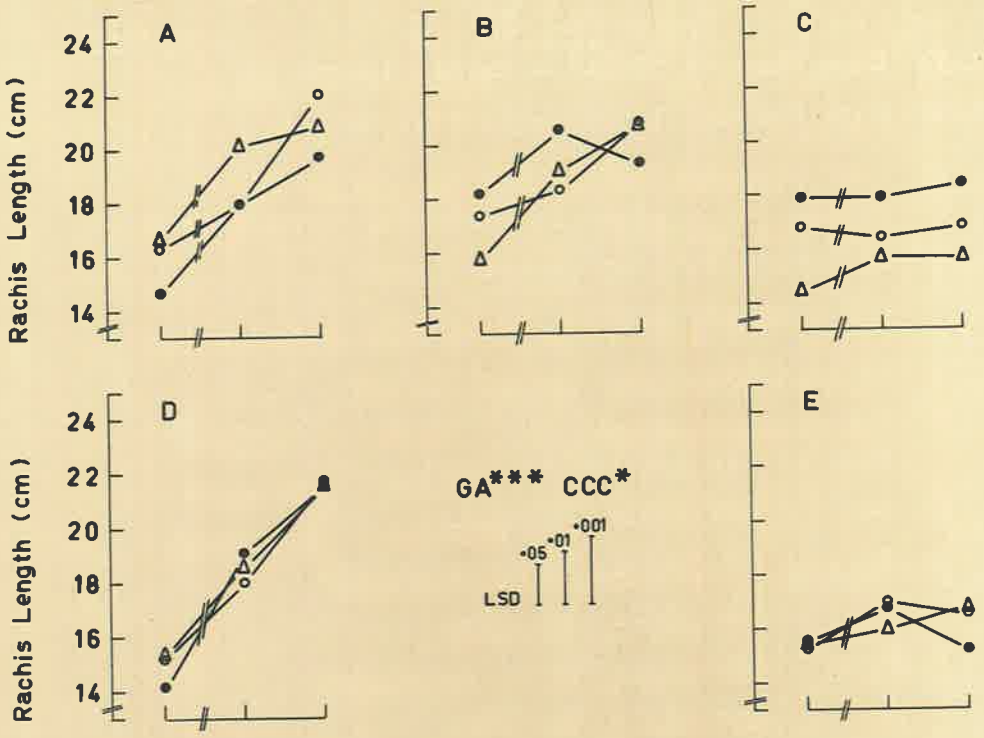



Figure 2.40

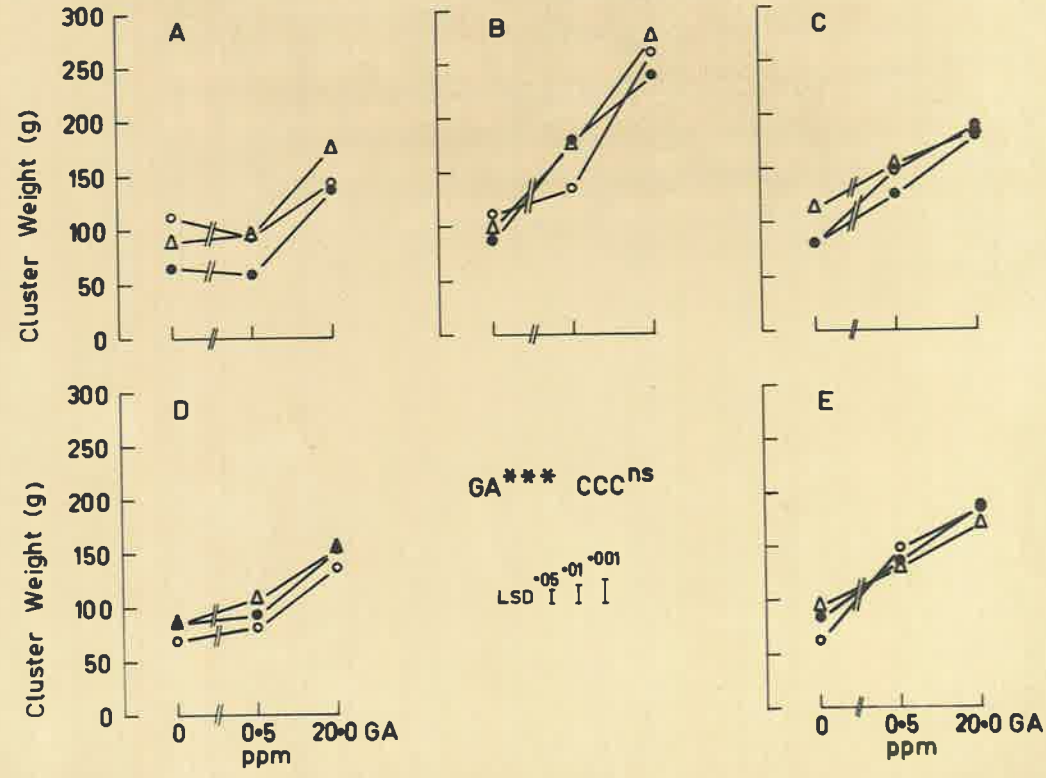


Fig. 2.41 Zante Currant 1967-8 - Number of seedless berries

Fig. 2.42 Zante Currant 1967-8 - Fresh weight of seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o____ o

1000ppm Δ —— Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus:

*P=0.05, **P=0.01, ***P=0.001.

# ZANTE

Figure 2.41

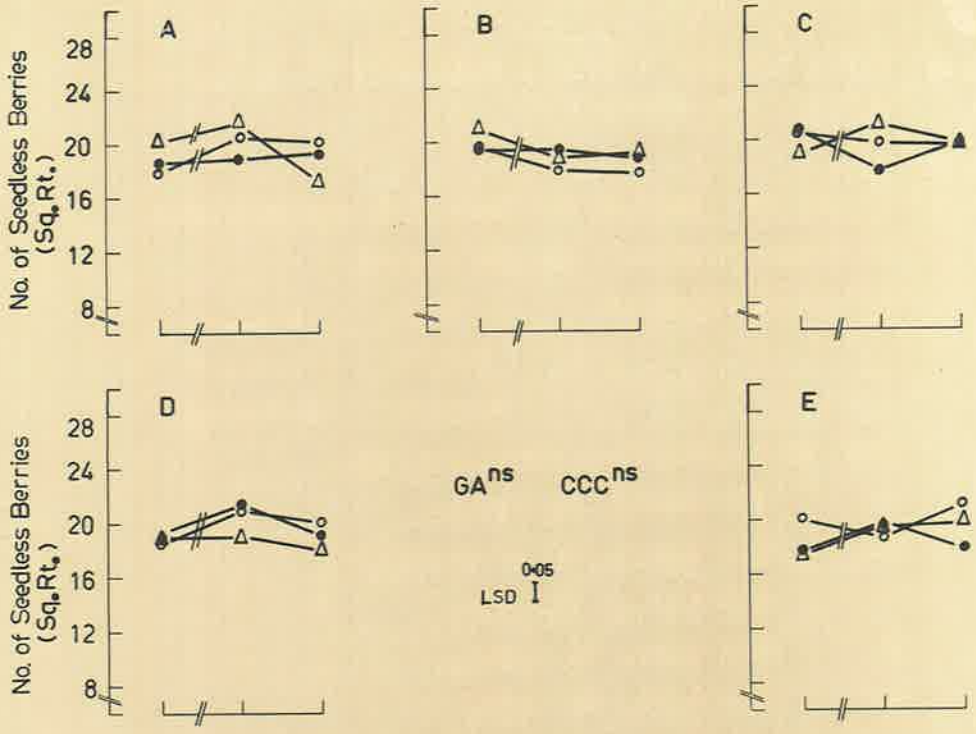



Figure 2.42

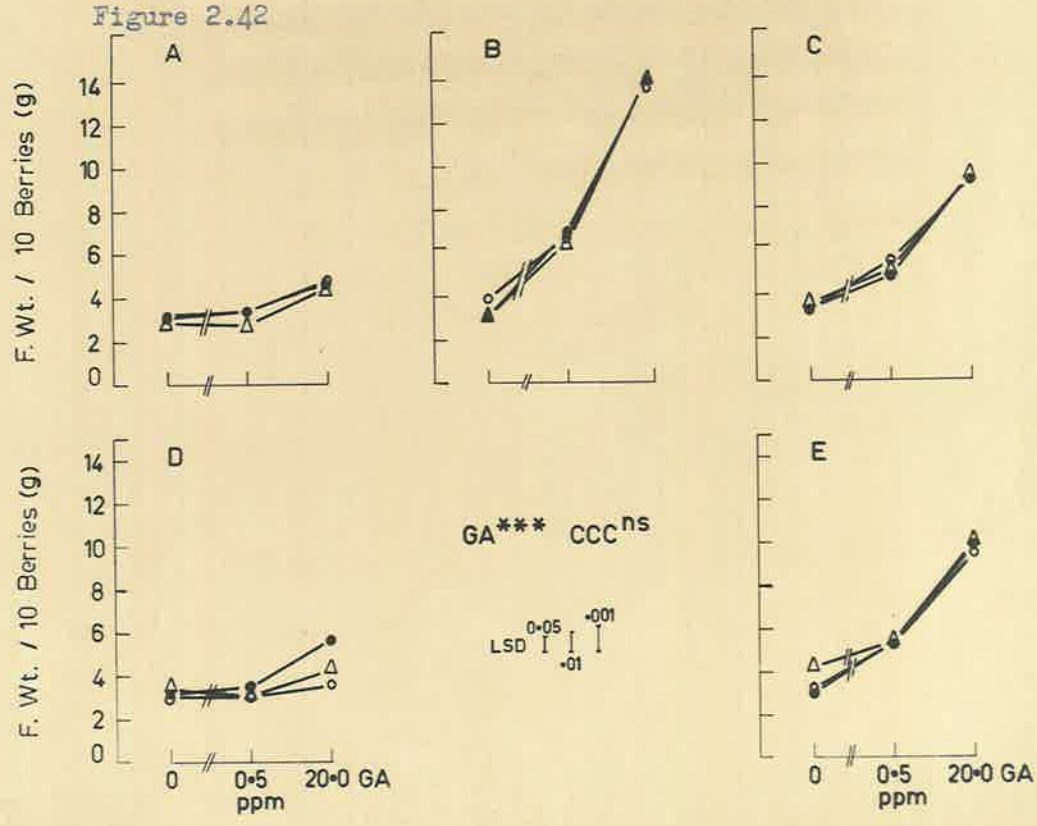


Fig. 2.43 Zante Currant 1967-8 - Length/width ratio of seedless berries

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .____.

100ppm o____o

1000ppm Δ____Δ

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

ZANTE

Figure 2.43

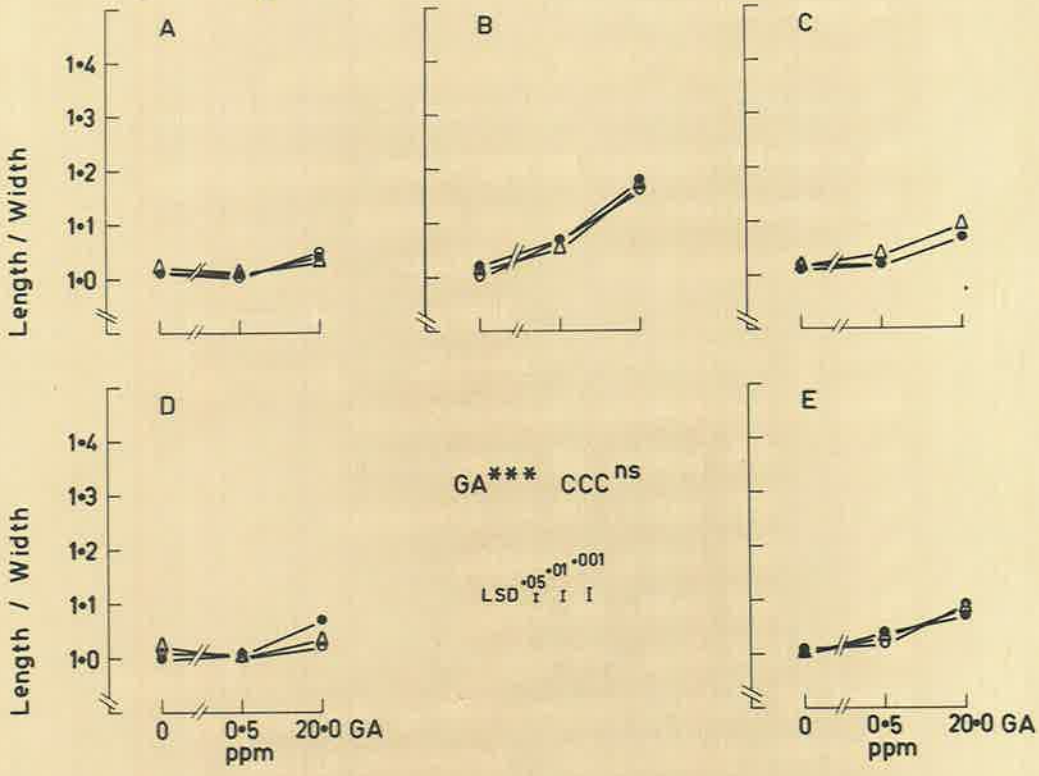


Fig. 2.44 Zante Currant 1967-8 - Refraction of expressed juice

Fig. 2.45 Zante Currant 1967-8 - Pedicel length

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm ._____.

100ppm o_____o

1000ppm △——△

Time of application combinations:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

# ZANTE

Figure 2.44

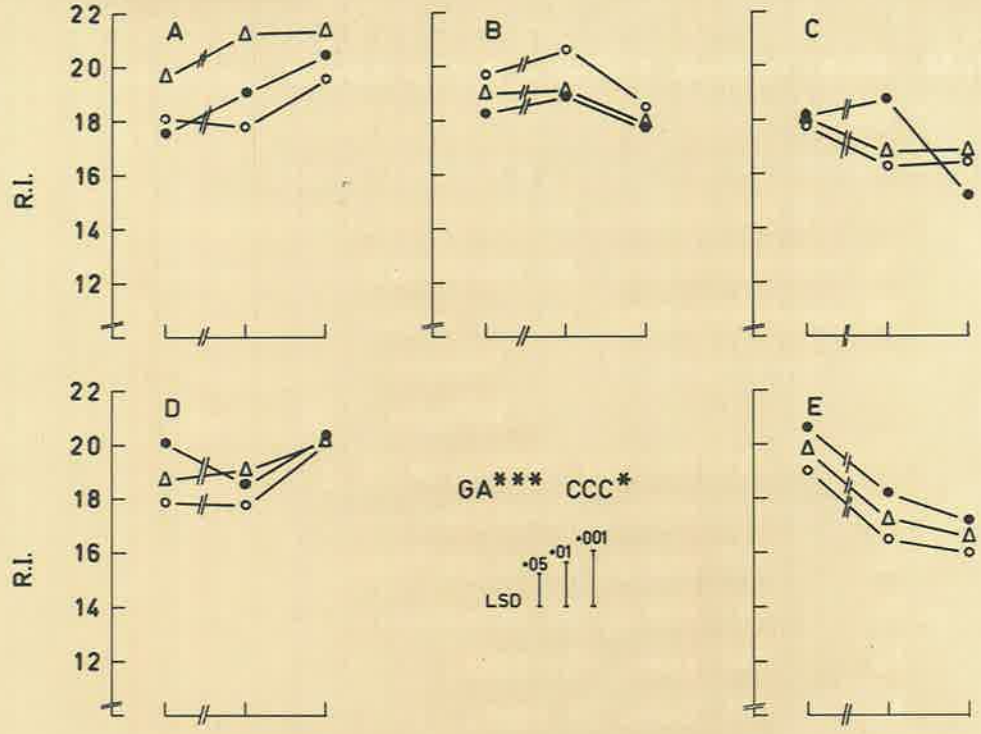



Figure 2.45

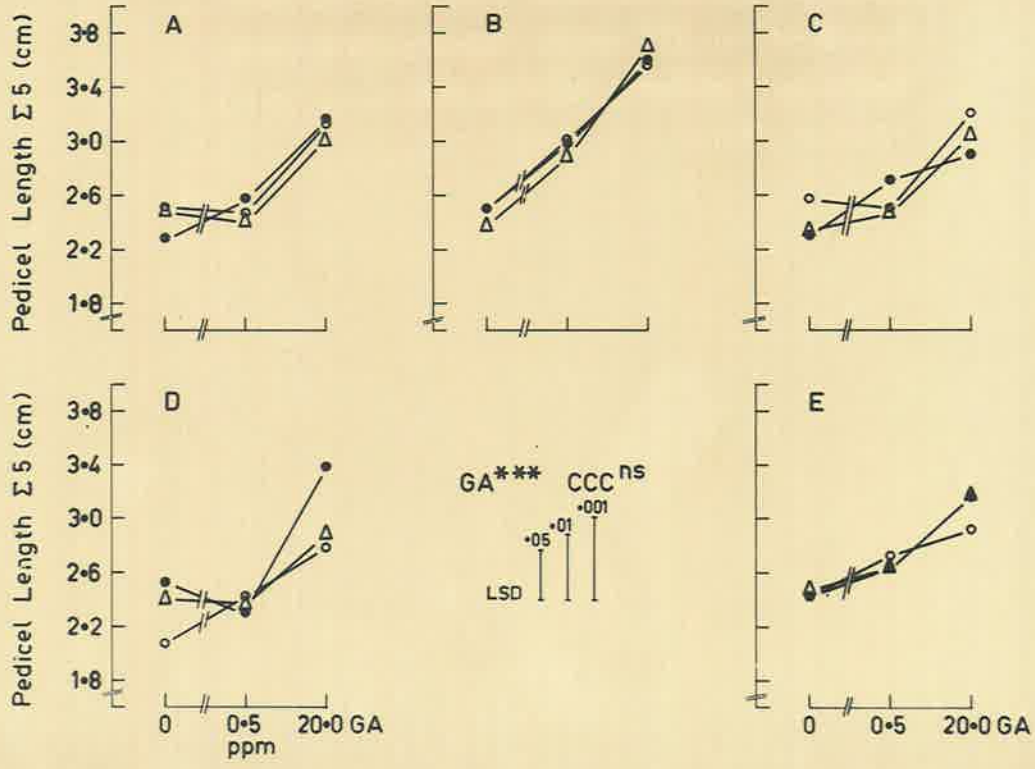


Fig. 2.46 Zante Currant 1967-8 - Rachis length

Fig. 2.47 Zante Currant 1967-8 - Cluster weight

GA and CCC applied in factorial combinations

GA concentration: as per legend

CCC concentration: 0ppm .——.

100ppm o——o

1000ppm Δ——Δ

Time of application combination:

A. CCC early, GA early;

B. CCC early, GA at anthesis;

C. CCC early, GA late;

D. CCC late, GA early;

E. CCC late, GA late.

Vertical lines represent LSD at the denoted level of significance. Asterisks denote the level of 'F' ratio significance thus: *P=0.05, **P=0.01, ***P=0.001.

# ZANTE

Figure 2.46

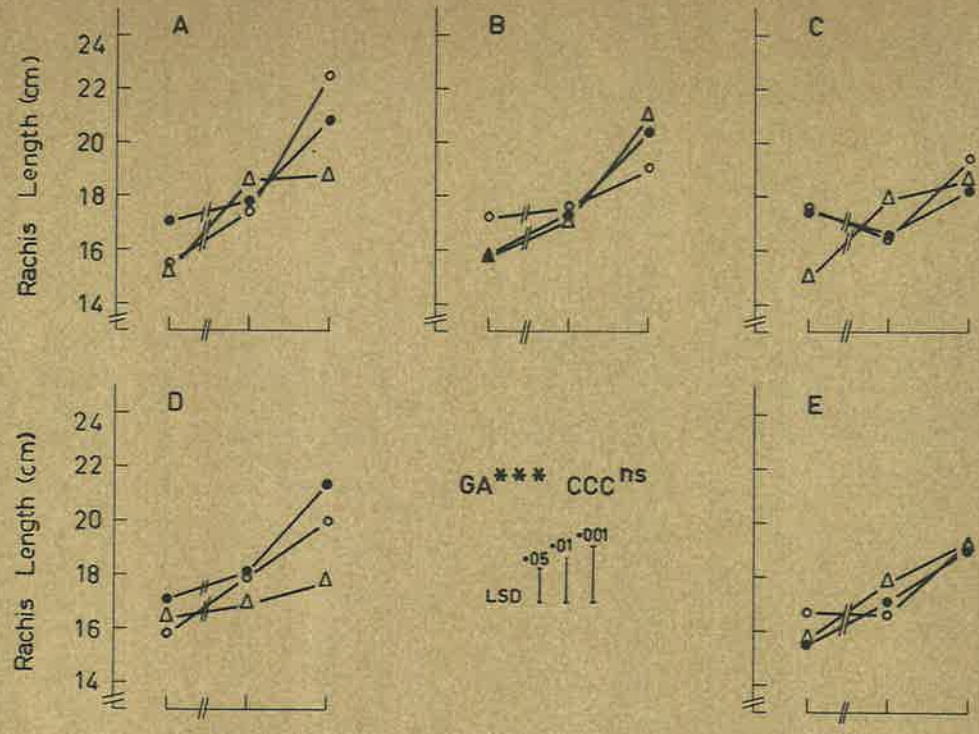



Figure 2.47

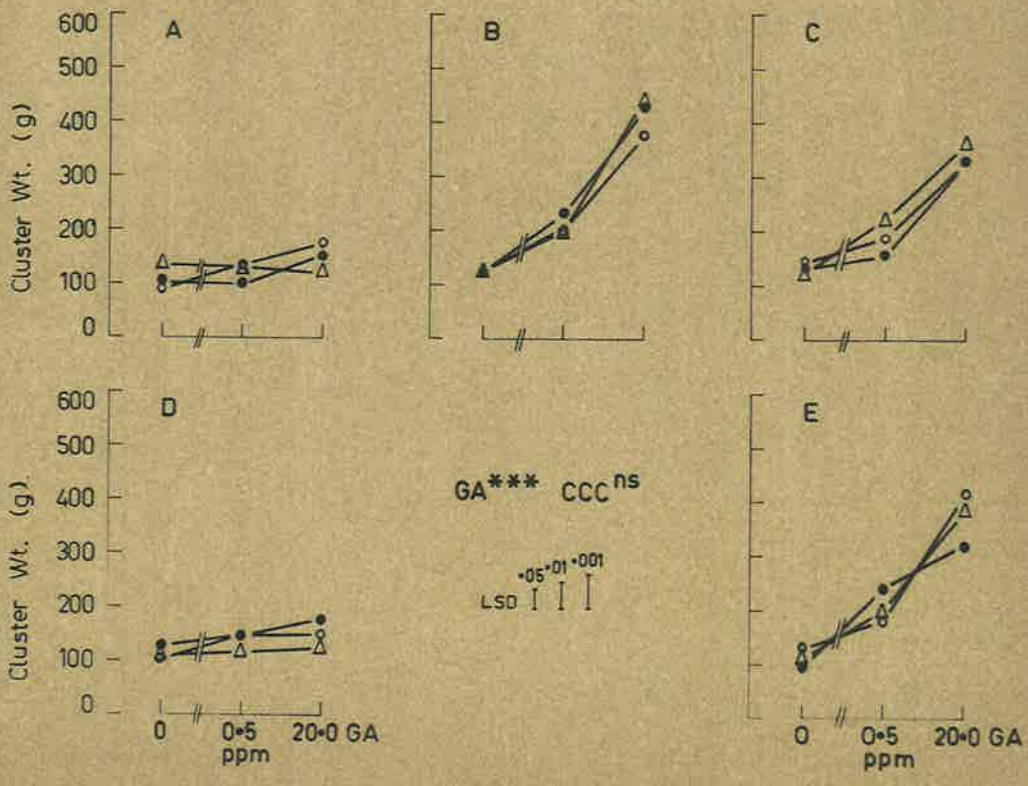


Table 2.1

## Summary of results for 1966-7 and 1967-8

| PARAMETER | Doradillo 1967-8 | | | Muscat 1966-7 | | | Muscat 1967-8 | | | Sultana 1967-8 | | | Cape Currant 1966-7 | | | Cape Currant 1967-8 | | | Zante Currant 1966-7 | | | Zante Currant 1967-8 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT | GA | CCC | INT |
| Cluster weight | ±*** | . | . | . | . | . | −*** | . | . | +*** | . | . | +*** | (+) | . | +*** | . | . | +* | . | . | +*** | . | . |
| Total no. berries | +*** | . | . | . | +*** | . | +*** | +* | . | | | | −*** | (+) | * | −*** | +*** | . | | | | | | |
| Rachis length | +** | . | . | +*** | −** | *** | +** | . | . | +*** | . | . | +*** | . | . | +*** | −* | . | +*** | . | . | +*** | . | . |
| Pedicel length | | | | +*** | . | ** | | | | | | | +*** | (−) | *** | | | | | | | +*** | . | . |
| **SEEDED BERRIES** | | | | | | | | | | | | | | | | | | | | | | | | |
| No. per cluster | −*** | . | . | . | +*** | . | −*** | . | . | | | | −*** | . | . | −*** | +** | . | | | | | | |
| Fresh weight | . | −*** | . | −*** | −*** | * | . | −*** | . | | | | . | −* | . | | | | | | | | | |
| Length | . | −*** | . | (−) | −*** | * | +*** | −*** | . | | | | . | . | . | | | | | | | | | |
| Width | . | −*** | . | −*** | −*** | . | −*** | −*** | . | | | | . | . | . | | | | | | | | | |
| L/W ratio | +*** | . | . | +*** | (−) | . | +*** | −*** | . | | | | +** | . | . | | | | | | | | | |
| Sugar(RI) | | | | . | . | . | | | | | | | . | +* | * | | | | | | | | | |
| **SEEDLESS BERRIES** | | | | | | | | | | | | | | | | | | | | | | | | |
| No. per cluster | +*** | . | . | −* | . | . | +*** | . | . | +*** | +* | . | −*** | (+) | * | −** | +*** | . | −* | +** | . | . | . | . |
| Fresh weight | | | | −*** | −*** | . | +*** | . | . | +*** | −*** | . | +*** | . | . | +*** | . | . | +*** | . | . | +*** | . | . |
| Length | | | | −* | −*** | . | +*** | . | . | +*** | −*** | . | +*** | . | . | +*** | . | . | | | | +*** | −** | . |
| Width | | | | −*** | −*** | . | +*** | . | . | +*** | . | . | +*** | . | . | +*** | . | . | | | | +*** | −* | . |
| L/W ratio | | | | +*** | . | . | +*** | . | . | +*** | −*** | . | +*** | . | . | +*** | . | . | | | | +*** | . | . |
| Sugar(RI) | | | | . | −*** | . | | | | −*** | . | . | +*** | . | . | | | | | | | +*** | −* | . |
| % seedless berries | +*** | . | . | −* | −** | . | +*** | . | . | | | | (+)[1] | . | . | +*** | . | . | | | | | | |

+,− = positive or negative response, i.e. the parameter increased or decreased with increasing concentration of GA or CCC

() = estimate of the response, although this is confused by non-parallel curves

* = statistical significance of the variance ratio; *5%, **1%, ***0.1%

. = not significant

blank spaces = not measured or absent

1 = not analysable because GA at 20ppm gave 100% seedless

(4) Discussion

Fruit set is considered by many authors to be that process which results in fruit development; retention (non-abscission) of the ovary is thought to be consequential to this. Substantiation of this point of view has come from a number of experimental observations in which the process of development has been separated from abscission: GA and auxin treated ovaries may be retained but fail to develop (e.g. Weaver and McCune 1957, 1959a & b), and development has been induced in abscissed ovules (Leopold and Scott 1952). Thus a berry which has been retained and has begun to grow is regarded as having set. The further development of the fruit to maturity is often considered separately.

There is ample literature suggesting that in many fruits both pollination and syngamy are processes essential to setting (e.g. Massart 1902; Luckwill 1957; Coombe 1960; Jackson 1966). Fruits that set without pollination (vegetative parthenocarpy) are exceptions to this concept.

The processes that are especially correlated with pericarp expansion, namely endosperm and embryo development, also play an important role in set. The work of Marré (1948) illustrates this point in that he was able to demonstrate that a perfect-seeded fruit was able to draw nutrients from a parthenocarpic fruit in close proximity to it. Nitsch et al. (1960) made a similar suggestion with reference to grape flower clusters. Further, since it has been suggested that one factor limiting fruit set is the level of available nutrients (Coombe 1960), it may be supposed that berries in which seed development is occurring will be set preferentially.

The results of the present investigations are discussed in relation to these concepts of fruit development.

The relationship between GA and fruit-set is poorly understood, and is clouded by the induction of parthenocarpy in seeded cultivars (Weaver and McCune 1959a & b, 1962; Crane 1964; Zuluaga and Lumelli 1966; Zuluaga et al. 1968). A reduction in the number of berries on certain seedless cultivars has been documented (Weaver and McCune 1959c; Lynn and Jensen 1966; Mosesian and Nelson 1968; Christodoulou et al. 1968). In the present investigation GA application often resulted in an increase in the total number of berries set on seeded cultivars. This effect was most pronounced in the cultivar Doradillo (Fig. 2.7), in which the later the treatment the greater the increase in set. Previous investigators have often overlooked this effect and have concentrated on the more spectacular reduction in seededness. Pre- or post-anthesis applications gave set increases in Sultana though no increase in set was noted in either of the two parthenocarpic cultivars, Zante and Cape. Anthesis application to Sultana and pre-anthesis application to Zante or Cape gave reductions in set.

Post-anthesis applications of GA have less effect on seed development than pre-anthesis applications (though the effect is still marked in Muscat), but are much more effective in stimulating the growth of seedless berries (Fig's. 2.18, 2.28, 2.37, 2.42). It seems reasonable to suppose that the increase in set of seedless berries is related to this stimulated growth and concomitant increase in the ability of these berries to attract nutrients without detriment to those berries that have already commenced to grow

actively. Other examples of GA-induced setting of parthenocarpic fruit have been cited by Crane (1964).

The set increase brought about by a pre-anthesis application of GA on Sultana is difficult to explain since it is known that applications at this stage of development induce atrophy of the embryo sac tissue (Nishitani et al. 1968), and thereby preclude the occurrence of syngamy in at least one portion of the berries. At the concentrations of GA used pollen germinability is probably not limiting (Weaver and McCune 1960; Nishitani et al. 1968).

Decreases in set have been varyingly described as being due to a reduction in pollen germination (Weaver and McCune 1960), interference with ovule development (Watanabe 1963a, cited by Sachs and Weaver 1968, 1963b; Smirnov and Perepelysina 1965; Nishitani et al. 1968), and unbalanced development within the ovary (Mosesian and Nelson 1968). It is noteworthy that pre-anthesis applications reduced set in the two parthenocarpic cultivars, but that only when applied at anthesis was GA effective in reducing set in Sultana. This suggests a different mode of action in each case. Most attention has been paid to Sultana and it has been found that time of application is critical and GA is only effective in reducing set for a short time during anthesis. This period may coincide with the time between anther dehiscence and syngamy. An alternative but less direct explanation than those already cited for this effect may be that a group of berries (pollinated ?) may be more responsive to gibberellin and begin to grow very rapidly, thus depleting a limited supply of organic nutrients. This would result in the starvation of the less responsive group and subsequently in their abscission (Sachs and Weaver 1968, have noted that berries treated with GA respond within 24 hours).

Reduced set in Zante cannot be explained in terms of impaired ovule development since this cultivar does not depend on the presence of a fecund ovule for set; it is, however, dependent to a large degree on pollination (Stout 1936) and therefore it seems likely that the mode of action of GA may be through reduced pollen viability.

The effect of CCC is to increase set regardless of the cultivar (Coombe 1965, 1967). This universal effect is regarded by Coombe (pers.comm.) as being a response to an increase in the level of nutrients available to the cluster. Support for this hypothesis comes from the interaction between time of application of CCC and set: CCC applied early increases set and it also decreases shoot growth and this corresponds with a time when the cluster's capacity as an active 'sink' is declining (Hale and Weaver 1962); CCC applied at set has no effect on the degree of set, though it still reduces berry size. Further Coombe (1967) has shown that CCC applied at anthesis has little effect on set. Thus the prime effect of CCC would appear to be the retardation of growth and there is probably a concomitant reduction in the demand for nutrients. This demand is probably expressed both between the sinks external to the cluster and the cluster, and between the developing ovaries within the cluster. That is, CCC treated berries would by this hypothesis have a lower demand for nutrients as well as having more nutrients available to them.

An interesting conclusion from these results and the observations of others (Weaver and McCune 1959a; Zuluaga et al. 1968), is that a pronounced mutual antagonism exists between the presence of seed and the effect of GA application on berry weight. Pre-set application of GA reduces seed development, but the seeded berries that survive the GA treatment show little weight change in response to the application of this growth regulator (the only exception was the reduction in weight of seeded Muscat berries observed in 1966-7, Fig. 2.1).

Coombe (1965, 1967) observed that berry size is reduced by early applications of CCC. CCC treatment of the seeded cultivars reduced berry size in every experiment, that is, the effect was independent of the times of application used here. It seems inconsistent that no response can be obtained from the application of a promoter (GA) while size is reduced in response to a supposed inhibitor of the synthesis of that promoter. Thus either gibberellins are normally not limiting growth in seeded cultivars but are none the less essential, or CCC acts in some other way.

Cape Currant and to a lesser extent Zante Currant were the only cultivars that were enlarged by pre-anthesis treatment with GA; little response was obtained from this treatment on the other cultivars and this agrees with published data (Weaver and McCune 1959a & c; Zuluaga and Lumelli 1966). From Table 2.2, which summarises the absolute and relative responses to GA, it can be seen that the cultivars fall into three groups: Group I, seeded berries of Muscat and Doradillo, which show little or no change in weight in response to GA at any time of application; Group II, seedless Muscat and Sultana, in which berry weight is increased to the greatest extent by GA applied at set;

and Group III, Zante and Cape Currant, in which the anthesis application of GA is the most effective in increasing berry weight. The time of optimum response to GA application for Zante and Sultana has been noted by Weaver and McCune (1959c), but no explanation was offered for it. In the context of this more comprehensive investigation it is evident that responsiveness is related to the degree of seed development. It is clear that the action of GA is largely repressed up to anthesis. It is probable that this repression is relieved by the process of anthesis, either by a decrease in the level of the inhibitor or by an increase in the level of a specific relieving compound. The lack of response of the Group I berries and the shifted response curve for the Group II berries may be explained in either of two ways: either sufficient gibberellin is synthesised endogenously after syngamy so that it does not limit growth, or GA action remains repressed as long as seed development continues.

It is clear that GA influences berry elongation (e.g. Weaver and McCune 1959a & c). All seedless berries treated with GA have elongated more than normal. However only one seeded cultivar has been documented as responding; Weaver and McCune (1959a) observed that Muscat berries treated with GA were elongated and this has been confirmed by this investigation. The effect of GA was to cause a redistribution of growth - the longitudinal axis was expanded and the equatorial axis was decreased (Fig's. 2.1, 2.23 and 2.24). An important supplement to this information is the observation that seedless Muscat berries show essentially the same qualitative response as the seeded berries. In all cases where increases were noted they were greatest when the GA was applied at anthesis.

Table 2.2

The effect of stage of development on the response of berry weight to the application of 20ppm GA.

| Cultivar and seededness | $\bar{x}$ [1] | Change in berry weight | | | | | |
|---|---|---|---|---|---|---|---|
| | | Pre-anthesis | | Anthesis | | Post-anthesis | |
| | g. | g. | % | g. | % | g. | % |
| Doradillo seeded | 3.77 | +.08 | + 2.2 | + .05 | + 1.2 | - .08 | - 2.2 |
| Muscat seeded | 4.48 | +.07 | + 1.5 | + .24 | + 5.3 | - .12 | - 2.8 |
| Muscat seedless | 1.68 | -.08 | - 4.5 | + .12 | + 6.9 | + .79 | + 46.9 |
| Sultana (steno-spermocarpic) | 1.98 | -.08 | - 4.1 | + .91 | + 46.2 | + 1.45 | + 73.2 |
| Cape Currant (partheno-carpic) | .60 | +.91 | +150.0 | + 1.29 | +204.0 | + .77 | +128.0 |
| Zante Currant | .32 | +.16 | + 52.2 | + 1.08 | +343.0 | + .61 | +194.0 |

1. Mean untreated weight at harvest

The degree of elongation in response to an anthesis application of GA bears no relation to the presence of seed or the normal L/W ratio (Table 2.3). The responses fall into three groups: seeded Doradillo berries which show a small but significant increase; Muscat seeded and seedless, Sultana and Zante, all of which respond equally (approximately a 15% increase), and Cape which shows a 38% increase in L/W in response to 20ppm GA. It can be seen that these groups are unrelated to the berry weight groups discussed above.

Table 2.3

The effect of GA applied at anthesis and CCC applied pre-anthesis on Length/Width ratio of berries.

| Cultivar and seededness | Δ L/W 20ppm GA | Δ L/W Nil | Δ L/W 2000ppm CCC | $LSD_{0.05}$ |
|---|---|---|---|---|
| Doradillo, seeded | +0.02 | 0.97 | 0.00 | 0.01 |
| Muscat, seeded | +0.17 | 1.11 | -0.02 | 0.03 |
| Muscat, seedless | +0.14 | 1.11 | +0.02 | 0.04 |
| Sultana | +0.20 | 1.23 | -0.05 | 0.03 |
| Cape Currant | +0.40 | 1.05 | -0.03 | 0.04 |
| Zante Currant | +0.16 | 1.02 | -0.01 | 0.02 |

CCC significantly reduced the L/W ratio of Sultana and seeded Muscat berries. It was most effective in this regard on Sultana but only when applied pre-anthesis; post-anthesis applications were without effect (Fig. 2.23 and 2.31).

Weaver and McCune (1959a) observed that the most elongated Sultana berries were produced by anthesis applications of GA. This result has been confirmed in this investigation and the generality of this statement extended to include Cape Currant, Zante Currant and Muscat. Coombe (1960) and Harris et al. (1968) noted that cell division occurred in the pericarp only for a short period after anthesis. That GA is most effective when applied shortly before or concurrently with this cell division indicates that GA may affect berry shape by increasing cell

division in the polar regions of the berry, relative to that in the equatorial regions. It may be relevant that Sachs (1961) demonstrated the ability of GA to influence the number of cell divisions occurring in apices of Samolus and Hyoscyamus.

Important practical implications were seen to arise from the observation that clusters treated with GA approximately two weeks prior to anthesis were elongated (Alleweldt 1959; Rives and Pouget 1959; Weaver and McCune 1959a, b & c, 1962). These authors reasoned that elongation of the rachis and pedicels would allow better aeration of the clusters and thus reduce the likelihood of damage due to moulds. This treatment has proved impractical due to the deleterious effect of GA on flower initiation in many cultivars (Weaver 1960; Julliard and Balthazard 1965; May pers. comm.). Sultana does not display this GA reduction of flower initiation but cluster compactness is not as serious a problem in this cultivar as in some of the seeded cultivars.

The observations of this investigation demonstrate that in all the cultivars tested, GA application resulted in increased rachis elongation, while in two, Muscat (Fig. 2.1) and Cape (Fig's. 2.2 & 2.39), rachises were significantly shortened by the application of CCC pre-anthesis. This substantiates the possible role of GA in the control of rachis elongation. An important observation and one also noted by Weaver and McCune (1959a) was that the application of GA in quantities greater than 20ppm tended to produce rachises that were coiled, toughened and thigmotropic. Since tendrils and reproductive clusters of Vitis originate by divergent development of the same structure this result may have some bearing on the physiology of tendril growth.

Weaver and McCune (1957, 1959c) have noted that the effect of exogenously applied auxin and GA on maturity of grapes appears to be related to the fruit load carried by the vine. Observations in this investigation support the proposition that vine shoots grown under Australian conditions can sustain load increases up to approximately 10 to 15% without deleterious effects on maturity (Antcliff 1967).

Yield per vine consists of a number of components: number of clusters, number of berries per cluster, the relative number of seeded and seedless berries, the density of the berry (sugar content) and the volume of the berry. An examination of Table 2.1 reveals a tendency for homeostacy in cluster weight. An increase in the number of berries is offset by a reduction in berry size and vice-versa. If the chemicals are applied at a time when the number of berries set cannot be influenced (e.g. post-anthesis application of GA to Sultana) then the level of sugars at harvest offsets to a degree the increase in size of the berries. This tendency is a major factor limiting yield increases in response to the application of exogenous growth regulators.

## (5) Conclusions

The results provide evidence for the following generalizations regarding cluster growth as influenced by gibberellin:

(a) GA is involved in the process of fruit set and exogenous treatment may, depending on stage of development, type of development, time of application and concentration, increase or decrease fruit set;

(b) GA is at least partly responsible for the expansion of the pericarp, but may not be limiting in the presence of seed;

(c) GA is responsible for the extension of the longitudinal axis of the berry;

(d) GA influences in a positive way rachis and pedicel elongation.

Further, the effects of GA and CCC are generally opposite. This is consistent with the hypothesis that CCC lowers the level of endogenous gibberellins in these tissues.

## III. GIBBERELLIN-LIKE AND ABSCISIC ACID-LIKE COMPOUNDS IN GRAPE OVARIES (AND POLLEN), AND THE EFFECT OF THE APPLICATION OF CCC.

### (1) Introduction

The presence of gibberellin-like activity within extracts of grape ovaries has been demonstrated by Coombe (1960, 1962) and Weaver et al. (1965). Coombe (1960) showed that in the cultivars of Seedless Emperor and Zante Currant (Black Corinth) there was a marked increase in the level of promotion in crude extracts of fruit taken at the setting stage. Weaver et al. (1965) demonstrated that seeded Tokay had more activity than seedless Tokay berries. Their results also indicated that ringing (girdling, cincturing) of the trunk of Zante Currant vines resulted in an increase of the level of normal acidic gibberellins in the fruit. Thus it would appear that the berry's content of gibberellin is related to its ability to grow, and also probably to its ability to set.

Although abscisic acid (ABA) has been shown to be present in a number of fruits (Ohkuma et al. 1963, Cornforth et al. 1966; Milborrow 1967; Pieniazek et al. 1967; Rudnicki et al. 1968), its presence in developing ovaries of Vitis vinifera has yet to be demonstrated. Rudnicki et al. (loc.cit.) postulate that the ABA is involved in the process of fruit ripening. It is also probable that ABA is intimately associated with the processes of flowering and fruit set since these processes involve the abscission of a number of organs (namely; the operculum, calyptra or cap; and the anthers). It may also be involved in the abscission of those ovules that fail to set (Ohkuma et al. 1963).

The relationship between endogenous gibberellins and ABA has yet to be studied, although Monselise et al. (1967) have demonstrated that an inverse relationship exists between the β Inhibitor (probably ABA) and auxin levels in a number of citrus tissues. Also the initial work on dormancy in Acer suggests that an inverse relationship may exist between gibberellin and ABA (Robinson et al. 1964). The role of abscisins in plant growth, as distinct from dormancy and abscission, has yet to be ascertained.

It has been established that the application of CCC to plants generally results in a decrease in normal gibberellin-like activity (e.g. Dennis et al. 1965, Zeevart 1967). Dennis et al. (loc. cit.) showed that the CCC inhibition of gibberellin was not related to the mechanism of action of the other growth retardants (e.g. Amo 1618 and Phosphon D), and Reid et al. (1968) and Bristow and Simmonds (1968 have shown that unusual gibberellins are produced in tissues treated with this growth retardant. Thus the exact nature of the relationship between CCC and endogenous gibberellins remains to be clarified.

No information has been published on the effect of CCC application on ABA or related compounds in spite of the possibility that an increased level of this type of compound could account for many of the effects of CCC.

## (2) Materials and Methods

### Cultivars of grape used

Three cultivars were chosen to span the range of ovule development: Doradillo (perfect), Sultana (stenospermocarpic), and Cape Currant (parthenocarpic).

The Doradillos and Sultanas were part of a commercial planting in the Waikerie District of South Australia. The Cape Currants were growing in Alverstoke Experimental Orchard at the Waite Institute.

Treatment

Vines treated with CCC were compared with untreated vines, and samples were taken at random from two treated rows in the commercial plantings and compared with random samples taken from two neighbouring untreated rows. For Cape Currant an A.B.B.A. design was used along a single row, ten vines for each treatment. CCC was applied as an aqueous solution at 300 ppm (w/v) plus Tween 20 at 0.05% , cover sprayed approximately 2 weeks before anthesis.

Sampling

Flowers or berries were sampled at five times:

(i) Just prior to treatment i.e. about 2 weeks before anthesis;

(ii) One week later when the calyptrae were yellowing but had not commenced abscission;

(iii) One week later, at anthesis;

(iv) Ten days after anthesis when the setting abscission was at its peak rate and rate of cell division was probably high in set berries;

(v) Two weeks after (iv), estimated to coincide with maximum rate of cell expansion in the first growth phase.

Samples (i)to (iv) consisted of forty clusters selected for equality of stage of development. Sample (v) was of twenty clusters. The sampled clusters were placed in plastic bags in an ice box. As soon as feasible (usually two hours for those sampled at Waikerie) the clusters were deep frozen, and stored at -15°C until they could be lyophylised. Prior to lyophylisation the berries or flowers were separated from the stems. Sample (iii) was sieved to separate pollen from the ovaries. Fresh and dry weights of each sample were measured. Sample (iv) of Sultana was destroyed when the lyophilizer overheated. At maturity samples were taken to ascertain the effect of CCC on set and pericarp development.

Extraction and purification

The procedure followed was as indicated in Fig. 3.1. The procedure was developed after the work shown in appendix A; it was selected for its good recovery of activity and the clear separation of the inhibitor from the promoters and for its rapidity and simplicity as it permitted a relatively large number of samples to be handled at one time.

1.0g d.wt. samples were ground in 20ml of 98% Methanol (MeOH) in a glass homogenizer (Kontes). This and all subsequent stages till the final rotary film evaporation were carried out in a cool room at 3 - 5°C. The resultant suspension was washed into a centrifuge tube with a further 30ml and then centrifuged at 3000 rpm for ten minutes. The supernatant was poured off, the residue taken up in a further 50ml of solvent and then shaken on a reciprocating shaker for 4-5 hours. This was then re-centrifuged, the supernatant decanted and the residue again shaken overnight in a further 50ml of 98% MeOH. The following morning the final centrifugation was carried out and the supernatants bulked. The residue was discarde

DEAE Sephadex (A 25) columns were prepared by rinsing Sephadex (2g d.wt. per column), with distilled water (100ml/g) in a large Buchner funnel. The gel was washed with 0.5N NaOH (approx 25ml/g) first under a slight vacuum and then allowed to drip through unaided.

The Sephadex was then washed with distilled water until the washings were neutral and dehydrated with a wash of absolute methanol. Finally the resin was equilibrated in 98% MeOH in the cold overnight. An appropriate portion of the bulk resin was poured into a 2.5 cm glass column, (total capacity approx. 7m. eq.). Prior to adding the extract, the resin in the column was backwashed to eliminate air.

The procedure was scaled down where pollen was used. 200mg d.wt. of pollen were ground and extracted in 15ml of MeOH (98%) and rinsed with a further 5ml. The combined supernatants (initial extraction plus 2 washings) were placed on a 1g column (1.0cm).

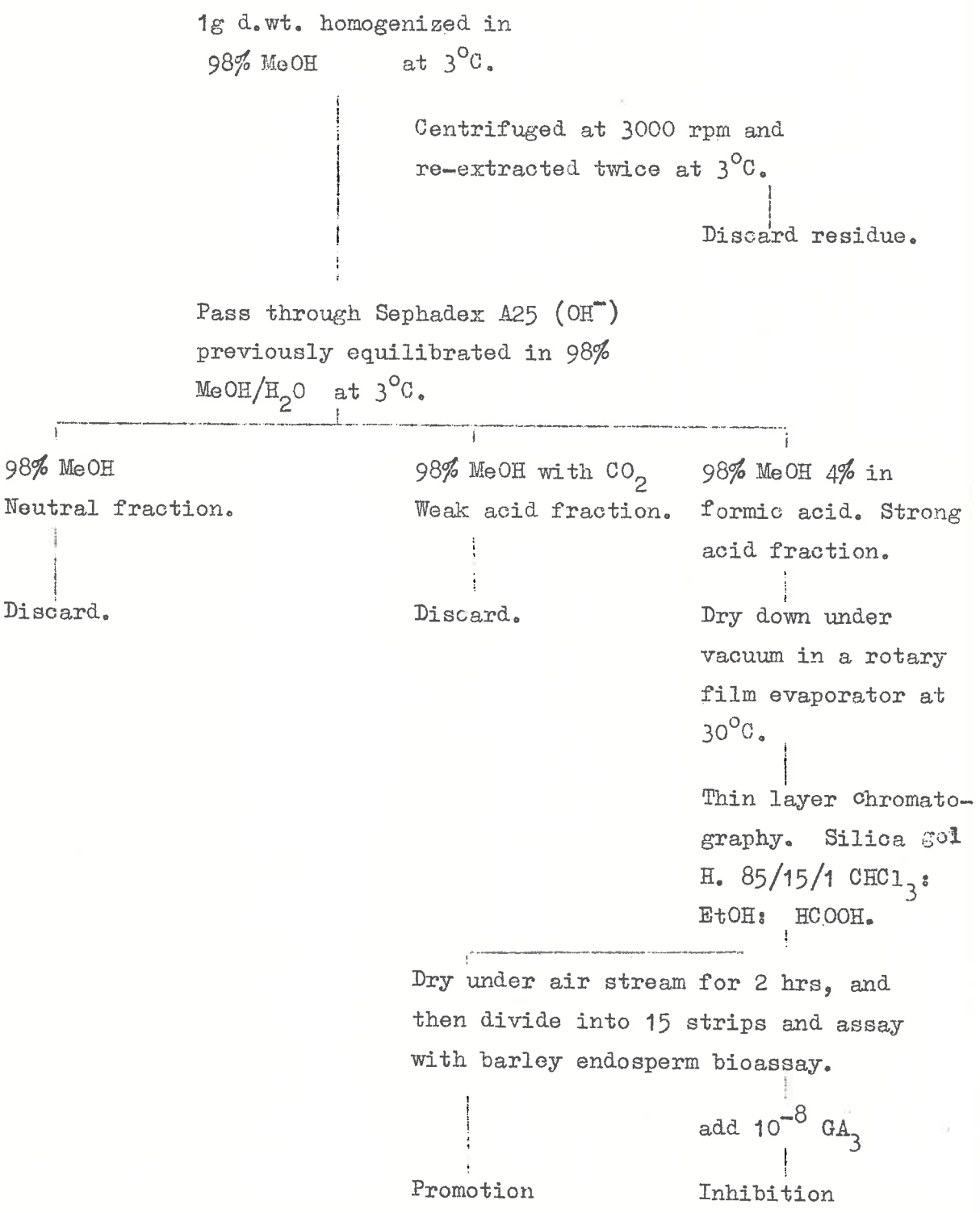
The extracts were added to the top of the columns and allowed to filter through under gravity. The extract was washed through with a further 50ml (30ml for pollen) of solvent to give a neutral fraction which was discarded. The column was then washed with 200ml (100ml) of $CO_2$ - saturated solvent. Excess $CO_2$ was removed from the column by passing 75ml (50ml) of neutral solvent through. This combined fraction was designated the weak acid fraction and contained phenolics and those pigments which were not removed by the neutral washings. This fraction was also discarded. The strong acid fraction obtained by passing 75ml (30ml) of solvent plus 4% formic acid through the column contained the biologically active components. This fraction was dried in a rotary film evaporator at 30°C and the residue dissolved in 1.0ml of absolute MeOH.

Fig. 3.1 Flow diagram of extraction technique

For further explanation see text

Fig. 3.1

FLOW DIAGRAM OF EXTRACTION PROCEDURE



Thin-layer chromatograms of approximately 0.3mm thickness were prepared from silica gel H (Merck) on 5 x 20 cm glass plates. The layers were divided into two strips by scraping a line up the middle, and the origin and front marked 15 cm apart (See Plate 3.1). Two spots containing 100mg d.wt. equiv. of berries (0.1ml) or 20mg of pollen (0.2ml) were applied on to each of two plates i.e. four for each extract.

The plates were run in two lots in a solvent of composition, 85:15:1, chloroform: ethanol: formic acid. The runs were split on the basis of replicate plates. In general the second replicates were not spotted until two days after the first. After running, the plates were dried under a fan for two hours (Skene pers.comm.). The plates were then marked into 15 x 1 cm sections except the first which was 1.5 cm and included 0.5 cm below the origin. The 15 sections of the chromatograms were scraped into 2" x 1" vials. One side of each plate was assayed for promotion and the other was assayed for inhibition using the barley endosperm bioassay (Nichols & Paleg 1963; Coombe et al. 1965).

Recovery with this method was judged to be complete within the limits of accuracy of the bioassay (See Appendix A).

Bioassay procedures

Barley seed (cv.'Prior' ex Queensland) was prepared by soaking for three hours in 50% $H_2SO_4$ then shaking vigorously in water to remove husks, first in distilled water (10 rinses), and then in autoclaved distilled - deionized water a further 10 times. The seed was then soaked in deionized

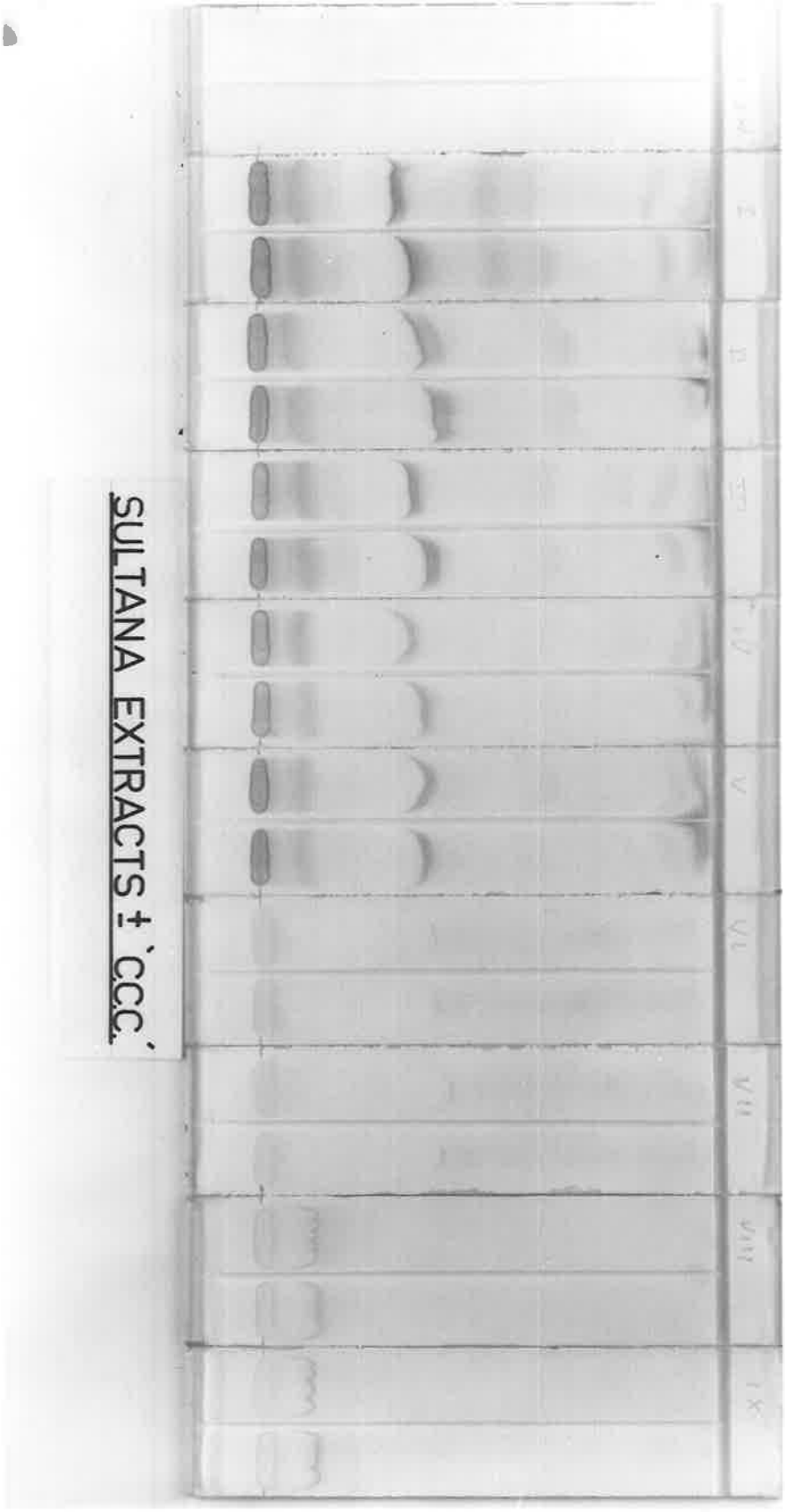
water at 3°C for 21 hours, after which it was cut on sterilized brass blocks as described by Coombe et al. (1965).

The incubation mixture for the promotion assay consisted of 0.5ml of 0.01M Phosphate buffer pH4.0 (autoclaved), plus 0.5ml autoclaved distilled - deionized water. In the assays for inhibitors 0.5ml of $2 \times 10^{-8}$ g/ml $GA_3$ replaced the water. Each assay included three replications of standard solutions. Concentrations of $GA_3$ (Merck 97%) were: 0.0; $10^{-10}$; $10^{-9}$; $10^{-8}$; and $10^{-7}$ g/ml. ABA supplied by the American Tobacco Co. was used as the standard in the inhibition assay and consisted of a mixture of 1:1 trans-trans : cis-trans ABA. Concentrations of 0.0; $10^{-9}$; $10^{-8}$; $10^{-7}$ and $10^{-6}$ g/ml based on a total composition of the ABA were used (See Appendix A).

The assays for promotion and inhibition were run on consecutive days, each assay including a chromatogram spotted with 0.1ml methanol, and totalled 195 vials. Two endosperm halves were added to each vial; the vials were then capped and incubated at 30°C for 42 hours.

The contents of each vial were diluted to 10ml with distilled water, and a 9cm No.1 filter paper cup placed in the vial. The refractive index of the solutions was measured with a Waters digital differential refractometer. This method was adopted following work done by Coombe (pers. comm.). The refractometer was modified by shortening the flow lines to reduce the resistance, and connecting the effluent line to a water vacuum pump to enable quick change of the samples sucked from each vial via polythene tubing.

Plate 3.1 One replicate of Sultana extracts after thin-layer chromatography


SULTANA EXTRACTS ± 'CCC'
I
II
III
IV
V
VI
VII
VIII
IX

It was found that with this method one person could read two vials per minute. Distilled water was used in the reference cell. Measurements were made at intervals on distilled water and these were subtracted from sample readings to give the parameter Δ refraction unit (ΔRU).

Statistical analysis of results

The first analysis carried out was the estimation of the confidence limits at the 5% level of probability which were calculated from the values of ΔRU for each MeOH plate to establish which readings on the TLC sections were significant.

In order to compare treatment effects an analysis of variance was carried out in the following manner: the plates were divided into three areas: area I fractions 1 - 5; area II fractions 6 - 10; area III fractions 11 - 15. Within these areas the highest value (lowest in the case of inhibition) was selected, and analysed. Thus the analysis included eleven treatments, three areas, two replications. In the case of the inhibition analysis only areas II and III were compared. In the analysis for promotion, area III was used as a nil comparison and in the inhibition analysis area II was used as the comparison. The analysis was run both unlogged and transformed to $\log_e$. It was decided to use $\log_e$ because it gave a more linear variance.

An analysis of variance was carried out on the inhibition data from the three cultivars for sample times (ii) to (v) to compare CCC and nil treatments.

Evaluation of the Effect of CCC on Cluster Development

Ten clusters were sampled from each of six representative vines for each treatment. The whole sample was used in the determination of cluster weight and length. The clusters were then separated into a number of pieces, and berry number was determined from a weighed sub-sample equivalent to about one tenth of the whole. A further sub-sample of 100 berries was used to estimate berry weight, length, width and sugar.

## (3) Results

### (i) Effect of CCC on fruit set and berry size (Tables 3.1 & 3.2)

Berry fresh weight was reduced in CCC treated berries in both Cape Currant and Sultana. Cluster weight and number of berries per cluster were higher in both these cultivars, but this effect was only significant in Cape Currant. Doradillo was not sampled, but Coombe (1967) has demonstrated that this cultivar responds to CCC in a manner similar to other cultivars (see also Section I, this thesis).

### (ii) Gibberellin-like activity

The results (Fig. 3.2) show there were four zones of promotive activity - promoter I at $RM_{GA_3}$ = 0.25, promoter II at $RM_{GA_3}$ = 0.65, promoter III at $RM_{GA_3}$ = 1.00, and promoter IV $RM_{GA_3}$ = 1.95. Results for promoter I should be treated with caution due to the wide difference that exists between the two replicates (Fig. 3.2). It is considered that breakdown of this compound occurred between the time the two replicates were assayed because little activity was present in the second replicate.

Promoters I, III and IV were evident in Cape Currant extracts (Fig. 3.2). The total activity of promoter III was similar to that of promoter I and no significant change in activity occurred during the period for assay for either compounds (Fig. 3.4). A third area of promotion (promoter IV) which ran slightly ahead of the inhibition was evident in the majority of the Cape Currant extracts (See Fig. 3.2).

Table 3.1
Cape Currant: The effect of CCC on cluster development at maturity, on vines that were sampled in the analysis of the effect of CCC on endogenous growth regulators

| Parameter | Nil | CCC | Level of significance |
|---|---|---|---|
| Cluster weight g | 93 | 150 | 0.01 |
| Cluster length cm | 15.9 | 16.3 | n.s. |
| No. of berries/cluster | 192 | 368 | 0.001 |
| Berry weight g | 0.49 | 0.41 | 0.05 |
| Sugar (ref. index) | 20.3 | 19.1 | n.s. |

Table 3.2

Sultana: The effect of CCC on cluster development at maturity, on vines that were sampled in the analysis of the effect of CCC on endogenous growth regulators

| Parameter | Nil | CCC | Level of significance |
|---|---|---|---|
| Cluster weight g | 496 | 532 | n.s. |
| Cluster length cm | 21.3 | 20.8 | n.s. |
| No. of berries/cluster | 271 | 309 | n.s. |
| Berry weight g | 2.00 | 1.75 | 0.05 |

Table 3.3

The level of the ABA-like compound present in grape extracts and the effect of CCC applied two weeks pre-anthesis (averaged over the three cultivars)

| CCC ppm | Sampl 2 | 3 | 4 | 5 | Mean |
|---|---|---|---|---|---|
| 300 | 1.847[1] | 1.790 | 1.838 | 1.952 | 1.858 |
| 0 | 1.820 | 1.843 | 1.830 | 2.118 | 1.910 |
| mean | 1.833 | 1.817 | 1.834 | 2.035 | 1.844 |

1 = ln Refraction Units

Difference between sampling times is significant P=0.001,

$LSD_{.05}$ =0.075

Difference between treatments (CCC) is significant P=0.1,

$LSD_{0.1}$ = 0.087

Two areas of promotion were evident in chromatograms of Sultana extracts, and these corresponded to promoters II and III (Fig. 3.2). Promoter II tended to increase with time though this trend was not significant (Fig. 3.5). Promoter III showed a small, insignificant increase in concentration at anthesis and declined thereafter.

Chromatograms of the cultivar Doradillo showed two significant areas of promotion corresponding to promoter II and promoter III as in Sultana (Fig. 3.2). However, unlike Sultana, highly significant changes in activity were revealed at the different sampling times (Fig. 3.6); promoter II increased in activity up to anthesis and declined thereafter. The amount of promoter III was greater (P=0.001) at the second and third sampling times than at any other time.

Promoters I, III and IV were present in Cape Currant anthers (Fig. 3.3), but only promoter III was evident in treated and untreated Sultana (Fig. 3.3), and untreated Doradillo anthers (Fig. 3.3).

(iii) Abscisic acid-like activity

A single area of inhibition ($RM_{ABA}$ 1.0) was present in all chromatograms assayed. There was a significant decline in inhibitive activity after anthesis in all cultivars. At the first sample time Cape Currant had a lower activity than the other two cultivars. Doradillo had a generally higher concentration (significant P = 0.05), particularly at fruit set (Fig. 3.8).

(iv) Effect of CCC

The application of CCC had no significant effect on the level of extractable gibberellins in developing flowers and ovaries, nor did it have any significant effect on the level of gibberellin in Sultana and Cape Currant anther extracts. But it had a remarkable effect on the level of gibberellin-like activity in both replicates of Doradillo anthers (Fig. 3.3). The activity was spread throughout the chromatogram, although there was an indication of several overlapping areas.

CCC treated Doradillo ovaries exhibited a higher level of inhibition ($P=0.05$) at the final sampling time (Fig. 3.6). A similar trend existed in the other cultivars (Figs. 3.4 & 3.5), and while this was not significant in the analyses for Sultana and Cape Currant, the overall analysis (Table 3.3) shows significance at the $P=0.1$ level of probability.

The level of inhibition from anthers was unaffected by the CCC treatment, but it is probable that the magnitude of the promotion in Doradillo anthers would mask any increase in inhibition.

Fig. 3.2 Chromatography of extracts

L.H.S. Assay for gibberellin-like substances
R.H.S. Assay for ABA-like substances (in the presence of $10^{-8}$g $GA_3$)

Horizontal lines represent the Rf of $GA_3$ and ABA respectively.
ln $\Delta$R.U. = $\log_e$ change in refraction in arbitrary units above distilled water.

The samples are extracts of ovaries collected from untreated vines at anthesis (anthers separated).

The horizontal dotted lines represent the LSD (P=0.05) calculated from the mean of two chromatograms spotted with 0.1ml methanol and assayed (L.H.S. plus water, R.H.S. plus $10^{-8}$g $GA_3$).

FIG. 3.2

CHROMATOGRAPHY OF EXTRACTS

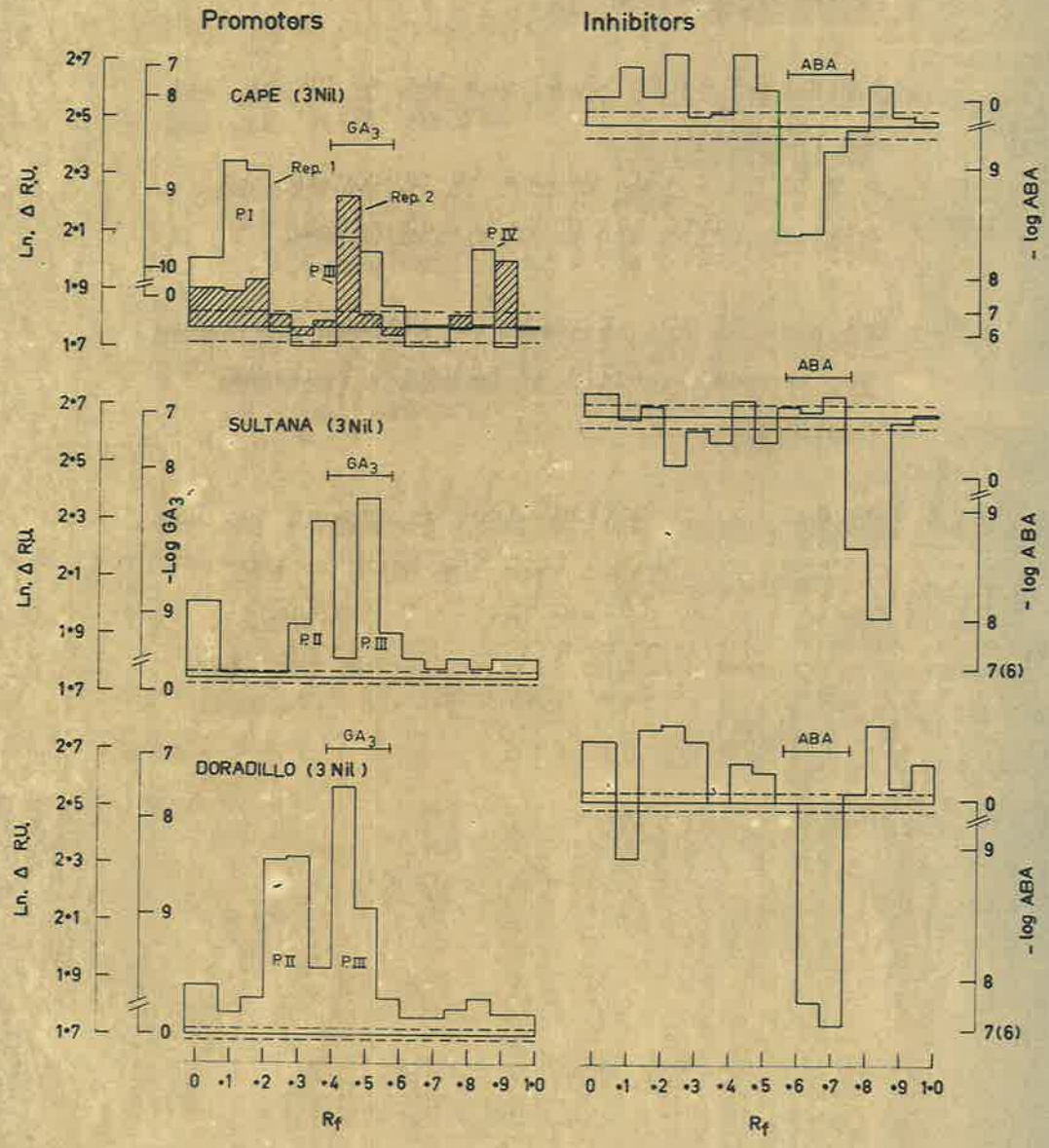


Fig. 3.3 Chromatography of anther extracts

L.H.S. Samples collected at anthesis from untreated vines.
R.H.S. Samples collected at anthesis from treated vines.

Horizontal lines represent the Rf of $GA_3$.

ln Δ R.U. = loge change in refraction in arbitary units above distilled water.

The horizontal dotted lines represent the LSD (P=0.05) calculated from the mean of two chromatograms spotted with 0.1ml methanol and assayed.

FIG. 3.3

ANTHER EXTRACTS

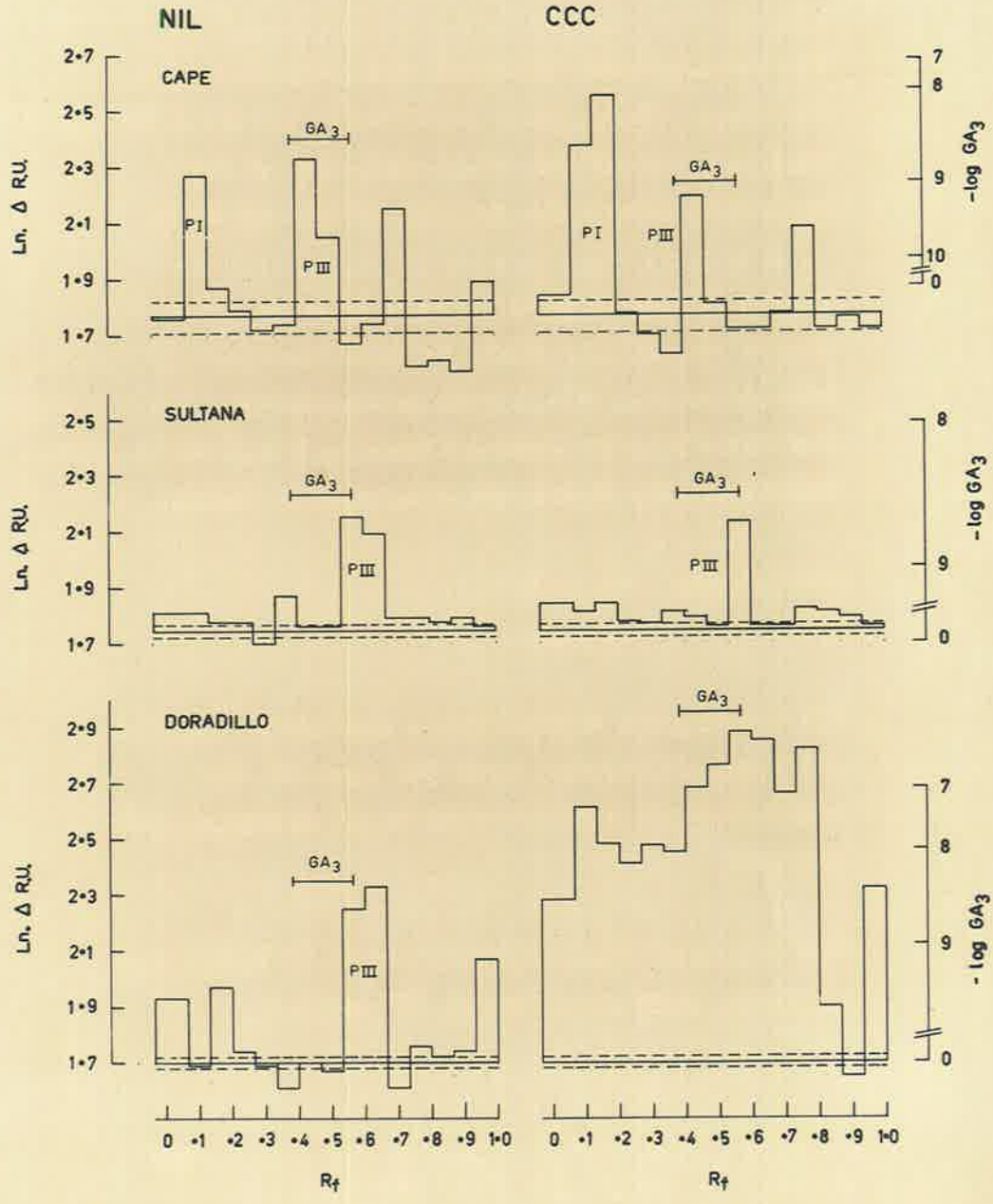


Fig. 3.4 Cape Currant: Changes in activity of endogenous regulators in the presence and absence of CCC

The samples were prepared and assayed as in the text. Each point represents the mean of two observations of the level activity of the principal compounds present in the extract.

Sample number represents the time at which the sample was taken, thus, 1= pre-treatment (2 weeks, pre-anthesis), 2 = 1 week pre-anthesis, 3 = anthesis, 4 = set (10 days post-anthesis), 5 = 3 weeks post-anthesis (all times are approximate only).

CCC 300ppm o_ _ _ o

Nil .——— .

The horizontal dotted lines represent the LSD (P=0.05) calculated from the mean of two chromatograms spotted with 0.1ml methanol and assayed.

The vertical line represents LSD (P=0.05) for extracts with significant 'F' ratios.

FIG. 3.4

CAPE CURRANT

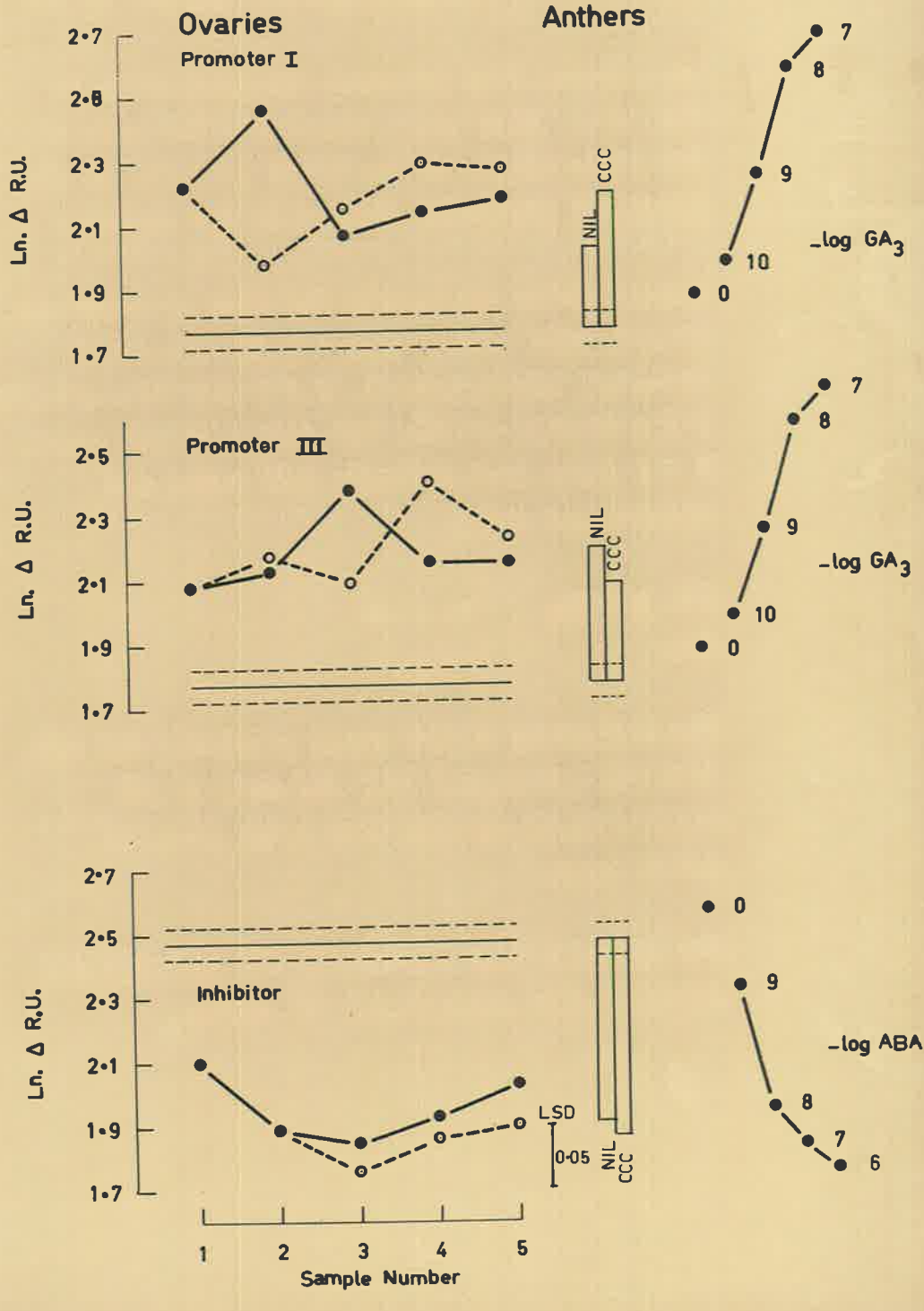


Fig. 3.5 Sultana: Changes in activity of endogenous regulators in the presence and absence of CCC

The samples were prepared and assayed as in the text. Each point represents the mean of two observations of the level activity of the principal compounds present in the extract.

Sample number represents the time at which the samples were taken, thus 1 = pre-treatment (2 weeks, pre-anthesis), 2 = 1 week pre-anthesis, 3 = anthesis, 4 = set (10 days post-anthesis), 5 = 3 weeks post-anthesis (all times are approximate only).

CCC 300 ppm o- - - o

Nil .———.

The horizontal dotted lines represent the LSD (P=0.05) calculated from the mean of two chromatograms spotted with 0.1ml methanol and assayed.

The vertical line represents LSD (P=0.05) for extracts with significant 'F' ratios.

FIG. 3.5

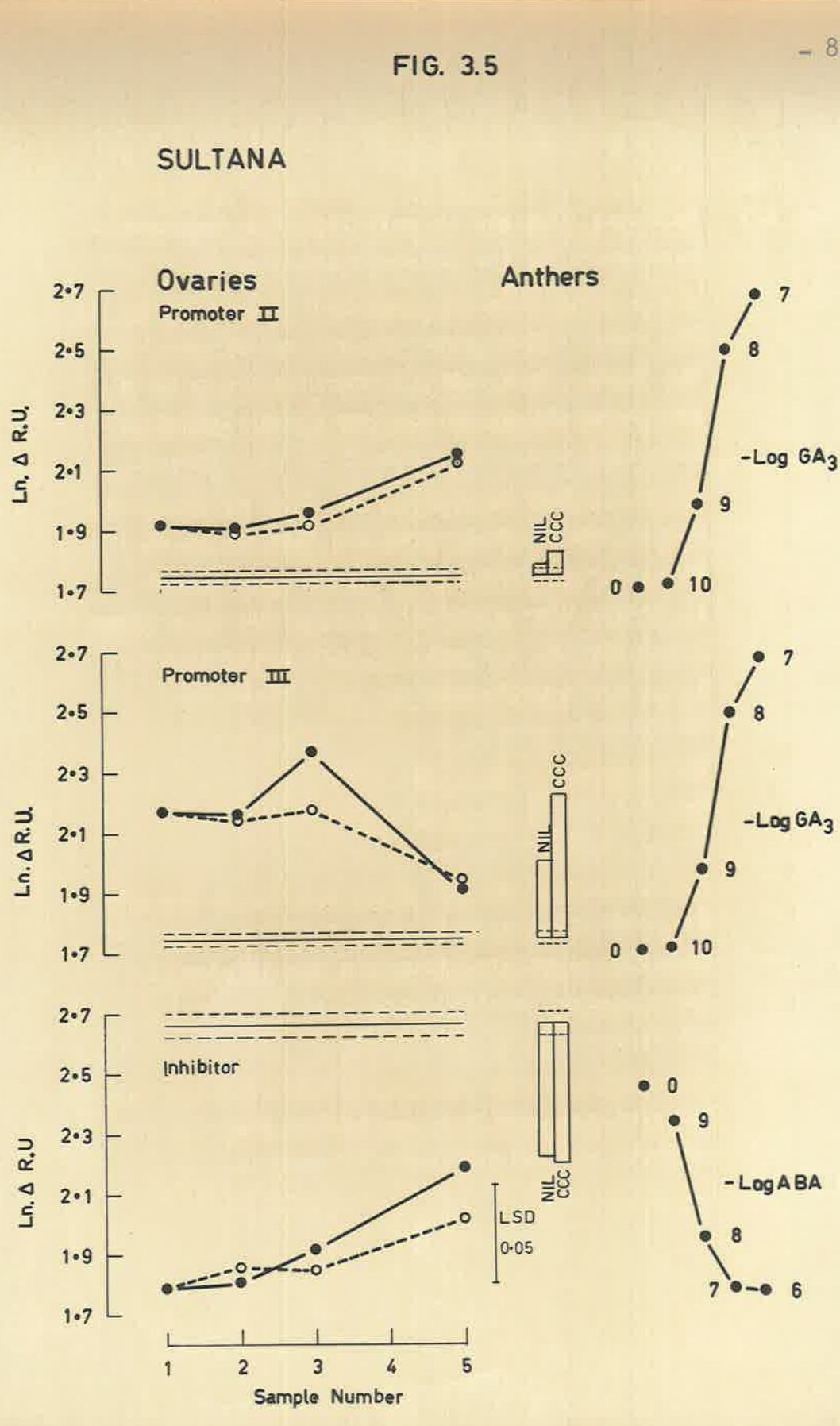

SULTANA
Ovaries
Anthers
Promoter II
Promoter III
Inhibitor
Ln. Δ R.U.
Ln. ΔR.U.
Ln. Δ R.U
2·7
2·5
2·3
2·1
1·9
1·7
NIL
CCC
LSD
0·05
-Log GA3
-Log ABA
0
7
8
9
10
6
1
2
3
4
5
Sample Number

Fig. 3.6 Doradillo: Changes in activity of endogenous regulators in the presence and absence of CCC

The samples were prepared and assayed as in the text. Each point represents the mean of two observations of the level activity of the principal compounds present in the extract.

Sample number represents the time at which the samples were taken, thus 1 = pre-treatment (2 weeks pre-anthesis), 2 = 1 week pre-anthesis, 3 = anthesis, 4 = set (10 days post-anthesis), 5 = 3 weeks post-anthesis (all times are approximate only).

CCC 300ppm o_ _ _o

Nil ._____.

The horizontal dotted lines represent the LSD (P=0.05) calculated from the mean of two chromatograms spotted with 0.1ml methanol and assayed.

The vertical line represents LSD (P=0.05) for extracts with significant 'F' ratios.

# DORADILLO

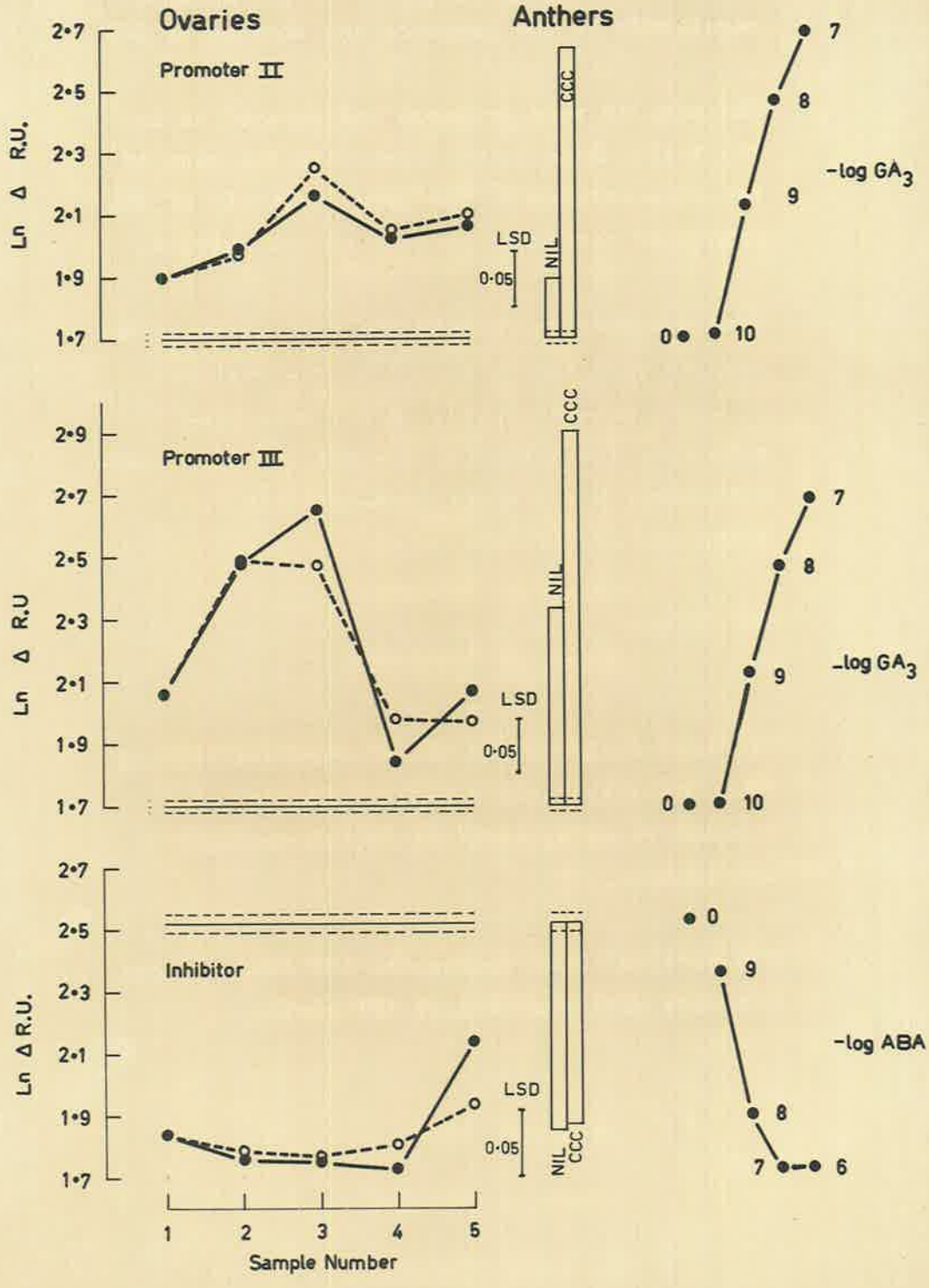

Ovaries
Anthers
Promoter II
Promoter III
Inhibitor
Ln Δ R.U.
2·7
2·5
2·3
2·1
1·9
1·7
2·9
LSD
0·05
NIL
CCC
-log GA3
-log ABA
0
7
8
9
10
6
1
2
3
4
5
Sample Number

Fig. 3.7 Changes in the concentration of promoter III with stage of development in untreated flowers and berries.

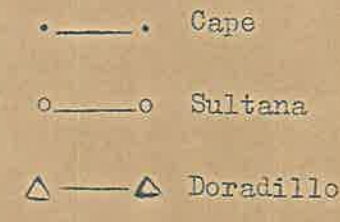



Fig. 3.8 Changes in the concentration of the ABA-like compound with stage of development in untreated flowers and berries.

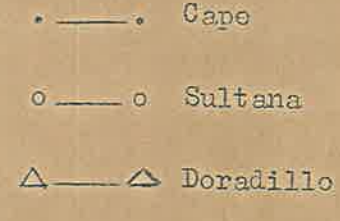



Fig. 3.9 The changes in the ratio of the concentration of the ABA-like compound and the promoter III with stage of development in untreated flowers and berries.

The dashed line indicates a theoretical equivalence of inhibitive to promotive activity.

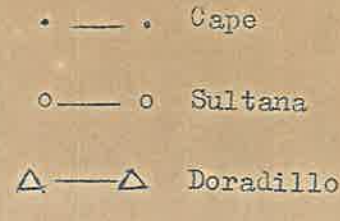


Figure 3.7

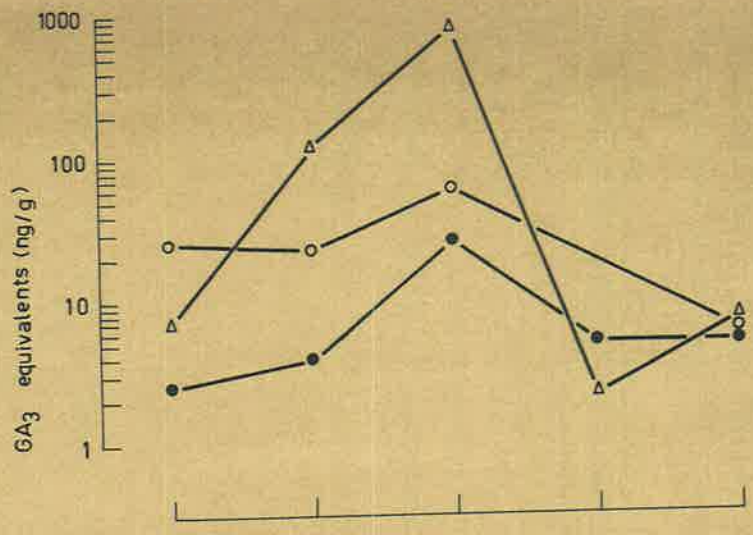



Figure 3.8

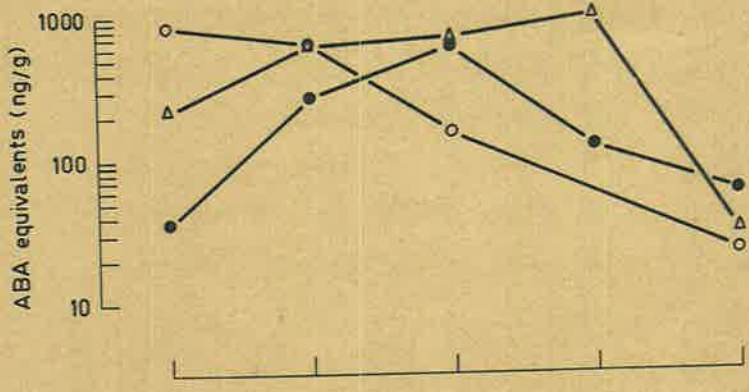
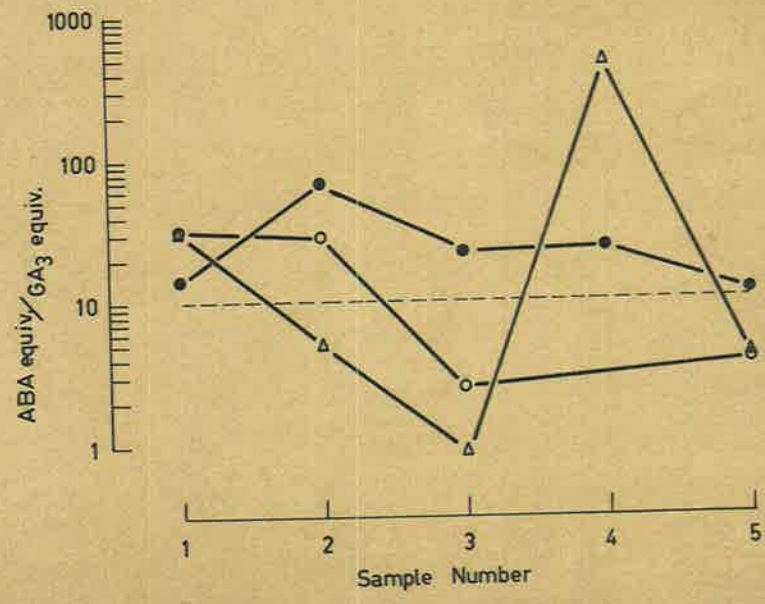



Figure 3.9



(4) Discussion and Conclusions

The suggestion that greater biosynthesis of gibberellins in seeded berries is responsible for the dissimilarity in size that exists between seeded and seedless cultivars is borne out by this experiment, however differences in detail are evident between published information and the data presented here.

A qualitative difference in gibberellin-like activity was noted between the parthenocarpic cultivar on one hand and the stenospermocarpic and seeded cultivars on the other. Cape extracts contained two and possibly three promoters (promoter I, III and IV), but did not contain activity in the region of promoter II. Sultana and Doradillo did not give any evidence for the presence of either promoter I or IV, but contained promoters II and III. The difference between the latter two cultivars was quantitative. Weaver and Pool (1965b) reported qualitative differences between seeded and seedless cultivars, but there is no clear relationship between their observations and the above results. Further work would be required to determine the importance of these differences.

The ion-exchange behaviour of the compounds indicates that they possess at least one carboxyl group. The polarity of promoter I compares with that of apricot gibberellin (Appendix A, Table A.1). Promoters II and III have an Rf similar to that of the unconjugated gibberellins: promoter II behaves chromatographically like $GA_8$ though this compound has low potency in the barly endosperm bioassay; promoter III is similar in behaviour to $GA_3$. The presence of significant promotive activity (promoter IV) near the solvent front in extracts of Cape Currant suggests the

presence of a compound with properties unlike that of the $GA_{1-8}$ series; it is more mobile with an $RM_{GA_3}$ = 1.95 than $GA_{4/7}$ $RM_{GA_3}$ = 1.4. However it is possible that this is due to the presence of native refraction. The amount of refraction is equivalent to 1.5mg glucose, though it could not be a sugar since sugars do not move from the origin in this solvent; furthermore, although approximately 10mg of extract are spotted, the majority of this is composed of acids, such as tartaric acid, which stay near the origin of the chromatogram. It is emphasized that this information by no means establishes the identity of the compounds mentioned.

Because promoter III was the most active and was common to the three cultivars, all further discussion is confined to it. A summation of total promotive activity was not used because of the almost total lack of information on the role of unusual gibberellins. Furthermore the chromatographic behaviour of this promoter is similar to $GA_3$, a gibberellin with known activity when applied exogenously.

Activity of promoter III at anthesis (Fig. 3.7) correlates well with final berry size, with Doradillo having higher activity than Sultana, which in turn was higher than Cape Currant. This result differs from the data presented by Iwahori et al. (1968) who found that the difference in gibberellin content of seeded and seedless variants of Tokay was not in the level of activity attained, but rather in the duration of that activity. This difference may be explained in one of three ways; it may be due to the different methods of measurement, it may be intrinsic in genetic differences in the cultivars, or it may be due to a different definition of anthesis.

The third is less likely but may contribute since in neither case was sampling precise and the samples were essentially mixed populations in which differences of as much as two weeks may have existed in the stage of growth of the individual flowers in each sample. They also found that in the seedless (stenospermocarpic) variant the second rise in activity occurred much earlier than in the seeded variant. Sampling in this experiment was not carried out sufficiently often nor for a long enough period to assess such a difference.

The changes in concentration of the ABA-like compound during berry development were as great as with the gibberellin-like compounds (Fig. 3.8). Activity was close to maximal at anthesis in the three cultivars. With Cape Currant, concentrations rose from a low level two weeks before anthesis to a maximum at anthesis, and declined thereafter. The same trends were evident in Doradillo except that the concentrations were generally higher, and the maximum concentration was achieved at set (Note: the upper values for ABA equivalents are at or close to the maximum response of the assay, particularly the latter, and should be treated with caution - they may in fact represent higher values). With Sultana the greatest concentration occurred earlier and declined steadily during development. Although there are differences in the form of the curves and the concentration of the ABA-like compound at the various stages of development it is difficult to connect these dissimilarities with cluster differences in the three cultivars.

It is possible that the presence of an ABA-like compound in developing flowers is related to abscission processes associated with anthesis, but it may also be true that for normal fruit-set and development a balance between promoters and inhibitors such as gibberellins & abscisins must be achieved for controlled development of the fruit. Jackson and Blundell (1966) found that ABA could induce fruit set in a species of Rosa.

The relationship between the concentrations of the ABA-like compound and promoter III is expressed in Fig. 3.9. The equivalence ratio of 10/1 is an assumption on the basis that the inhibitor is equivalent in activity to ABA, and that the ratio of activity within the ovary is similar to that in the barley endosperm bioassay ($1 \times 10^{-7}$g ABA almost totally offsets the response to $1 \times 10^{-8}$g $GA_3$ - see standard curves in Fig's 3.4, 3.5 & 3.6). It is notable that the ratio of ABA-like activity to promotive activity is always greater than 10 in Cape Currant, but that in both Sultana and Doradillo, the promoter dominates at anthesis. This correlates with final berry size as does the concentration of promoter III. An extraordinary result is the ratio at set in Doradillo (because of non-linearity of the ABA response at the concentration represented, this value may be even higher). Unfortunately no corresponding value is available for Sultana, and so the importance of this cannot be assessed, but it may be that ABA plays an important role in berry set and/or growth. It has been noted (Gaylor and Glasziou 1969) that ABA can stimulate the synthesis of an enzyme necessary for sugar uptake by storage cells in sugar cane. If both ABA and gibberellin are important we must either consider sequential action or compartmentation. Both may in fact operate. More information is required on the metabolic roles and sites of action of both hormone classes before this question can be resolved.

Contrary to work done on other plants (e.g. Dennis et al. 1965; Zeevart 1966; Radley 1967; Reid and Carr 1967; Bristow and Simmonds 1968), CCC had no consistent qualitative or quantitative effect on extractable gibberellin-like compounds. Dale and Fillipe (1968) found that although CCC reduced the level of gibberellin present in young Phaseolus vulgaris seedlings, it did not prevent the appearance of appreciable quantities of gibberellin. It is possible that a small change may have occurred which was not picked up in the assay, either because of variation or the relatively long interval between sampling times.

There was one notable exception to the above discussion: extraction of CCC treated Doradillo anthers showed much greater activity than all other extracts. This result is difficult to reconcile with the results of previous work on the effect of CCC on endogenous gibberellins, for although there was an increase in the level of promotion in the areas that could be attributed to polar gibberellins (c.f. Reid and Carr 1967; Bristow and Simmonds 1968), the greatest increase in activity was in the region of the normal acidic gibberellins such as $GA_3$. Since this effect occurred in only one cultivar it is probably not relevant to the effect of CCC on fruit set and requires further substantiation.

Evidence that ABA does play a role in pericarp development is given by the correlation between berry size and the level of ABA-like compound in treated and untreated berries (Tables 3.1, 3.2, 3.3 and Fig's 3.4, 3.5 & 3.6). Berries from CCC treated clusters are smaller than untreated berries and contain higher levels of the ABA-like compound. Furthermore it has been demonstrated that ABA inhibits cell expansion in Avena coleoptiles and in tissue cultures (Thomas et al. 1964). However no such correlation

exists between cultivars, indicating that between cultivar differences in size are due to another factor, perhaps gibberellin as discussed above.

The conclusions that may be drawn from the above data are as follows:

(a) That qualitative differences in endogenous gibberellins exist between the berries from the parthenocarpic cultivar and berries from two cultivars whose berries normally have some degree of seed development;

(b) That for the latter, the difference between the stenospermocarpic berried cultivar and the cultivar with seeded berries in terms of endogenous gibberellins is quantitative rather than qualitative;

(c) That the concentration of promoter III, which was common to the three cultivars, at anthesis was correlated with the final size of the berry;

(d) That there was an ABA-like compound present in developing flowers and fruit, the concentration of which was close to maximal at anthesis and declined thereafter;

(e) That the concentration of this inhibitor was generally higher in the seeded cultivar, particularly at fruit set;

(f) That the effect of CCC in reducing berry size may be related to a decreased rate of decline of the ABA-like compound;

(g) That CCC had no significant effect on extractable gibberellins within the ovary;

(h) That the levels of endogenous gibberellins and the endogenous ABA-like compound are probably under independent control since no obvious relationship existed between the level of the extractable compounds of the two hormone classes.

## IV. GENERAL DISCUSSION

It is widely accepted that the processes involved in fruit-set and pericarp development are under hormonal control (~~e.g. Nitsch 1953, 1962;~~ Crane 1964), although it is also recognised that nutrient availability can be a limiting factor (e.g. Coombe 1962; Mullins 1967). The aim of this work was to study the relationship between gibberellins and fruit-set and growth in the grape in two ways: firstly by examining the effect of exogenous applications of a gibberellin ($GA_3$) and a growth retardant (CCC), and secondly by examining the content of endogenous gibberellins and ABA-like compounds in the presence and absence of this growth retardant.

### (1) Set

The factors involved in fruit-set are complex and a change in set could be due to one or more of the following factors:

(i) a direct effect on the processes controlling abscission;

(ii) an indirect effect on the processes controlling abscission such as:-

a. a direct alteration of the ability of the ovary to attract nutrients (e.g. an alteration of the viability of the pollen or the fecundity of the ovary);

b. a direct alteration of the ability of the ovary to utilize nutrients;

c. an indirect effect on nutrient supply such as an alteration of the competitive balance between the growing shoot tip and the developing cluster.

What role does GA play in fruit-set ? The results presented here suggest that GA is not primarily involved in the process of fruit-set. In no situation did GA at the concentrations used here dramatically alter the number of berries set. The decreases in fruit set on the two parthenocarpic cultivars obtained when GA was applied two weeks prior to anthesis were probably due to a reduction in pollen viability as has been suggested by Weaver and McCune (1960). A similar effect may be responsible for the reduction of set in Sultana by anthesis applications, or it may be due to enhanced competition between individual flowers on the cluster. It has been noted that berries derived from flowers sprayed with gibberellin before anthesis are no larger than their unsprayed counterpart, but that large increases in weight occur if the flowers are treated during anthesis (presumably after pollination). Thus the pollinated portion at the time of spraying may utilize the whole of the limited nutrient supply.

The increase in set produced by post-anthesis applications of GA (notably in Doradillo) is probably due to promoted growth of the seedless portion of the cluster, thus enabling them to compete more ably for nutrients with berries that have already set and commenced to grow (Coombe 1962).

It is likely that increases in set attributed to the application of CCC are the result of an action of CCC at a site distant from the cluster. From the extraction experiments there was no evidence that CCC significantly altered the level of extractable gibberellin-like compounds or ABA-like compounds during the period of anthesis and fruit-set. Changes were only obvious after set had occurred. Further evidence for this hypothesis comes from a number of sources: removal of the shoot tip and young leave

at anthesis increases fruit set (Coombe 1962). CCC application to any part of the shoot results in decreased shoot growth and increased fruit-set (Coombe pers.comm.), and further that CCC applications to the cluster in low concentrations are more effective in decreasing berry size than in increasing berry set (Coombe 1967). Thus it is likely that CCC acts by altering the competitive balance that is thought to exist between shoot growth and reproductive growth. Further, a large reduction in shoot growth is required, and for maximal effect the CCC must be applied two to three weeks before anthesis. An increase in the level of ABA-like compounds such as occurs in the berry may be responsible for the reduction of shoot growth after CCC application.

The reduction in the number of berries with seeds in the seeded cultivars after treatment with GA can be explained on the basis of GA induced embryo abortion (Watanabe 1963a, cited by Sachs and Weaver 1968, 1963b; Smirnov and Perepelisyna 1965; Nishitani et al. 1968). The measurements here made show no evidence for an excess of endogenous gibberellins being responsible for defective embryo development in seedless cultivars.

(2) Berry size and shape

The effectiveness of GA in increasing berry size depended on four things; the cultivar, the degree of seed development, the time of application, and the concentration applied. At a given GA concentration the most important factor was the degree of seed development, though there were differences between the cultivars in each group (parthenocarpic, stenospermocarpic and perfect).

Thus pre-anthesis applications were more effective on Cape than they were on Zante. The degree to which seed growth was perfect also determined the optimum time of application. Thus parthenocarpic berries responded maximally to anthesis applications, stenospermocarpic berries to applications approximately ten days after anthesis and seeded berries did not respond markedly at any time of application (in one instance a reduction in berry size was recorded - Muscat 1966-7, pre-anthesis application).

At the pre-anthesis time of application, gibberellin-like activity was low in the three cultivars tested. On the basis of this evidence it is unlikely that the unresponsiveness of Sultana and Doradillo is due to sufficient gibberellin. However two differences were noted between the parthenocarpic Cape and the other two cultivars which were correlated with responsiveness to pre-anthesis applications; (a) a low concentration of ABA-like activity in Cape compared with a high concentration in the others and (b) the presence of promoter II in Sultana and Doradillo and its absence in Cape. Either of these two observations could conceivably account for the observed differences in response, but the difference in ABA-like levels is the more likely since it has been observed that ABA and GA are antagonistic (e.g. Thomas et al. 1964 ; Chrispeels and Varner 1967). Little is known of the role of different gibberellins in these tissues.

However, at anthesis the three cultivars all had high concentrations of the ABA-like compound, and thus the correlation breaks down. Response to GA now becomes inversely correlated with the concentration of endogenous gibberellin. To account for the lack of antagonism between the ABA-like compound and externally added GA at anthesis and after anthesis an alternative hypothesis must be considered: it is suggested that the tissue must be prepared before GA can act, and the presence of a specific predisposing compound may be the determinant factor. There is a distinct possibility that this compound may be a cytokinin (e.g. Weaver et al. 1966; Zuluaga et al. 1968). It has been demonstrated a number of times in different tissues that cytokinins can over-ride inhibition of response due to the presence of ABA or coumarin (Khan 1967; Overbeek 1967; Knypl 1967; Khan and Downing 1968; Letham 1969), and that cytokinins predispose barley aleurone tissue to respond to GA (Eastwood et al. 1969).

There are two possible ways of accounting for the lack of response to GA if seed development continues; either, in the presence of seed growth, gibberellin is non-limiting or GA action remains repressed. Evidence in Sections II and III suggests that the more likely situation is that GA is non-limiting. Thus Cape Currant has no promoter II and has a low level of promoter III, and responds maximally to GA applied at anthesis; Sultana has a low concentration of promoter II and a low level of promoter III (maximal at anthesis?) and responds maximally to GA applied after anthesis, while Doradillo has marked promoter III activity at anthesis and fails to respond to the application of GA. However better data would be required on both the quantitative and qualitative changes of gibberellins and their roles in development before full confidence could be taken in

the above hypothesis.

The high inhibitor to promoter ratio evident at fruit set in Doradillo is puzzling, and lack of a value for Sultana at this stage of growth makes it difficult to assess its importance. It may be related to the growth of berries that have set, but further data will be required in order to examine its function and its relationship to response to externally applied gibberellin. The importance of the generally higher ABA-like activity in Doradillo also requires assessment.

The ability of CCC to reduce berry size irrespective of the presence of seed could be interpreted as meaning that GA was essential for berry growth, but not limiting. However the evidence presented in section III suggests that this effect is due to a sustenance of high concentrations of the ABA-like compound in the berries during their early growth. This also suggests a role for ABA in the control of berry size.

Berry size is determined by two factors, the number of pericarp cells and their size. No quantitative measurements have been made of the relative importance of each process in determining cultivar differences. Similarly little work has been done to determine whether differences in shape are due to different numbers of cells or different size of cells in the different regions of the berry. Sachs and Weaver (1968) demonstrated that the effect of GA on berry size was primarily due to increased cell size and further that the cells at the distal pole were most affected.

The relationship between the extractable growth substances assayed in this work and berry shape is obscure. Although gibberellin applied at anthesis is effective in increasing the length of the berry versus the width, the spherical seeded berry, Doradillo, possessed more gibberellin-like activity at this stage of development than the elongated Sultana. An adequate explanation of control of berry shape will probably involve a balance between two or more growth regulators. It has already been noted that auxin application results in berries with decreased L/W ratios (Sachs and Weaver 1968; Coombe pers.comm.), and thus an auxin-gibberellin ratio may be the important factor.

Two important observations on the relationship between gibberellin and berry shape are: (a) it is more effective when applied at anthesis than at any other time and (b) the effectiveness of GA in this regard is not related to seed growth. That GA is only effective early in berry growth suggests either an effect on cell number or that expansion in the various directions is programmed early in the expansion of the cells and, once set, cannot be altered. Sachs and Weaver (1968) noted differences in cell size - the polar cells were larger in GA treated berries than the equatorial cells - although they did not discount an effect on cell number. Differences in responsiveness between the cultivars requires further investigation (seeded Doradillo berries were unaffected, Muscat seeded and seedless, Sultana and Zante showed a 15% increase in length/width ratio in response to 20ppm, while Cape showed a 38% increase with the same concentration).

CCC, only when applied before anthesis, decreased the length/width ratio of Sultana and Muscat (both cultivars have relatively large and elongated berries). It is difficult to explain this observation on the basis of the data on extractable ABA-like compounds or gibberellin-like compounds because no significant differences between CCC treated and untreated berries were noted in either of these classes of compounds at anthesis. But if the differences in shape are due to cell expansion then the higher concentrations of the ABA-like compound at the stage of maximal rate of cell expansion in the first growth phase would be a better explanation. However this does not explain why CCC applied at fruit set was equally as effective as CCC applied pre-anthesis in reducing berry weight, yet had no effect on berry shape.

(3) GA and CCC interaction

The effects of the two compounds, GA and CCC are generally opposite and, where parallel, are probably so for different reasons. A number of parameters showed a significant response to GA only, notably those concerned with pericarp growth in parthenocarpic cultivars. A few showed significant response to CCC only; these were, without exception, parameters measured on seeded cultivars and may be treated as special cases. The latter effects may be explained as being situations where GA is required but is not limiting if one accepts that CCC inhibits the synthesis of endogenous gibberellins. However all these responses could be explained on the basis of a totally different concept, a concept which is based on the effect of CCC being directly antagonistic to GA through promoting the build-up or maintainance of high levels of a natural

gibberellin antagonist, ABA. Although ABA has been shown to prevent gibberellin responses in a number of systems (e.g. Chrispeels and Varner 1967) it is probably better conceived of as being an anabolic hormone which both prevents the action of the catabolic hormone GA and promotes anabolistic processes such as the storage of reserve products. Recent evidence for such a concept has been published by Gaylor and Hatch (1969). This type of concept could explain some of the interactions that have been described, and why CCC has effects on some parameters and not others, and why GA has effects on some parameters and not others. Furthermore this type of concept enables such synergistic interactions as reported by Guttridge (1966) to be more readily accommodated.

A complete explanation of the interaction of these two compounds, GA and CCC, in this system is not yet possible because critical data on the precise site and mode of action of each is unavailable. It seems likely from what has been discussed that CCC is effective as a setting agent at a site distant from the cluster; there was no significant effect of CCC on either GA-like or ABA-like compounds during the period of anthesis to fruit-set. On the other hand it is probable that GA acts on set within the cluster. There is reasonable evidence that GA applied to the cluster does not move out of the cluster although it may move into the cluster from outside (Weaver and McCune 1959c). When one considers the effects of GA and CCC on parameters of pericarp growth, however, it seems more likely that their effects are direct and due to the presence of the compounds in the ovaries.

## 4) Conclusions

Based on the results of this investigation and the observations of other authors a reasonable hypothesis of the control of fruit set and development would seem to be as follows: as the flower matures an inhibitor (anabolic hormone) builds up (e.g. ABA) and causes the observed decline in growth and nutrient importation, and probably in the promotion of the processes that lead up to anthesis. Pollination and perhaps syngamy result in the production of a compound (a cytokinin) that relieves the inhibition of growth and nutrient importation, and thus disposes the pericarp tissues to respond to hormones such as gibberellin synthesised in the developing tissues of the seed. This relieving compound may also be responsible for the shutting down of the synthesis of the inhibitor although accumulation continues in Doradillo up until fruit set. The process essential for set is the relief of the inhibition. Whether or not set occurs then depends on the availability of organic nutrients and the ability of the ovary to gain its nutrient requirement in competition with neighbouring ovaries.

While this hypothesis does not pretend to describe the whole of the control of berry set and pericarp growth it should provide a sound working hypothesis on which to base further studies.

## V. ACKNOWLEDGMENTS

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# VI. APPENDICES

# APPENDIX A

## EXPERIMENTS ON EXTRACTION AND ASSAY TECHNIQUES

### Introduction

Work published by Coombe (1960) and Weaver and Pool (1965b) on gibberellin-like activity in Vitis is difficult to interpret due to the poor definition of their methods of purification. Coombe's (loc.cit.) data from crude extracts may, as he suggests, represent the promotor/inhibitor balance rather than total gibberellin-like activity. The data of Weaver and Pool (loc.cit.), while demonstrating the presence of a number of gibberellins with widely varying solubility properties, is difficult to interpret because of the likelihood that one compound may be present in more than one fraction. Furthermore no account was taken of the possibility of an inhibitor being present. For the work described in section III it was necessary to separate three groups of compounds: conjugated (water-soluble, polar) gibberellins, unconjugated gibberellins, and ABA-like compounds. Such a separation would enable simpler interpretation of results.

Abscisic acid and the known gibberellins exhibit the behaviour typical of organic acids, and in fact possess one or more carboxyl groups. Polarity within the unconjugated gibberellins depends mainly on the number of oxygen containing groups - hydroxyls and epoxides; they are marginally soluble in water but are soluble in the more polar of the organic solvents (e.g. the alcohols and ethyl acetate). The solubility characteristics of ABA are similar to those of the less polar of

the unconjugated gibberellins ($GA_{4/5/7}$). Conjugated gibberellins such as those suggested by Murakami (1961), Sembdner et al. (1964), and described by Schreiber et al. (1967) and Tamura et al. (1968), are glycosides of gibberellin and also possess a free carboxyl group. However, only a few of the reported conjugated gibberellins have been fully characterised, and claims have been made that some of these are neutral compounds (e.g. Hashimto and Rappaport 1966), that is, either the conjugation has taken place via the carboxyl group or the compounds do not possess a carboxyl group.

This information was used to design a system of purification and analysis based on the barley endosperm bioassay for analysing the three groups of compounds mentioned above namely, conjugated gibberellins, unconjugated gibberellins and ABA-like compounds.

Partitioning of extracts

Throughout these experiments one method of sample preparation and extraction was used. The tissue was lyophilised and then homogenised at 3°C in 98% methanol (MeOH) with a glass homogeniser (Kontes). The homogenate was centrifuged, the supernatant decanted and the residue was then re-extracted twice during the next twenty four hours with 98% MeOH by shaking on either a reciprocating or a wrist-action shaker, and then centrifuged. The supernatants were combined, and reduced in volume in a rotary film evaporator under reduced pressure at 30 to 35°C. No soluble activity remained in the residue after this extraction.

Ten grams d.wt. of two weeks pre-anthesis Cape Currant flowers were extracted in the above manner, dried in a rotary evaporator and then taken up in 250ml of 70mM phosphate buffer (pH 8.0) and partitioned three times with equal volumes of petroleum spirit (BP 40-60°C). The aqueous phase was adjusted to pH 3.5 with 10% HCl and partitioned four times with equal volumes of ethyl acetate (Merck, Pro - Analyst). The aqueous residue was dried in a rotary evaporator, lyophylised, and re-extracted with absolute MeOH (freshly prepared by treatment with magnesium turnings and iodine, refluxed and then distilled). All fractions were rotary evaporated to dryness and then taken up in 10ml of distilled water. A dilution assay of 250, 25 and 2.5mg equivalent of tissue was carried out in triplicate using the barley endosperm bioassay (Coombe et al.1967). An assay for the presence of inhibitors was carried out by adding $5 \times 10^{-10}$ g/ml $GA_3$ to one set of vials (also three dilutions and three replicates).

The results (Fig. A.1) show the presence of a reasonably high level of inhibitor in the ethyl acetate phase, and a low level of promotor in the methanol triturate. There was no activity in the petroleum spirit phase.

Although the system showed some promise it was evident that an extra step would be needed to separate the components more clearly. Furthermore the method was time consuming and did not effect a good purification. The formation of a denatured protein emulsion added to the difficulties.

Fig. A.1 Partition analysis: pre-anthesis Cape Currant

For explanation see text

Fig. A.2 Anion exchange chromatography: post-anthesis Doradillo and $GA_3$

For explanation see text

NOTE: log $GA_3$ equivalent is the calculated amount of $GA_3$ that would be present if all activity were eluted in a particular fraction (at the lowest dilution 1/20th of the $GA_3$ added).

FIG. A1

PARTITION ANALYSIS: Pre-anthesis CAPE CURRANT

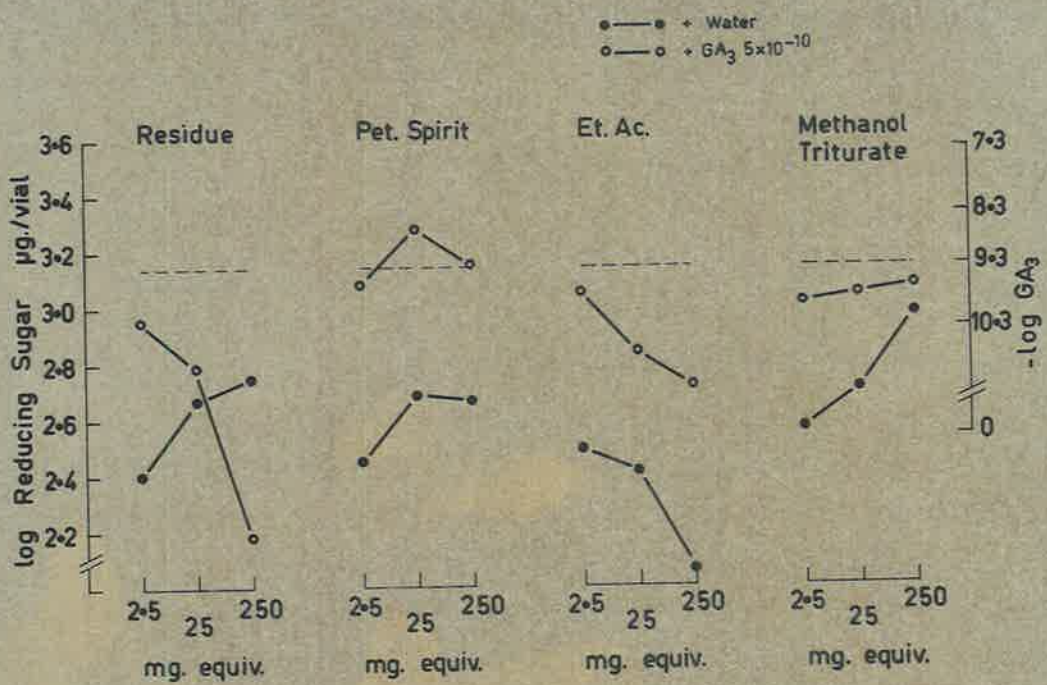
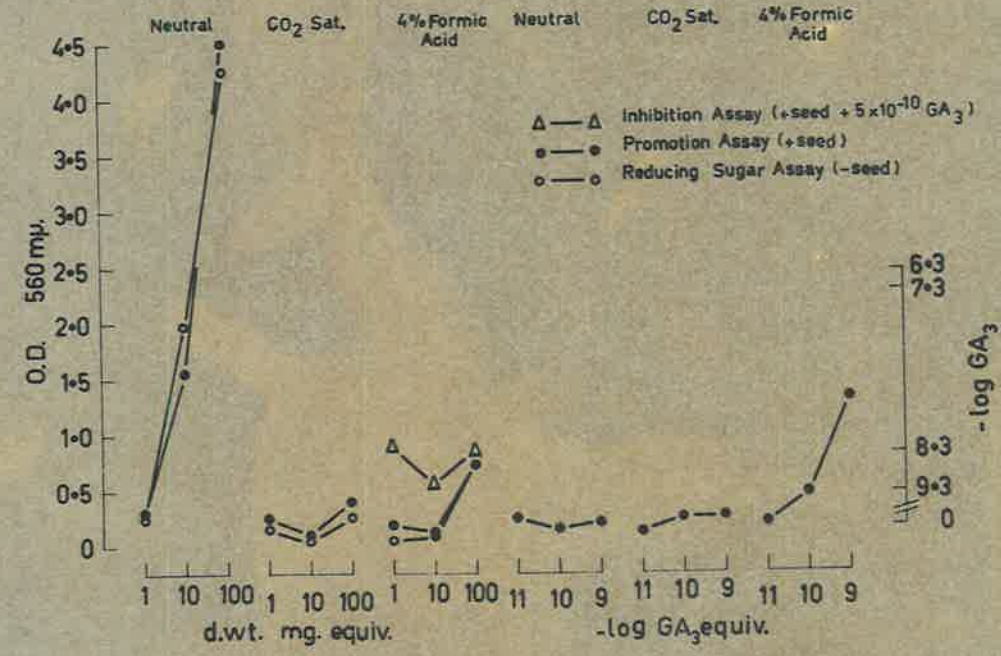



FIG. A2

ANION EXCHANGE CHROMATOGRAPHY

Doradillo: post-anthesis Standard: $GA_3$ $2 \times 10^{-8}$ g.



Anion Exchange chromatography

The use of anion exchange chromatography for the purification of plant hormones was first described by Jones (1964); however, under his conditions (quaternary ammonium anion exchange resin), approximately one third of the gibberellin was hydrolysed.

A preliminary experiment based on his technique confirmed the hydrolysis of $GA_3$. It was felt that anion exchange chromatography still held potential as a means of preparation of carboxylated plant hormones. A milder anion exchange resin was chosen (Sephadex A25) in which the active group was a mild base (diethylaminoethyl ether, DEAE). A method based on that of Zinkel and Rowe (1964) was used, and for simplicity 98% MeOH (the extracting solvent) was used instead of their 89ether:10MeOH:1$H_2O$ mixture.

Two columns, each containing ten grams of Sephadex A25 were prepared as described in Section III. To column A the MeOH extract of 4g d.wt. of Doradillo (two weeks post-anthesis) berries was added. To column B, $2 \times 10^{-8}$ g/ml $GA_3$ was added in 100ml of MeOH. The chromatography was carried out at room temperature. Three fractions were collected from each column: (1) 600ml 98% MeOH, (2) 750ml of 98% methanol saturated with $CO_2$, (3) 750ml 98% MeOH with 4% formic acid. The eluted fractions were dried in a rotary evaporator, redissolved in 10ml of distilled water, and three 10 fold serial dilutions made. A barley endosperm assay was carried out in triplicate on these fractions, 0.5ml samples of each fraction and dilutions were added to vials and incubated with; (i) 0.5ml distilled water, no seed; (ii) 0.5ml distilled water plus seed; (iii) 0.5ml $GA_3$ ($5 \times 10^{-10}$ g) plus seed.

The results in Fig. A.2 demonstrate the presence of reducing substances (sugars) in fractions (1) and (3) of the Doradillo column (cf. extract plus seed and extract minus seed). Recovery of the $GA_3$ added to column B was complete within the accuracy of the assay ($2 \times 10^{-9}$ versus $1 \times 10^{-9}$g theoretical). Fraction (3) of the Doradillo column contained an inhibitor and so gave no indication of the presence of any promotors (Note: subtract + seed + GA from - seed).

The results of this experiment were encouraging because of the good recovery of $GA_3$ and the cleanliness of the active fraction (No.3) of the Doradillo extract. This method of purification was easier to perform than partitioning and was apparently purer.

Anion exchange chromatography of ABA and apricot gibberellin

Although a preliminary examination using electrophoresis (Coombe pers.comm.) indicated that the polar gibberellin-like compound from apricots was monocarboxylic, no information was available on its ion exchange properties. The aim of this procedure was to determine the fraction in which it was eluted, and whether or not any gross reduction in activity occurred during elution. It was further decided to investigate whether ABA was, in fact, eluted with 4% formic acid, and whether or not there was any possibility of separating ABA and GA at this stage.

Two 10 gram Sephadex columns were prepared as previously described: to column "A" 20$\mu$g of active apricot extract was applied and to column "B" a mixture of 5$\mu$g ABA and 1$\mu$g $GA_3$. The columns were then eluted in six batches with 98% methanol containing increasing proportions of formic acid: 500ml 0.0M; 250ml 0.05M; 250ml 0.1M; 250ml 0.25M; 250ml 0.5M; and 250ml 1.0M (approximately equal to 4%). The resultant

Fig. A.3 Anion exchange chromatography: Apricot extract and ABA plus $GA_3$

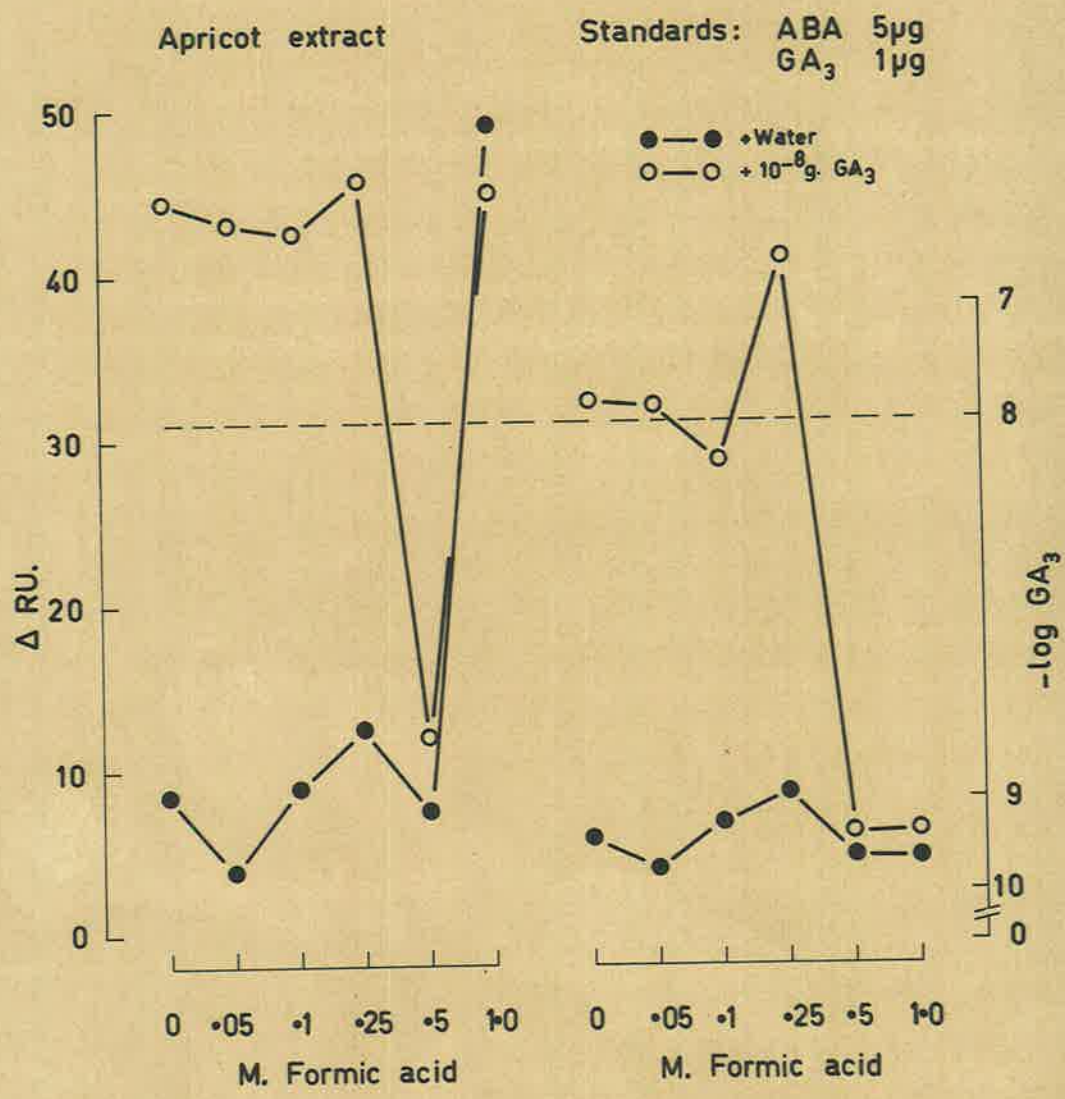
For explanation see text

The horizontal dotted line represents the mean of 3 replicate vials containing $10^{-8}$ g $GA_3$

M. Formic acid = the molarity of formic acid in 98% (w/v) methanol/water eluting solvent

FIG. A3

ANION EXCHANGE CHROMATOGRAPHY



fractions were lyophylised and taken up in a total of 5ml of 0.062M citrate/phosphate buffer pH4.0. The assay was carried out on 0.8ml of this solution plus 0.2ml autoclaved distilled-deionized water, or, in the case of inhibition assay, 0.1ml of autoclaved distilled-deionized water plus 0.1ml 5 x $10^{-8}$ $GA_3$. The assay was carried out in duplicate. A check was made on the presence of refractive compounds by running a series using extract plus water as in the promotion assay but omitting seed.

The results (Fig. A.3) show that the activity from the partially purified apricot extract was not eluted until a concentration of 1M formic acid was reached. There was also an indication of an inhibitor in the 0.5M fraction but this was not checked further. ABA was eluted by both the 0.5M and 1.0M fractions. This masked any promotive activity due to $GA_3$ but it is presumed that $GA_3$ was eluted with ABA.

These results confirm that the active compound from the apricot extract behaves as a carboxylated compound. Further, the ABA behaves similarly to the inhibitor that is present in extracts of grape berries (see above and Section III).

Thin layer chromatography (in association with B.G. Coombe)

Both paper and thin layer chromatography have been used in the preparation of plant extracts for hormone assays. Thin layer chromatography was chosen as it generally results in more compact spots and is better suited for the separation of a range of closely allied compounds (Randerath 1966). A search was made for a solvent mixture with the following properties: (i) readily removable to minimise interference with the assay; (ii) move $GA_3$ to approximately Rf 0.5; (iii) separate ABA from the gibberellins and (iv) separate polar gibberellins from sugars.

## SUMMARY OF CHROMATOGRAPHIC DATA FOR SILICA GEL THIN LAYER PLATES

| References | $C_6H_6$ | Et. Ac. | Isoprop. | $CHCl_3$ | EtOH | $CH_3COOH$ | HCOOH | Glucose | Apricot "Gibb." | $GA_3$ | $GA_{4/7}$ | $GA_8$ | ABA | IAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | SILICA GEL G (Merck.) | | | | | | |
| (a) | 4 | 6 | 9 | | | 1 | | 0.15(5)[1] (.14-.23)[2] | | 0.85(5) (.80-.88) | 0.89(5) (.85-.92 | | 0.83(2) (.81-.84) | 0.84(2) (.82-.85) |
| | 20 | 30 | 50 | | | | 1 | 0.35 | | 0.81 | 0.81 | | | |
| | | 30 | 40 | 30 | | | 1 | 0.18 | | 0.80 | 0.80 | | | |
| (b) | | 50 | | 50 | | 5 | | 0.0 | | 0.30 | 0.49 | | | |
| | | 50 | | 50 | | 1 | | 0.0 | | 0.15(2) (.09-.20) | 0.40(2) (.33-.47) | | | |
| | | 50 | | 50 | | | 5 | 0.02 | | 0.35 | 0.61 | | | |
| (b) | | 50 | | 50 | | | 1 | 0.0(3) | | 0.17(3) (.15-.20) | 0.39(3) (.34-.47) | | 0.37(2)* 0.45(2)** | 0.47(2) |
| | | | | 95 | 5 | | 1 | 0.0 | | 0.08 | 0.21 | | | |
| | | | | 92 | 8 | | 1 | 0.0 | | 0.28 | 0.49 | | | |
| | | | | 90 | 10 | | 1 | 0.01(7) (.00-.04) | | 0.40(8) (.28-.55) | 0.53(7) (.39-.77) | | 0.55(5) (.53-.59) | 0.50(4) (.49-.50) |
| | | | | 85 | 15 | | 1 | 0.02(6) (.02-.03) | 0.13 | 0.38(6) (.24-.47) | 0.54(6) (.49-.60) | | 0.63 | |
| | | | | 80 | 20 | | 1 | 0.06(7) (.04-.12) | 0.34(2) (.30-.38) | 0.69(7) (.54-.91) | 0.76(7) (.64-.94) | | 0.70(4) (.66-.73) | 0.65(4) (.61-.70) |
| | | | | 70 | 30 | | 1 | 0.14(3) (.11-.17) | | 0.71(3) (.68-.73) | 0.74(3) (.71-.76) | | 0.78(2) (.74-.82) | 0.74(2) (.73-.76) |

References

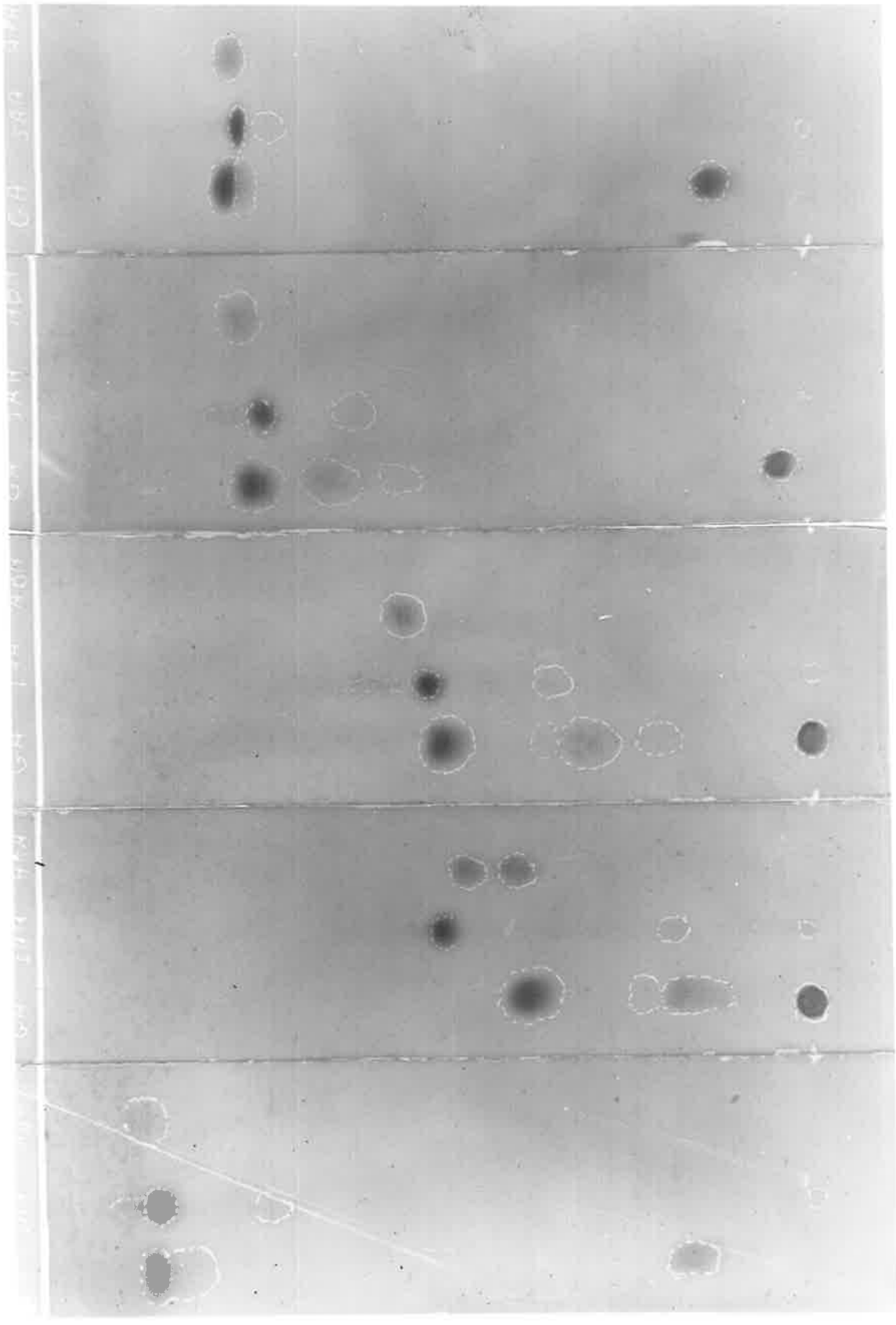
| $C_6H_6$ | Et.Ac. | Isoprop. | $CHCl_3$ | EtOH | $CH_3COOH$ | HCOOH | Glucose | Apricot "Libb." | $GA_3$ | $GA_{4/7}$ | $GA_8$ | ABA | IAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 60 | 40 | | 1 | 0.31(2) (.30-.31) | | 0.81(2) (.77-.85) | 0.83(2) (.79-.87) | | | |
| | | | 50 | 50 | | 1 | 0.40 | | 0.79 | 0.82 | | | |
| | | | 90 | 10 | | 0.5 | 0.02 | | 0.36 | 0.52 | | | |
| | | | 70 | 30 | | 0.5 | 0.15(2) | | 0.71(2) (.71-.72) | 0.83(2) (.80-.85) | | | |
| | | | 50 | 50 | | 0.5 | 0.41 | | 0.71 | 0.73 | | | |
| | | | 90 | 10 | | 0.1 | 0.02(2) | 0.15 | 0.30(3) (.28-.33) | 0.45(3) (.41-.55) | | | |
| | | | 70 | 30 | | 0.1 | 0.14 | | 0.30 | 0.79 | | | |
| | | | 50 | 50 | | 0.1 | 0.25 | | 0.27 | 0.28 | | | |
| | | | | | | | | Silica Gel H (Merck) | | | | | |
| | | | 85 | 15 | | 1 | 0.02(3) | | 0.45(8) (.38-.51) | 0.63(8) (.53-.74) | 0.33 | 0.60(2) (.56-.64) | |
| | | | 70 | 30 | | 1 | | | 0.67 | 0.77 | 0.67 | 0.72 | 0.76 |
| | | | 60 | 40 | | 1 | | | 0.71 | 0.79 | 0.70 | 0.75 | 0.79 |
| | | | | | | | | GELMAN ITLC | | | | | |
| | | | 99 | 1 | | 1 | 0.0(2) | | 0.37 | (0.53(0.76) ($GA_4$?)($GA_7$?) | | | |

Footnotes: 1. The mean Rf of the compound followed by the number of observations in brackets.
2. The rango of Rf values measured.
* ± cis trans Abscisic acid.
** trans trans Abscisic acid.

Ref. (a) Coombe pers. comm.
(b) Skene " "

Plate A.1 Chromatograms of plant hormones ($GA_{3/4/7}$, IAA, t.ABA and ± ABA) on silica gel G in solvents of composition from left to right; 50:50:1, $CHCl_3$: Et.Ac.: HCOOH; 90:10:1; 85:15:1; 80:20:1; 70:30:1, $CHCl_3$: EtOH: HCOOH.

Detected by spraying with 95:5 (v/v) EtOH/$H_2SO_4$ and heating at 120°C for 10 minutes.



Glass plates (5 x 20 cm) were coated with silica gel G (0.3mm) and then air dried overnight. Usually two spots containing a mixture of 5$\mu$g of each compound in methanol (see Table A.1) were applied to each chromatogram. The plates were then placed in solvents of varying composition as shown in Table A.1 and developed to 15cm. The compounds were made visible by spraying with 5% ethanolic sulphuric acid, and heating at 110° C for 10 minutes (Jones et al.1963). Apricot gibberellin (supplied by Coombe) was used as an example of a polar gibberellin-like compound and was detected by bio-assay.

The results of these and later experiments are summarised in Table A.1. A solvent of composition 85:15:1 chloroform ($CHCl_3$): ethanol (EtOH): formic acid (HCOOH) was chosen as being most suitable. With this solvent ABA usually ran half to one Rf unit ahead of $GA_{4/7}$, $GA_3$ was well separated from $GA_{4/7}$ (one to one and a half Rf units) and the apricot gibberellin was moved about half way between glucose and $GA_3$. Solvents with a lower proportion of EtOH gave better resolution within the unconjugated group of gibberellins but did not distinguish between glucose and apricot gibberellin. Solvents with a polar content higher than 15% tended to form a secondary front with which all of the unconjugated gibberellins and ABA and IAA were associated. Such mixtures, in particular those with 20 or 30 % EtOH, gave excellent resolution of the polar gibberellin, interfering substances such as glucose, and the unconjugated gibberellins.

Examination of the interference of silica gel G with refraction read

Preliminary experiments showed that results based on the measurement of refractive index were highly variable when either the eluant from silica gel G was assayed or when silica gel G was included in the vial.

TABLE A.2

The effect of silica gel G on the change in refractometer reading of glucose solutions with water in the reference cell

| Gel area sq cm | Glucose $\mu$g/ml | | |
|---|---|---|---|
| | 0 | 300 | 1500 |
| | $\Delta$ RU | | |
| 0 | 0.0 | 3.0 | 14.3 |
| 1 | 2.4 | 5.5 | 16.8 |
| 5 | 11.5 | 15.5 | 26.3 |
| | $(\Delta RU + \Delta RU_{glucose}) - \Delta RU_{gel + glucose}$ | | |
| 0 | 0.0 | 0.0 | 0.0 |
| 1 | 0.0 | 0.1 | 0.1 |
| 5 | 0.0 | 1.0 | 0.5 |

The magnitude of this effect and whether or not the refraction due to the presence of silica gel was additive to that of reducing sugar (glucose) was examined. The refraction of filtrates of three concentrations of glucose, 0, 300, or 1500µg/ml, combined with 0, 1.0 sq cm or 5.0 sq cm of 0.3mm thick silica gel was measured on a Waters digital differential refractometer. There were two replications of each combination.

The results (Table A.2) indicate that the response was additive and that the refraction due to the presence of silica gel G was of such a magnitude that large errors could be introduced in readings by minor differences in gel thickness or area scraped.

Similar tests carried out on "Gelman ITLC" and silica gel H, neither of which contain binders, showed that the binder was responsible for most of the refraction: Five square cm of silica gel H had $\Delta$RU of 3.2 while an equivalent area of silica gel G had $\Delta$RU of 11.5. Thus by substituting a medium without a binder an acceptably low level of background refraction could be obtained.

Test of chromatography media for assay purposes

Using the above information four chromedia were tested for suitability for hormone assay purposes. Four chromatograms of silica gel H, Gelman ITLC, thin layer cellulose (MN300), and paper were prepared and spotted in duplicate with either $10^{-9}$g $GA_3$ or $10^{-7}$g ABA. The solvents were as follows:

| | | |
|---|---|---|
| Silica gel H : | 85/15/1 | ($CHCl_3$/EtOH/HCOOH) |
| Gelman ITLC : | 99/1/1 | (" " " ) |
| MN300 : | 2% aqueous formic acid | |
| Paper : | " " " " | |

Fig. A.4 Test of chromatography media for suitability in the bioassay of plant hormones

For explanation see text

Solvents: Silica gel H 85:15:1 $CHCl_3$/EtOH/ formic acid

Gelman ITLC 99:1:1 $CHCl_3$/EtOH/ formic acid

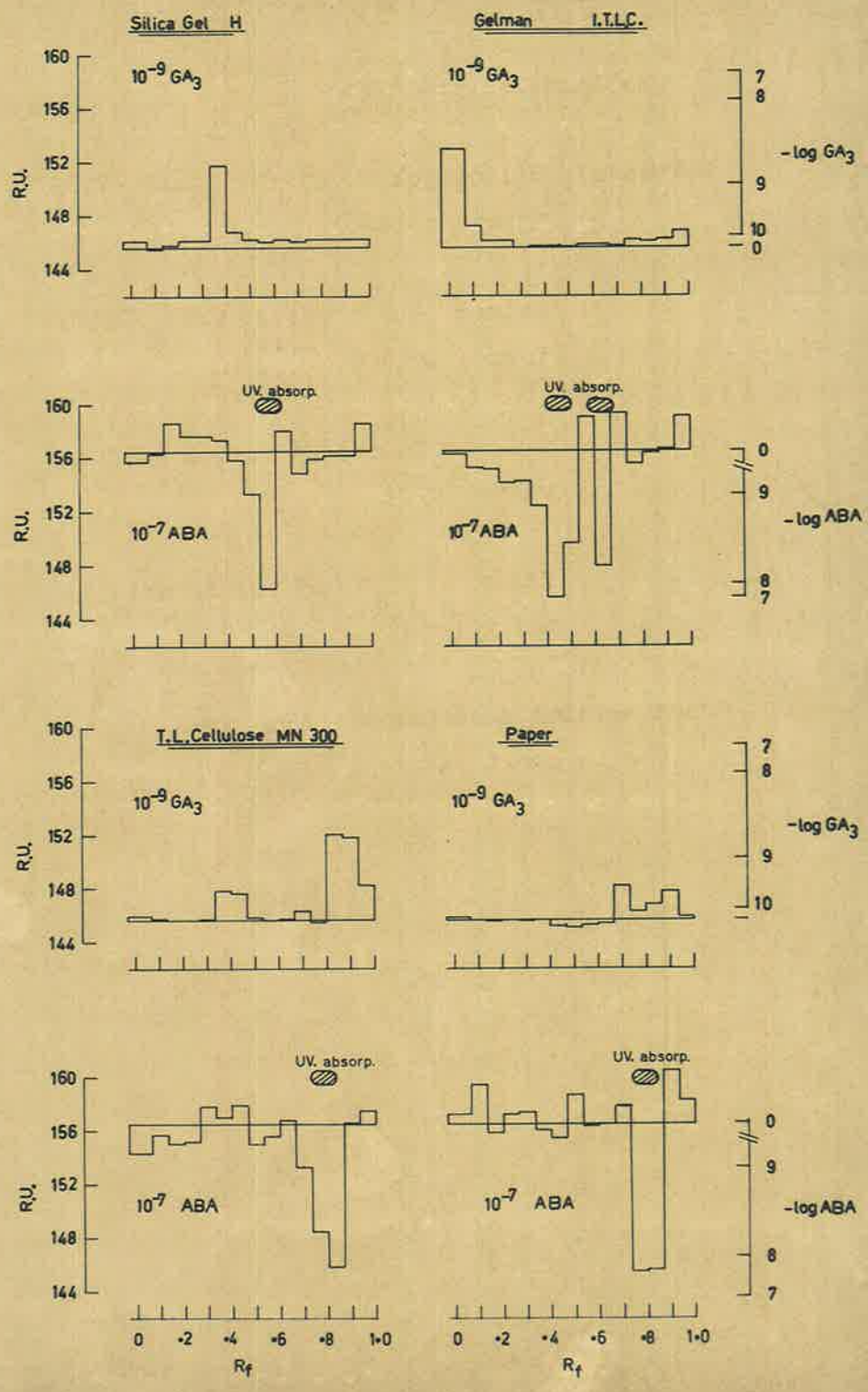
T.L. Cellulose 2% formic acid in water (v/v)

Paper 2% formic acid in water (v/v)

Cross hatched areas represent the absorption visible in short wavelength U V light.

FIG. A4.

TEST OF CHROMATOGRAPHY MEDIA



The chromedia were dried under a fan for two hours, divided into 15 sections and then assayed using the barley endosperm bioassay (see section III for details of procedure).

The results as indicated in Fig.A.4 (only one replicate presented) show that silica gel H performed better than the other media in that the areas of activity were confined to a single Rf unit, the response was of the same order as indicated by the standard curves, and in the assay for $GA_3$ a constant value was obtained for the baseline.

When viewed under short wavelength UV light two absorbing areas were discernible in the Gelman plates spotted with ABA. These corresponded to the two inhibitory areas as detected by the assay. It is possible that one of these was trans trans ABA and the other $\pm$ cis trans ABA. Both of these compounds have been found to have biological activity (Milborrow 1966, Bellin pers.comm.).

# APPENDIX B

## COLUMN STUDIES CHROMATOGRAPHY

### Introduction

Preliminary studies indicated that the incorporation of an extra step in the purification procedure might be worthwhile to enhance the purity of the samples being assayed. It would also enable the assay of larger dry weight equivalents of extracts and thus permit the determination of minor constituents. Further, such a step seemed to be essential in the preparation of samples for gas liquid chromatography.

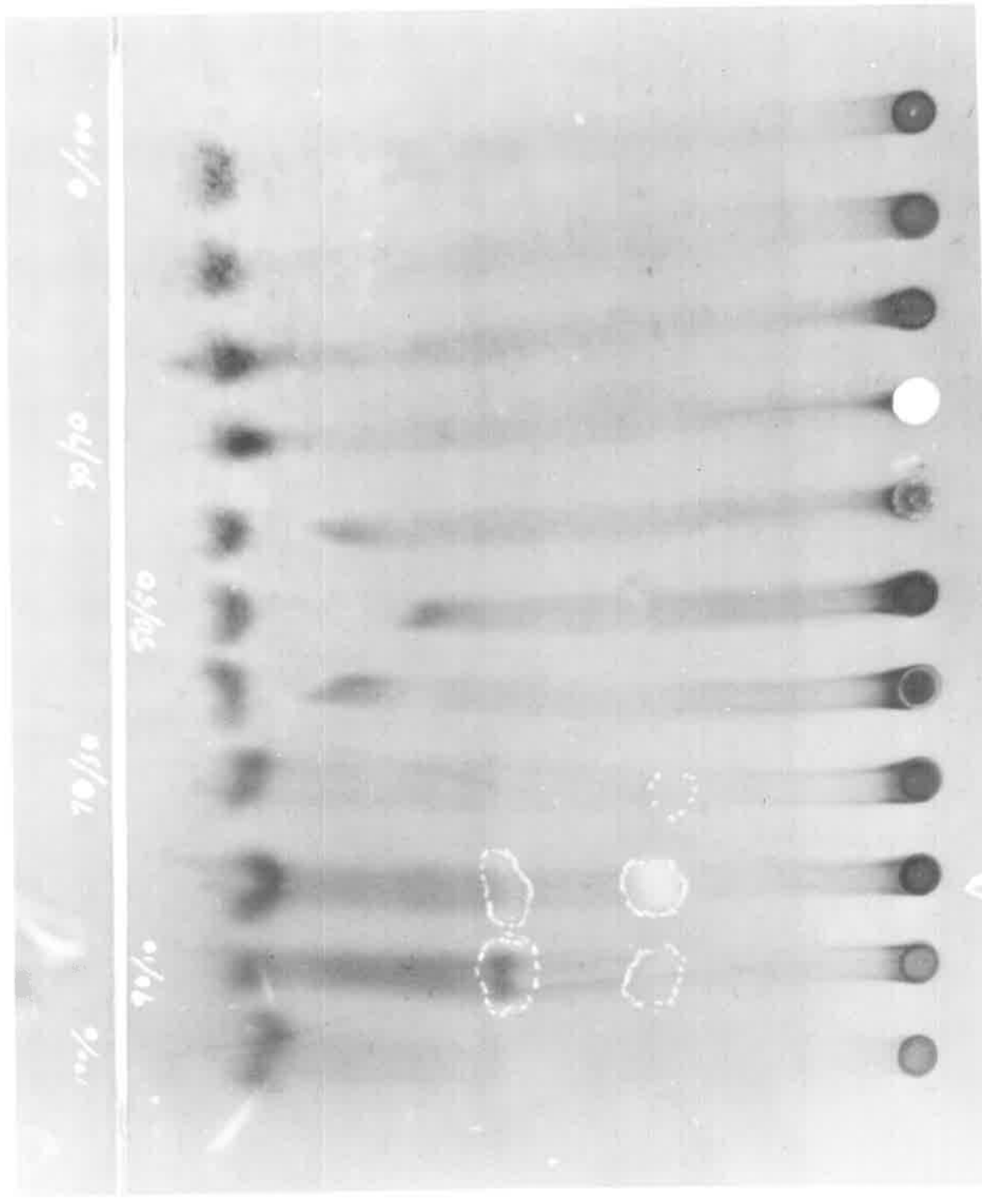
Thin layer chromatography on a preparative scale was tried and discarded as a technique because of the large number of steps involved in chromatographing the samples, scraping and eluting, and reducing the sample in volume prior to respotting on another chromatogram. Also inconsistent recoveries were obtained. Column chromatography using batch elution techniques seemed preferable.

### Methods and results

Attempts were made to imitate thin layer chromatography conditions in a column but these were unsuccessful due to retarded elution of gibberellins when extract was added. Attention was then turned to partition chromatography using water as the stationary phase on silica gel.

The gel, Silica Gel for Chromatography, M & B, was pre-washed in a large column using a gradient of methanol to chloroform. Thirty grams of silica gel was triturated with distilled water (100g silica gel :62.5 ml $H_2O$) then added to the columns in chloroform saturated with water and packed (Powell 1960).

Plate B.1 Chromatograph of fractions eluted from a silica gel column with mixtures of chloroform to methanol (see text for detail)


1%
10/1o
50/50
90/10
1/99

Air bubbles were removed by backflushing the column with chloroform. Samples and extracts were taken up in methanol and applied to the triturated silica gel in a round bottomed flask and the methanol was then removed under vacuum.

It was found that $GA_{4/7}$ were eluted with chloroform while $GA_3$ was eluted with 90:10 chloroform/methanol (Plate B.1). The behaviour of the column system was further tested by including 100 $\mu$g each of $GA_3$ and $GA_{4/7}$ with 5g pre- and post-anthesis extracts of Cape Currant. The results indicated that the effect of the extract was to increase the mobility of the gibberellins in the system. This was probably due to the presence of organic acid which would reduce the pH of the aqueous phase. It could also be due to the presence of methanol residues.

The behaviour of a polar gibberellin extracted from apricot seed was studied by using a graded series of chloroform to butanol mixtures. All activity was eluted with 50:50 chloroform/butanol, but due to difficulty encountered in removing all traces of butanol for bioassay purposes the technique was abandoned. Instead, a graded series of chloroform to methanol was tried. This was successful and all activity was eluted with 70:30 chloroform/methanol (Fig. B.1). A disadvantage was that this proportion of methanol tended to remove the stationary phase from the column.

Preliminary attempts were made to apply this system to the assay of extracts but the results were inconclusive. Due to lack of time the system was not fully exploited.

Fig. B.1 Silica gel partition chromatography of apricot extract

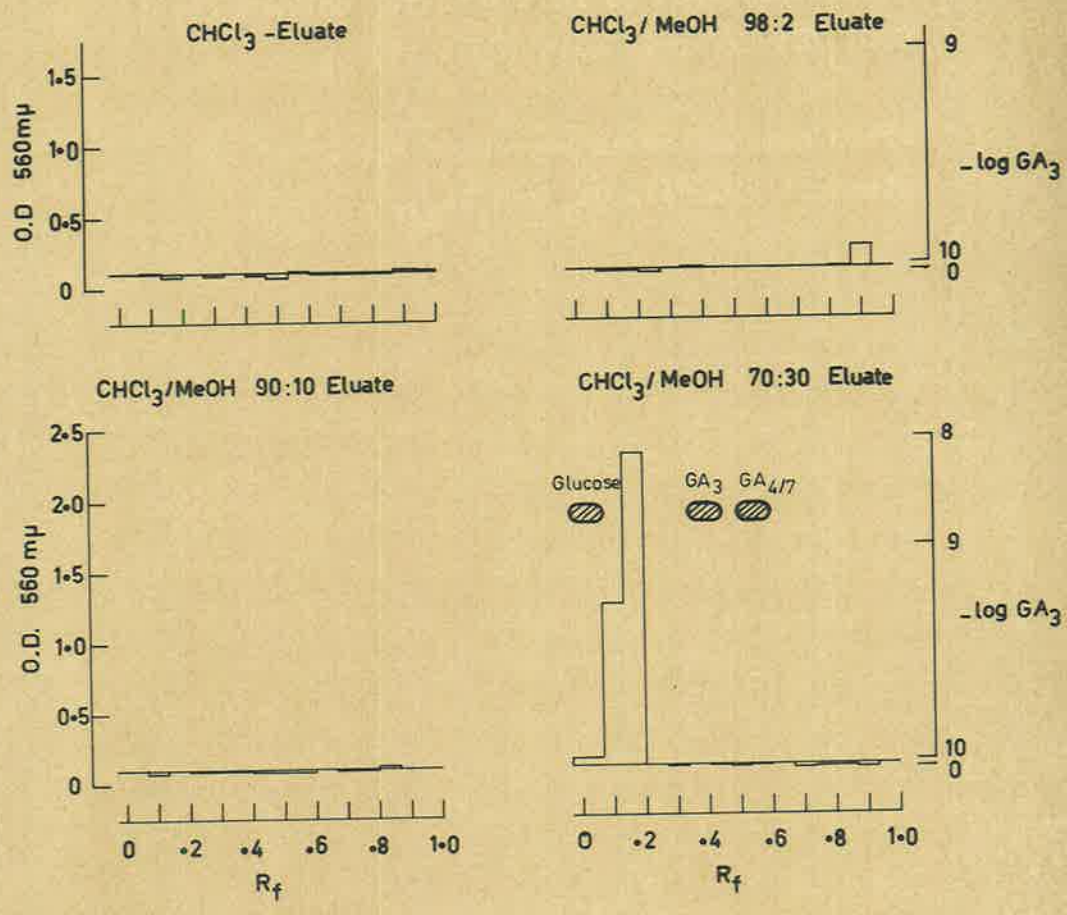
Thin layer chromatography on silica gel of the fractions eluted from a silica gel column prepared as in the text. Solvent $CHCl_3$/EtOH/Formic acid (85:15:1)

Cross hatched areas represent the Rf of standard glucose, $GA_3$ and $GA_{4/7}$

FIG. B1

SILICA GEL PARTITION CHROMATOGRAPHY

APRICOT EXTRACT



Conclusions

A system of column chromatography suitable for preliminary purification of large quantities of extracts was developed. Unconjugated gibberellins and abscisins (also auxins) were eluted together while the polar gibberellins could be eluted separately.

# APPENDIX C

## EXPERIMENTS RELATED TO THE GAS LIQUID CHROMATOGRAPHY OF PLANT HORMONES

(Some of this work was done in conjunction with Mr R.D. Firn)

### Introduction

Gas liquid chromatography (GLC) provides a means of quantitating small amounts of volatile substances. It is suitable for the assay of biologically active compounds because of its sensitivity and specificity. This in part overcomes the problem of relating activity in one biological system to activity in another. At best biological assay systems can be only regarded as being semi-quantitative unless elaborate precautions are taken with the methods.

Ikekawa *et al.* (1963) demonstrated the GLC of the methyl esters of $GA_{1-9}$. This work was extended by Cavell *et al.* (1967) to include a further eight gibberellins; these authors prepared the trimethyl silyl ethers of the methyl ester derivatives and the methods were then successfully applied to crude plant extracts. A further improvement in the technique was the combination of gas liquid chromatography with mass spectrography; this permits unequivocal identification of known compounds and provides an insight into the identity of unknown compounds (Pryce and MacMillan 1967 a & b; MacMillan 1967).

A number of authors have studied the techniques related to the GLC of indoles (Stowe and Schilke 1964; Powell 1964; De Moss and Gage 1965; Dedio and Zalik 1966; Koisumi *et al.* 1966; Grunwald *et al.* 1967; Brook *et al.* 1967), and several of these authors have applied the technique to the analysis of plant extracts (Powell *loc.cit.*; Dedio *et al.* *loc.cit.*; Koisumi *et al.* *loc.cit.*). The great sensitivity of this method was demonstrated

by Brook et al. (loc.cit.); they reported a response to $5 \times 10^{-8}$ g of methyl ester indole-3-acetic acid (IAA) when using a flame ionization detector, and $5 \times 10^{-11}$ g when using an electron capture detector.

An attempt was made to employ GLC techniques in a study of the hormones present in developing flower clusters of Vitis vinifera.

## Materials and Methods

A Shimadzu GC - 1C gas chromatograph fitted with dual flame ionization detectors was used. To facilitate use at high sensitivity the leads from the detectors to the electrometer were double shielded to reduce noise. Dual glass columns, 1875 mm long and 4 mm internal diameter provided with facilities for "on - column" injection of samples were fitted.

The chemicals used are listed below:

| | |
|---|---|
| SE-30 liquid phase | Applied Science Lab. |
| QF1 " " | " " " |
| Anachrom ABS, 80-90 mesh Column support material | |
| Hexamethyldisilazane (HMDS) | Wilkens |
| Trimethylchlorosilane (TMCS) | Applied Science Lab. |
| Carbitol (diethylene glycol monoethyl ether) | Ajax Chemicals |
| N-methyl-N-nitro-p-toluenesulphonamide "TONY" | Koch-light |
| Gibberellin $A_3$ (98%) | Merck |
| Gibberellin $A_{4/7}$ | ICI |
| Abscisic acid (1 part trans trans to 1 part ± cis trans ABA) | American Tobacco Co. |
| Indole-3-acetic acid | BDH |
| Indole acetonitrile | Light & Co. |

The glass columns were silanized by pouring hot (80°C) 1% HMDS in benzene through the clean dry columns. The solution was left in the columns for 3-4 minutes and then drained off. The column was then oven dried and the procedure repeated four times. Glass wool for packing the ends of the columns was similarly prepared.

To coat the support with QF-1 the following procedure was used: A 2% (w/v) solution of QF-1 in methyl ethyl ketone was prepared and 80ml was slurried with 20g of support material. The slurry was then thoroughly degassed, allowed to stand for five minutes and then filtered under reduced pressure in a Buchner filter until foaming had ceased. The support was then spread on filter paper and dried for four hours at 120°C. An acetone extract revealed 2.1% QF-1 (w/w). The same procedure was used for SE-30 except that toluene was used as the solvent.

To pack the support into the columns a glass wool plug was introduced near the injection port end of the column which was then connected to a vacuum pump. Support was added through a small funnel to the other end, and compaction was achieved by tapping the columns with a spatula. When full, a glass wool plug was introduced at the top of the column and the vacuum was then released gently.

The columns were conditioned overnight by passing a low flow of nitrogen ($N_2$) at a supra-optimal temperature (250°C for QF-1, 300°C for SE-30). Further conditioning was carried out in two ways: the first was to inject several 5 µl aliquots of HMDS on to the columns at a temperature of 120°C and a flow rate of 10ml $N_2$ per minute. The second method which was sometimes used in conjunction with the first was to overload the column with $GA_3$ at 200°C and 60ml $N_2$ per minute to ensure that any sites that would bind gibberellins

irreversibly would be filled (injected approximately 200µg $GA_3$ - methyl ester). After this operation the columns were again conditioned in the oven overnight, but at a lower temperature than was used in the initial conditioning (approximately 230°C).

Methylation was carried out with diazomethane produced by the method of Schlenk and Gellerman (1960) as modified by Powell (1964). Three rubber-stoppered tubes were connected in series with small bore teflon tubing; tube 1 contained methylene chloride, tube 2 contained the reaction mixture, and tube 3 contained the sample in 10% methanol in methylene chloride. The reaction mixture consisted of 2ml 60% KOH, 2ml carbitol and approximately 100mg "TONY" in 2ml methylene chloride which was added last. Prior to the addition of the "TONY" the nitrogen flow through tube 1 was adjusted so that when the "TONY" was added and tube 2 stoppered, the diazomethane produced was immediately removed to tube 3. This prevented any dangerous build up of diazomethane. As further safety precautions, the tubes were placed in a shielded perspex stand, and the whole process was carried out in a fume hood. Methylation was considered complete when the solution in tube 3 turned yellow. This was confirmed by TLC on silica gel G using di-isopropyl ether/acetic acid (95:5) as a solvent (MacMillan 1963).

The TMS ethers were prepared as described by Cavell et al. (1967). The dry sample was taken up in 0.1ml of redistilled dry pyridine and treated in a small capped vial (dry) with 0.1ml of a mixture consisting of pyridine, HMDS and TMCS in the ratio of 10:2:2. After standing for at least 10 minutes an aliquot of the sample was chromatographed. For small samples ($< 10\mu g$), the mixture was added directly to the dry sample.

The preparation of the plant extract was as follows: two lots of 4g d.wt. of Cape Currant berries collected two weeks post-anthesis were homogenised in 100ml of methanol, centrifuged and re-extracted twice. The combined supernatants were then dried down on to 25g washed dry silica gel (see Appendix B) and placed in 30cm x 2.5cm glass columns and eluted with 70:30 chloroform / methanol (300ml, fraction A) and then anhydrous MeOH (150ml, fraction B). Fraction A was then chromatographed on DEAE Sephadex as in section 3 (fraction $A_1$ = neutral; $A_2$ = weak acid; $A_3$ = strong acid). Fraction $A_3$ was taken up in water, pH adjusted to 3.0 and extracted with three equal volumes of peroxide-free redistilled ether (fraction $A_{3.1}$). This fraction and fraction B were further subjected to TLC on silica gel G (Skene 1967) in chloroform/ethyl acetate/formic acid (50:50:1).

The IAA used in this investigation was of horticultural quality and was purified by recrystallization prior to use. To effect recrystallization, 0.25g of the IAA was added to a few millilitres of chloroform and warmed on a hot plate. Methanol was added in sufficient quantity to dissolve the IAA. This process was carried out in a dark room illuminated with a green safe-light. After cooling, the solution was placed in the deep freeze overnight. The resultant faintly pink crystals were filtered off and rinsed with a small amount of chloroform. Chromatography in a number of systems on thin-layer cellulose gave only one spot when sprayed with a number of reagents (e.g. ferric chloride/ orthophosphoric acid reagent).

Results

Attempts were made to separate the three groups of compounds being studied, namely, auxins, abscisins and gibberellins. It was found that no one set of conditions was adequate. Further, under the conditions described by Ikekawa et al. (1963) or Cavell et al. (1967), different retention times were obtained. (This was probably due to an equipment idiosyncrasy since with the same support and liquid phase on another machine, a Packard, results comparable with those reported were obtained).

$GA_4$ and $GA_7$ could only be adequately separated if chromatographed on 2% QF-1, whilst recovery of $GA_3$ was low on this liquid phase (Figs C.1 and C.2). Adequate recovery of $GA_3$ was best achieved at high temperatures but at these temperatures the more volatile components (IAN, IAA, ABA) were difficult to distinguish from the solvent peak (Fig. C.3). Some of these difficulties were overcome by the use of linear temperature programming (Fig. C.4), however under these conditions recovery of methyl $GA_3$ was still poor. The use of trimethylsilyl ether derivatives of $GA_3$ to a large extent overcame this problem. It was not found necessary to use the TMS of the methyl ester as was used by Cavell et al. (loc.cit.). A further advantage of using the TMS of the free acid was the lability of the TMS group in water which permitted the bioassay of collected fractions. This was tested and found to be so although detailed studies were not made.[1]

1. Subsequent to this work a paper was published by Davis et al. (1968) on the preparation, gas liquid chromatography and collection of TMS ether derivatives of "Abscisic acid and other plant hormones".

It was found that under most conditions tried the cis trans (± ABA) and trans trans (t.ABA) stereo-isomers of ABA could be adequately separated. The identity of each peak was established by the use of (+) cis trans ABA (courtesy of F.T. Addicott). Difficulty was encountered with $GA_8$ under the GLC conditions used here.

A dilution bio-assay of fractions $A_{1,2,3}$ and B revealed inhibition in fraction $A_3$. On TLC and bioassay, the activity in $A_3$ was resolved into three zones of inhibition (Fig. C.5). The zone of inhibitive activity which coincided closely with the reported Rf of the inhibitor in grape sap (Skene 1967) and with the Rf of authentic ABA (Appendix A) was scraped off the remaining area of the plate, eluted with methanol and methylated. Chromatography of 0.1g d.wt. equiv. gave the chromatogram illustrated in fig. C.6. Though the chromatogram is complex, one of the resolved peaks coincided almost exactly in several runs with the retention time of standard ± ABA (Fig. C.4). A further two peaks coincided closely with known compounds and could possibly be IAA and IAN (both run close to ABA in the TLC solvent system used). While this information cannot be taken as an unequivocal identification of the compounds in question, the presence of a compound with a retention time similar to that of authentic ABA does support other information obtained in this study (Section III and Appendix A).

Conclusions

Methods were developed to chromatograph and separate on one GLC column a variety of acidic plant growth substances, namely auxins, abscisins and gibberellins, though better results may be achieved by the use of a separate method particularly when considering the more polar gibberellins. Trimethyl silyl ether derivatives were superior for the analysis of gibberellins and have the advantage of lability which

facilitates bioassay of chromatographed fractions after collection.

Biological activity, Rf on TLC and retention time on GLC suggest that inhibition in grape extracts is due at least in part to the presence of ABA.

Figure C.1 GLC of plant hormones with QF.1. as a stationary phase

| | | |
|---|---|---|
| Sample | - | methyl esters, 2 $\mu$g ABA (mixed isomers), 1 $\mu$g $GA_3$, and 2 $\mu$g $GA_{4/7}$ |
| Temperature | - | 223°C |
| Column | - | all glass - 1875 x 4 mm |
| Injection | - | on column |
| Stationary phase | - | 2% QF.1 |
| Support | - | Anachrom ABS, 80 - 90 mesh |
| Reference column | - | 2% SE 30 |
| Carrier gas | - | nitrogen |
| Flow rate | - | 105 ml/min atmos. |
| Inlet pressure | - | 0.8 kg/cm$^2$ |
| Hydrogen | | |
| Flow rate | - | 65 ml/min |
| Air pressure | - | 0.8 kg/cm$^2$ |
| Range | - | 12.8 m V |
| Sensitivity | - | $10^4$ |
| Detector temperature | - | 280°C |
| Chart speed | - | 2.5 mm/min |

Figure C.2 GLC of plant hormones with SE 30 as a stationary phase

| | | |
|---|---|---|
| Sample | – | methyl esters, 2 $\mu$g ABA (mixed isomers), 1 $\mu$g $GA_3$, 2 $\mu$g $GA_{4/7}$ |
| Temperature | – | 225°C |
| Column | – | all glass, 1876 x 4 mm |
| Injection | – | on column |
| Stationary phase | – | 1.5% SE 30 |
| Support | – | Anachrom ABS, 80 – 90 mesh |
| Reference column | – | 2% QF1. |
| Carrier gas | – | nitrogen |
| Flow rate | – | 90 ml/min atmos. |
| Inlet pressure | – | 0.7 kg/cm$^2$ |
| Hydrogen | | |
| Flow rate | – | 50 ml/min |
| Air pressure | – | 0.8 kg/cm$^2$ |
| Range | – | 3.2 m V |
| Sensitivity | – | $10^4$ |
| Detector temperature | – | 255°C |
| Chart speed | – | 2.5 mm/min |

FIG. C1

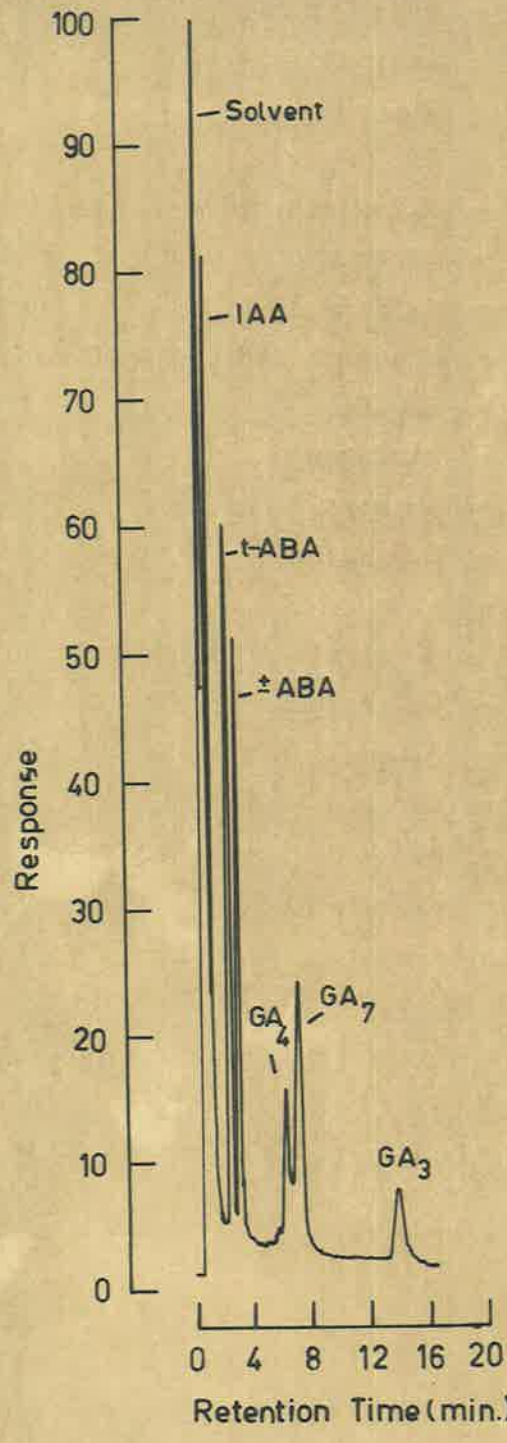

Response
100
90
80
70
60
50
40
30
20
10
0
Solvent
IAA
t-ABA
±ABA
GA4
GA7
GA3
0 4 8 12 16 20
Retention Time (min.)

FIG. C2


S
t-ABA
±ABA
GA4/7
GA3
0 4 8 12 16 20 24 28 32 34
Retention Time (min.)

Figure C.3 GLC of plant hormones: improved recovery of $GA_3$ at higher temperatures

| | | |
|---|---|---|
| Sample | - | methyl esters, 2 $\mu$g ABA (mixed isomers), 1 $\mu$g $GA_3$, 2 $\mu$g $GA_{4/7}$ |
| Temperature | - | 250°C |
| Column | - | all glass, 1875 x 4 mm |
| Injection | - | on column. |
| Stationary phase | - | 2% QF1 |
| Support | - | Anachrom ABS, 80 - 90 mesh |
| Reference column | - | 1.5% SE 30 |
| Carrier gas | - | nitrogen |
| Flow rate | - | 90 ml/min atmos. |
| Inlet pressure | - | 1.10 |
| Hydrogen | | |
| Flow rate | - | 65 ml/min |
| Air pressure | - | 0.8 kg/cm$^2$ |
| Range | - | 1.6 m V |
| Sensitivity | - | $10^4$ |
| Detector temperature | - | 280°C |
| Chart speed | - | 5 mm/min |

FIG. C3

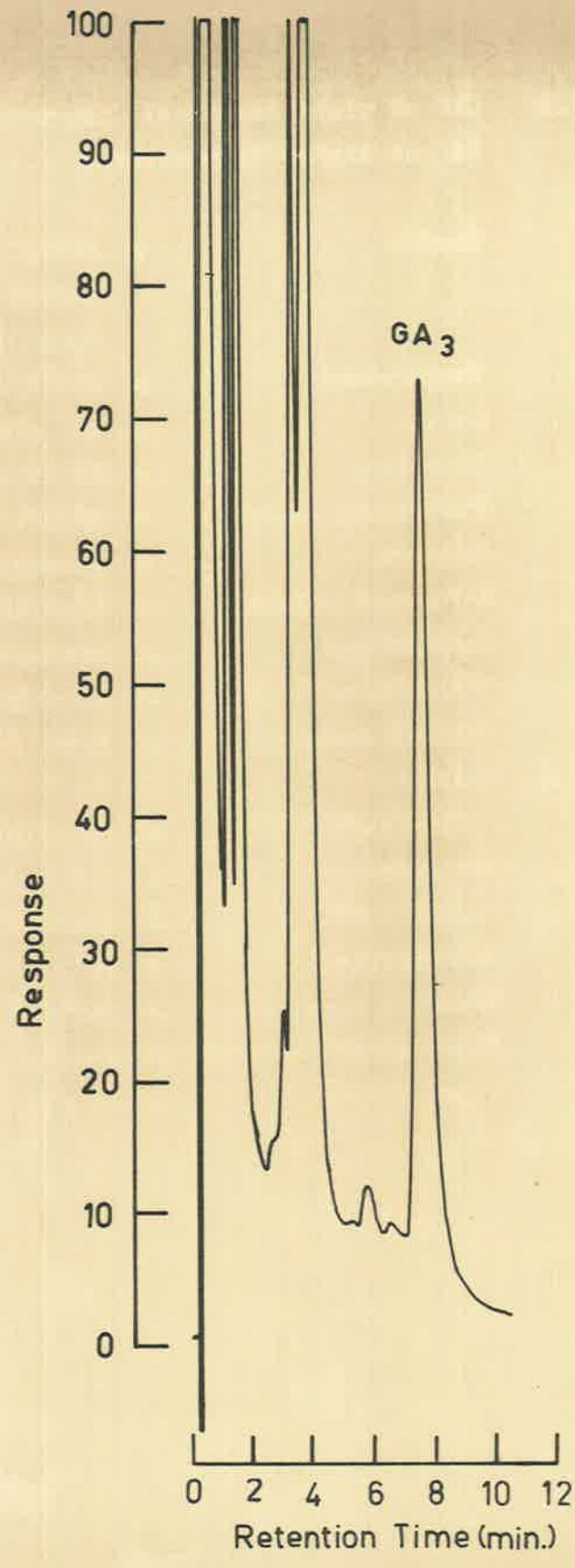

100
90
80
70
60
50
40
30
20
10
0
Response
GA 3
0
2
4
6
8
10
12
Retention Time (min.)

Figure C.4 GLC of plant hormones: the influence of temperature programming.

| | | |
|---|---|---|
| Sample | - | methyl esters, 2 $\mu$g ABA (mixed isomers), 1 $\mu$g $GA_3$, 2 $\mu$g $GA_{4/7}$ |
| Temperature | - | 6 minutes at 100°C, then 20° increase per minute to 250°C |
| Column | - | all glass, 1875 x 4 mm |
| Injection | - | on column |
| Stationary phase | - | 2% QF1 |
| Support | - | Anachrom ABS, 80 - 90 mesh |
| Reference column | - | 1.5% SE 30 |
| Carrier gas | - | nitrogen |
| Flow rate | - | 95 ml/min atmos. |
| Inlet pressure | - | 0.8 kg/cm$^2$ |
| Hydrogen | | |
| Flow rate | - | 55 ml/min |
| Air pressure | - | 0.8 kg/cm$^2$ |
| Range | - | 3.2 m V |
| Detector temperature | - | 280°C |
| Chart speed | - | 2.5 mm/min |

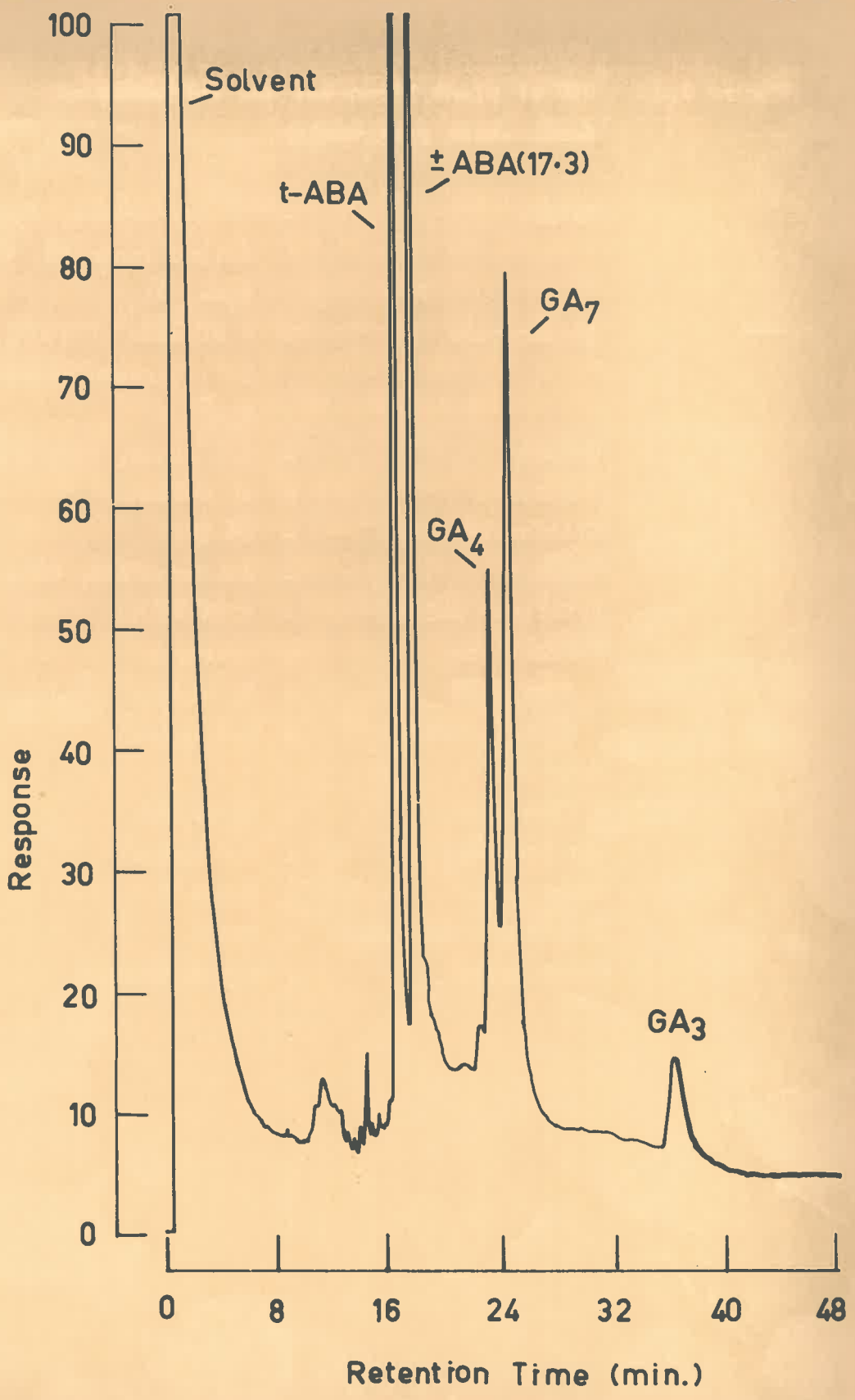

100
90
80
70
60
50
40
30
20
10
0
Response
Solvent
t-ABA
± ABA(17·3)
GA7
GA4
GA3
0
8
16
24
32
40
48
Retention Time (min.)

Figure C.5 Assay for inhibitors in post-anthesis Cape Currant extract

0.5 g d.wt. equivalent of Cape Currant extract, prepared as in text was spotted on silica gel G and chromatographed in a solvent of composition 50:50:1 chloroform : ethyl acetate: formic acid. Assay for the presence of inhibitors was carried out in the presence of $4 \times 10^{-8}$ g $GA_3$.

NOTE: The inhibition at the front of the chromatogram may have interferred with the sugar assay since there were no measurable sugars, although there is normally a small background level of sugars even in the absence of GA.

FIG. C.5.

CAPE CURRANT: 0•5g equiv. of post-anthesis extract
Assay for inhibitors

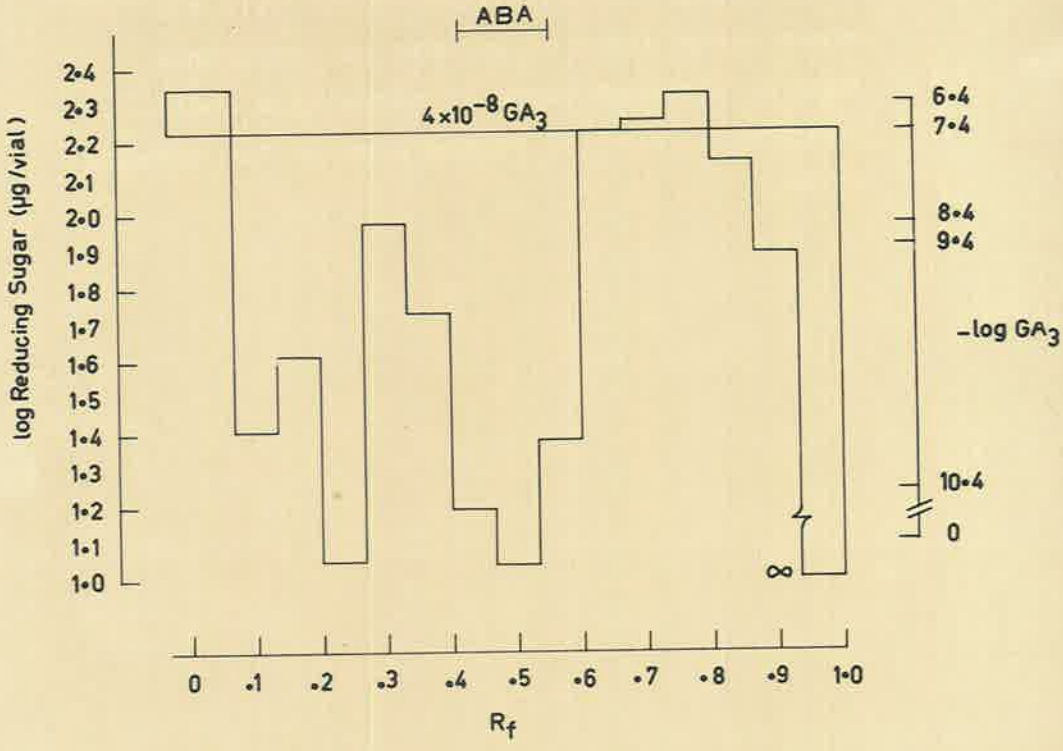
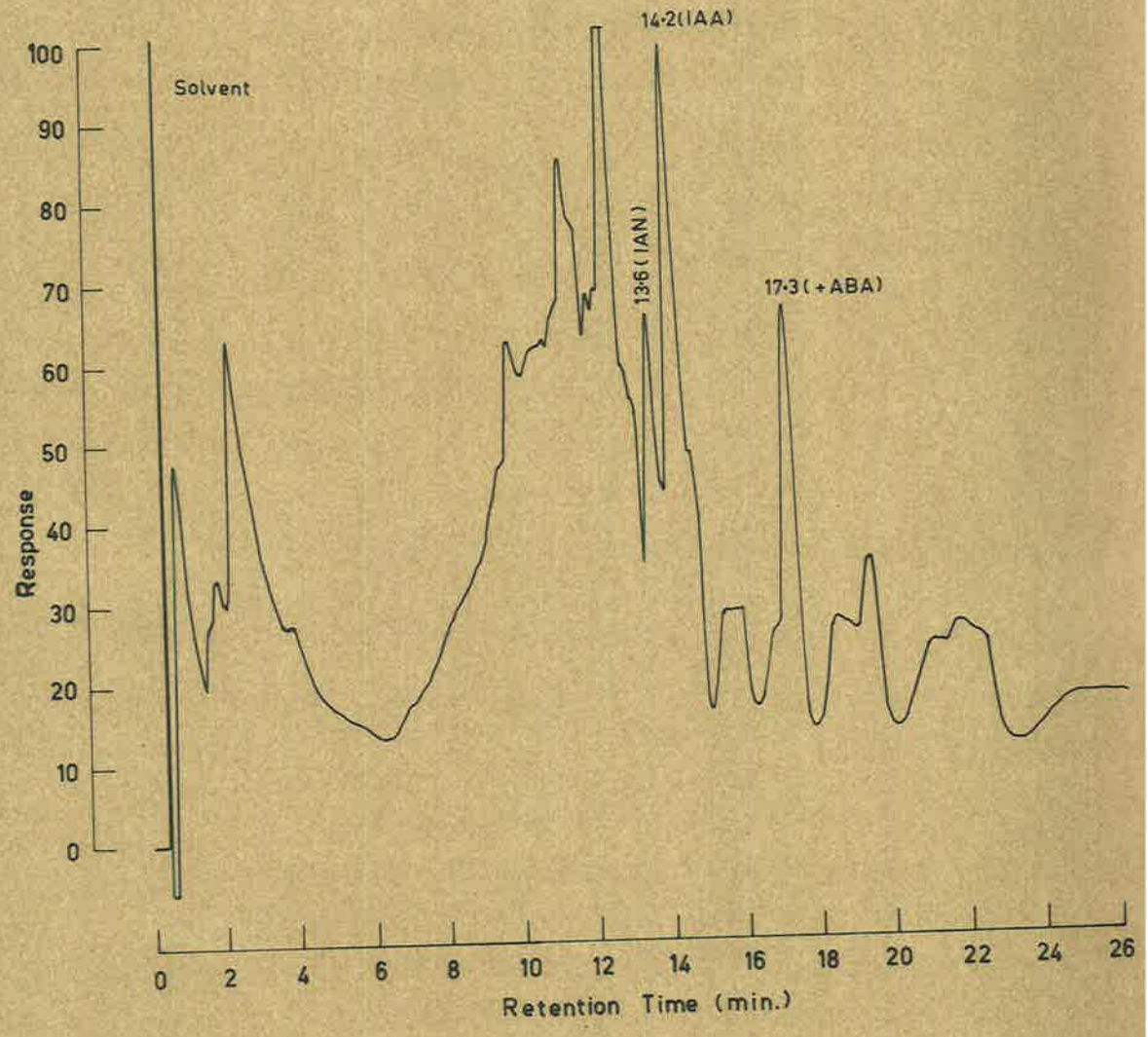


Figure C.6 GLC of inhibitive TLC zone of Cape Currant extract that corresponded to the Rf of authentic (+) ABA

0.1 g d.wt. equivalent of post-anthesis Cape Currant extract prepared as in the text was injected in methylene chloride.

Conditions of chromatography were as for figure C.4 except that the range used was 6.4 m V and the chart speed was 10 mm/min.

FIG. C. 6.


14·2(IAA)
Solvent
13·6 (IAN)
17·3 (+ABA)
Response
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Retention Time (min.)

# APPENDIX D

## EXPERIMENTS RELATING TO THE DETECTION OF GROWTH SUBSTANCES ON CHROMATOGRAMS

### Introduction

A summary of detection methods for gibberellins reveals that it is possible using spectrophotofluorimetry to distinguish between four groups of gibberellins. It is theoretically possible to distinguish between gibberellins 1-9 using the spray reagents listed(Paleg 1965). However the latter methods were limited to silica gel chromatograms and so a preliminary survey was undertaken of a wider range of reagents to see if plant hormones could be detected with equal sensitivity on paper and whether more specificity could be introduced.

### Materials and Methods

The reagents used were as follows:

1. Folin; BDH, applied and then placed in an oven at 80°C for 15 min.
2. Ethanolic sulphuric acid, 95:5(Jones *et al.* 1963); applied and examined immediately.
3. $FeCl_3/H_3PO_4$(Glick *et al.* 1964); a stable stock solution of 2.5% (w/v) $FeCl_3$ in 86% $H_3PO_4$ was made up. When required 0.8ml of this reagent was made up to 10ml with conc. $H_2SO_4$. For application to chromatograms this reagent was diluted with ethanol(95:5 v/v).

4. Ascorbic acid/HCl/$H_2O_2$(Jelliffe 1967); 20mg of ascorbic acid was dissolved in a mixture of 19ml methanol and 30ml conc. HCl. To this 2.1$\mu$l of fresh 30% aqueous $H_2O_2$ was added. The solution was then mixed, applied thoroughly and allowed to dry in the dark for 50 min. before examination.
5. Alkaline blue tetrazolium(Bush 1963); qualitative reagent: Solution I, 0.05%(w/v) blue tetrazolium (3,3-dianisolebis 4,4- (3,5-diphenyl)- tetrazolium chloride) in ethanol.
   Solution II, 2N NaOH. Immediately prior to use 1 volume of solution I was mixed with 4 volumes of solution II. The mixed reagents were applied, the chromatogram was then left at room temperature in light for 1hr and dried before examination(see also Bush 1961). A quantitative version is described by Bush(1963).
6. NaOH(Bush 1961); 2N NaOH(carbonate free) was applied to the chromatogram which was then left at room temperature for 1hr in light and dried before examination.
7. Trichloroacetic acid(Bush 1961); 20-33%(w/v) trichloroacetic acid in ethanol(or chloroform) was prepared. The chromatogram was dipped in the reagent and then dried at 80-100°C for 20 min.
8. Antimony trichloride($SbCl_3$) (Kagawa et al.1963); a 30%(w/v) solution of $SbCl_3$ in chloroform was applied to the chromatogram. The chromatogram was then placed in an oven at 120°C for 10 min. before examination.

9. $FeCl_3/HClO_4$ (Gordon and Weber 1951); the reagent was prepared by adding 5.4g $FeCl_3$ to 2 litres of 35% $HClO_4$. This solution was diluted and applied directly to the chromatogram.

10. Dimedone (Vurck and Pegram 1966); a solution of 0.25g dimedone (5,5-dimethylcyclohexane 1,3-dione) in orthophosphoric acid was applied to the chromatogram, which was then heated at 110°C for 10 min. prior to examination.

11. Sulphanilic acid - diazotised;
Solution A, 1 vol. of 9g sulphanilic acid in 900ml distilled water plus 90ml conc. HCl.
1 vol. sodium nitrite, 5% w/v in water.
Solution B, sodium carbonate (anhyd), 10% w/v in water.
Solution A was sprayed onto the chromatogram. Immediately following this solution B was applied by spraying. The chromatograms were examined immediately.

5 micrograms of the following compounds were applied as spots onto pieces of Whatman No, 3 chromatography paper: IAA (recrystallised), ABA (American Tobacco Co.), phloretic acid, glucose, $GA_3$, $GA_{4/7}$.

Results

See Table D.1

Reagent 3 was found to be sensitive; it was possible to detect 0.1$\mu$g of $GA_3$ and $GA_{4/7}$ on either paper or silica gel and the limit for detection of IAA on both chromedia lay between 0.1 and 1.0$\mu$g while 1$\mu$g of ABA could be detected on silica gel though larger quantities seemed necessary for detection on paper.

Results

| Reagent | Compound | Colour | | |
|---|---|---|---|---|
| | | White light | Long λ UV | Short λ UV |
| 1. Folin | IAA | Purple (Pink before heating) | Absorb - blue black | Absorb - violet |
| | ABA | - | - | - |
| | PA | - | - | - |
| | Gluc | - | - | - |
| | $GA_3$ | Blue - green | Blue - grey | - |
| | $GA_{4/7}$ | " " | " " | - |
| 2. Ethanolic $/H_2SO_4$ 95% | IAA | - | Green yellow fl. | - |
| | ABA | - | Green fl. marked | Absorb - blue purple |
| | PA | - | - | " " |
| | Gluc | - | Yellowish fringe fl. | " " |
| | $GA_3$ | - | Yellow fl. | Green fl. |
| | $GA_{4/7}$ | Light green | " " | Yellow green fl. |
| 3. $FeCl_3$ $H_3PO_4$ | IAA | Magenta | Orange fl. | Orange fl. |
| | ABA | Yellow | Green fl. | Green fl. |
| | PA | - | - | - |
| | Gluc | - | - | - |
| | $GA_3$ | - | Bright yellow fl. | gr. yellowish fl. |
| | $GA_{4/7}$ | Green | Orange | yellow fl. |

| Reagent | Compound | Colour | | |
|---|---|---|---|---|
| | | White light | Long λ UV | Short λ UV |
| 4. Asc rbic acid/HCl/ $H_2O_2$ | IAA | Faint pink | Lt. blue fl. | Lt. blue fl. |
| | ABA | - | Faint yellow green | - |
| | PA | - | - | - |
| | Gluc | - | - | - |
| | $GA_3$ | - | Yellow green | Lt. blue |
| | $GA_{4/7}$ | - | Yellow | Blue Yellow |
| 5. Alkaline blue tetra- zolium | IAA | Light pink | Blue fl. | Blue fl. |
| | ABA | Faint yellow | Green | Faint purple absorb |
| | PA | - | - | " " " |
| | Gluc | - | - | - |
| | $GA_3$ | - | - | - |
| | $GA_{4/7}$ | - | Faint blue fl. | Faint blue fl. |
| 6. NaOH | IAA | - | Lt. blue fl. | Blue fl. |
| | ABA | Yellow after UV exposure | Yellow gr. fl. | Gr. yellow centre fl purple absorb |
| | PA | - | - | Purple absorb |
| | Gluc | - | - | " " |
| | $GA_3$ | - | - | Mauve absorb |
| | $GA_{4/7}$ | - | - | - |

| Reagent | Compound | Colour | | |
|---|---|---|---|---|
| | | White light | Long λUV | Short λUV |
| 7. Trichloro-acetic acid | IAA | Brown yellow | Orange fl. | Orange yellow fl. |
| | ABA | - | - | - |
| | PA | - | - | - |
| | Gluc | - | Bright orange fringe fl. | Faint yellow fring |
| | $GA_3$ | - | " " | " " " |
| | $GA_{4/7}$ | - | Blue green fl. | Green fl. |
| 8. Antimony tri-chloride | IAA | Br. - grey | Absorb dark on orange back-ground | Dark absorb |
| | ABA | Greyish | Tend green | " " |
| | PA | " | dark orange | " " |
| | Gluc | Dark grey | " " | " " |
| | $GA_3$ | " " | " " | " " |
| | $GA_{4/7}$ | Dark blue | " " | " " |
| 9. $FeCl_3$ $HClO_4$ | IAA | Pink (yellow background) | Dark red absorb (green back-ground ) | - |
| | ABA | - | - | Blue absorb |
| | PA | - | - | - |
| | Gluc | - | - | - |
| | $GA_3$ | - | - | - |
| | $GA_{4/7}$ | - | - | - |

| Reagent | Compound | Colour | | |
|---|---|---|---|---|
| | | White light | Long $\lambda$UV | Short $\lambda$UV |
| 10. Dimedone 110°C for 10 min. | IAA | . | . | . |
| | ABA | . | . | . |
| | PA | . | . | . |
| | Gluc | Yellow | - | - |
| | $GA_3$ | - | Dark green | fl. dark green |
| | $GA_{4/7}$ | - | Intense bright mauve | Bluish |
| 11. Sulphanilic Diaxotised | IAA | - | - | - |
| | ABA | - | - | - |
| | PA | Purple | Purple absorb | Purple absorb |
| | Gluc | - | - | - |
| | $GA_3$ | - | - | - |
| | $GA_{4/7}$ | - | - | - |

NOTE

- : no observable reaction
- . : not tried
- PA : phloretic acid
- Gluc : glucose
- fl. : fluoresence

Discussion and Conclusions

It is clear that the plant hormones examined (gibberellins, abscisins and auxins) can be detected with a wider range of compounds than was previously thought to be the case(Paleg 1965). Two reagents appear to be particularly sensitive: ferric chloride/phosphoric acid(3) and dimedone(10).

In practice detection of substances on thin layer chromatograms is generally more sensitive than on paper because of the diffuseness of the spots on the latter; however a number of reagents appeared to be suitable for detecting substances on paper chromatograms viz. $FeCl_3/H_3PO_4$, dimedone, ascorbic acid/HCl/$H_2O_2$.

Many of the reagents tested are used for the detection of steroids and the testing of other steroid detecting reagents may be worthwhile, particularly as some of these are specific for steroids possessing a particular side group. For example the alkaline reagents tested are specific for $\Delta^4$ 3 keto steroids, while isonicotinic hydrazide(not tested) is specific for $\Delta^4$ and $\Delta^{1-4}$ 3 keto steroids(Bush 1961).

Although analyses of the fluorescence spectra induced by these reagents were not carried out, it is felt that such analyses could yield useful information on the identity of unknown compounds, particularly if used in conjunction with established reagents.

However a wider range of plant compounds would need to be tested before these reagents could be recommended for general use on either a qualitative or quantitative basis.

## APPENDIX E

## ANATOMICAL STUDIES OF OVULE DEVELOPMENT IN CAPE CURRANT

### Introduction

Tunn Currant is thought to be synonymous with the Cape Currant of South African origin(Boehm and and Tulloch 1967; Antcliff pers.comm.; Coombe pers.comm.), although no direct comparison has been made of the two cultivars. There may be two variants in Australia differing mainly in vigour but with similar cluster and leaf morphology. The material used for these studies was the less vigorous variant known as Tunn Currant in South Australia. The vigour of Cape Currant as described by Perold(1927) seems similar to that of the variant grown at the CSIRO Horticultural Research Station, Merbein. Perold(loc.cit.) describes Cape Currant as, "purely and simply a Red Muscadel, in which total Millerandage has become hereditary".

Anatomical studies made on such seedless cultivars as Zante and Sultana(Pearson 1932; Stout 1936) have revealed that ovule development was in one or more respects imperfect. It was thought important to study the ovule development of Cape to obtain information on the source of parthenocarpy and thus to provide background knowledge for the interpretation of the results in Sections II and III.

### Materials and methods

Samples of flowers and developing ovaries were taken at the same time that samples were collected for the examination of endogenous growth regulators(see Section III).

These samples were placed in FAA solution until they were embedded for examination. Dehydration was carried out in a tertiary butanol series as per Johansen(1940). Infiltration with histological grade wax(MP 56°C) was also carried out by the method of Johansen (loc.cit.), but because of difficulty in obtaining serial sections with this wax, the wax was changed to a commercial preparation of paraffin wax(MP 60°C) containing ceresin. Sections(12μ) were cut on a rotary microtome and stained with safranin and fast green and then mounted in Canada balsam.

Results

Sections from samples collected 2-3 weeks pre-anthesis show that the development of the ovule and associated tissues was apparently normal. The nucellus and the inner and outer integuments were at an early stage of development with only approximately three layers of cells being apparent in the nucellus(Plates E.1 & E.2). The megaspore mother cell was present; Plate E.2 displays what appears to be a division of this cell. Plate E.3 shows the presence of pollen tetrads which have probably formed from recent reductive divisions of the microspore mother cells.

By one week before anthesis development of the ovule was substantially complete although in some the nucellus had not yet attained its full size.

Deficiencies apparent in samples collected one week prior to anthesis were even more obvious in the anthesis samples. In the majority of cases the embryo sac had failed to develop(Plates E.4 & E.5), though in a few it developed normally(Plates E.6 & E.7). Insufficient samples were examined to determine accurately the relative proportion of incompletely developed ovules but there may be more than half. The embryo sac appeared to be replaced by files of elongated cells(Plate E.5). The source of these cells is unknown and no transitionary stages between the division of the megaspore mother cell(Plate E.2) and the formation of these cells was noted. Only one pollen tube was noted(Plate E.7).

Examination of samples taken at fruit set two weeks after anthesis showed in general little further development of the ovule(Plate E.8), but in two of the ovaries examined(CCC treated) the nucellus had expanded abnormally and burst through the integuments(Plate E.9). Insufficient samples were examined to establish whether this was an effect of CCC.

Discussion

Seedlessness of Cape is apparently related to the failure of embryo sac development. A similar situation occurs in Zante although in this cultivar embryo sac development apparently proceeds a little further than in Cape(Pearson 1932). It is apparent from Plates E.6 and E.7 that the megaspore mother cell is differentiated and does undergo at least one reductive division, and from plates E.6 and E.7 that in some instances megaspore formation can proceed to completion.

The presence of *columnar* cells in the position normally taken by the embryo sac has not been described before in the grape. In other parthenocarpic cultivars, either the embryo sac develops but the nuclei degenerate(Zante) or, as in White Corinth, it is replaced by a thin line of dead cells.

The possibility that pollen viability or compatibility may be limiting has not been studied for Cape, nor is it known whether pollination is necessary for set, but seeded berries do occasionally develop and a pollen tube was noticed in one section(Plate E.7). Formation of abundant pollen appears to proceed normally. However large numbers of pollen tubes such as has been described for Zante by Pearson(1932) were not observed within the ovaries.

Conclusions

Parthenocarpy in Cape is apparently generally obligate and is associated with defective embryo sac formation.

Plate E.1

L.S. developing ovule, 2-3 weeks pre-anthesis(phase contrast, x160).

Plate E.2

L.S. developing ovule, 2-3 weeks pre-anthesis(transmitted light x160).

Plate E.3

Anther, L.S., 2-3 weeks pre-anthesis showing pollen tetrads(phase contrast, x160).

Abbreviations:

c = calotta; c.p. = chalazal pad; e.c. = elongated cells; e.s. = embryo sac; f = funiculus; i.t. = inner integument; m = micropyle; m.m.c. = megaspore mother cell; m.s. = megaspore; n = nucellus; o.t. = outer integument; p.t. = pollen tube; p.tet. = pollen tetrads.

PLATE E.1

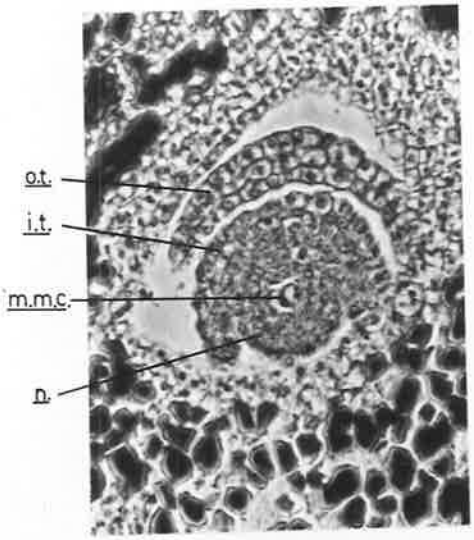



PLATE E.2

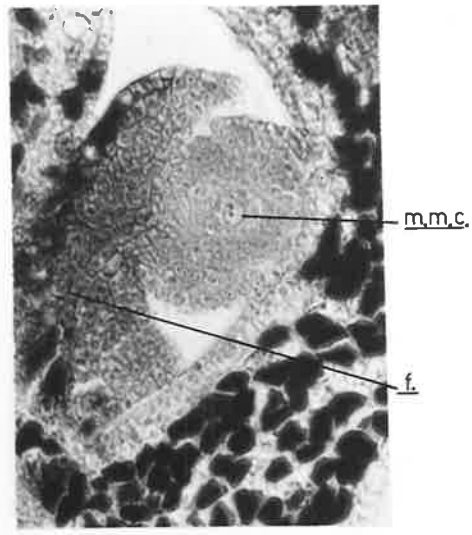



PLATE E.3

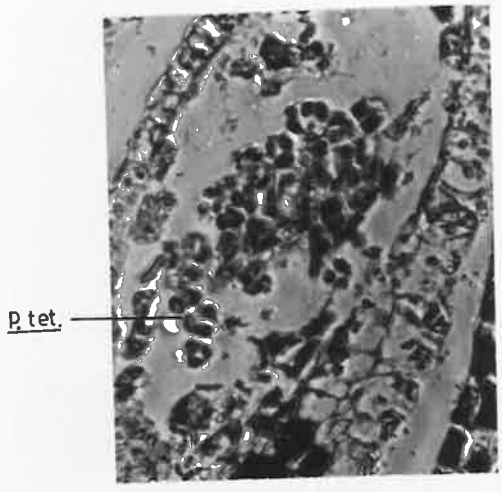


Plate E.4

T.S. of ovary at anthesis showing the absence of the embryo sac(phase contrast, x160).

Plate E.5

L.S. of ovary at anthesis showing the absence of the embryo sac(phase contrast, x160).

Plate E.6

T.S. of an ovule at anthesis with a fully developed embryo sac(phase contrast, x160).

Plate E.7

L.S. of the micropylar end of a fully developed ovule at anthesis demonstrating the presence of an egg nucleus(phase contrast, x320).

PLATE E.4

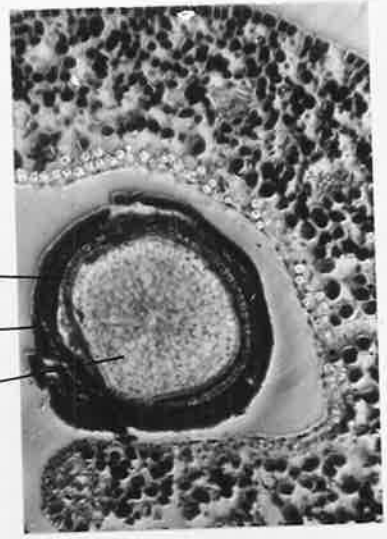



PLATE E.5

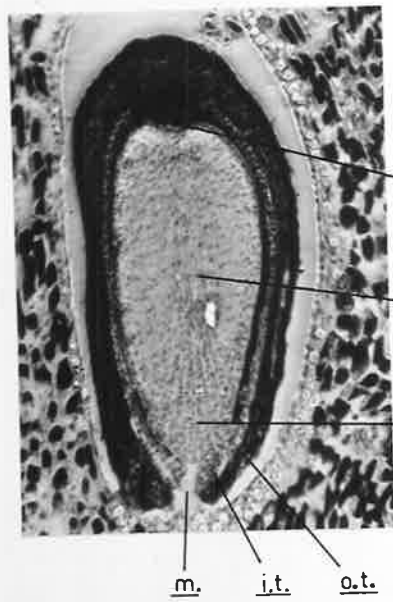



PLATE E.6

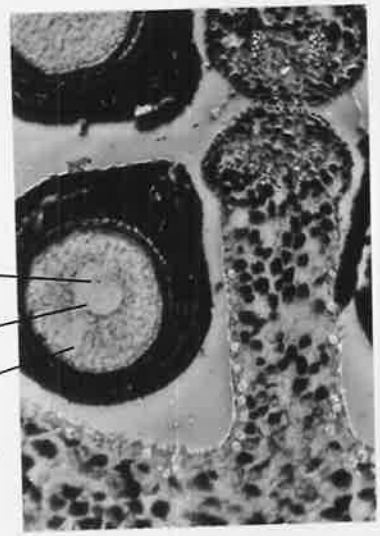



PLATE E.7

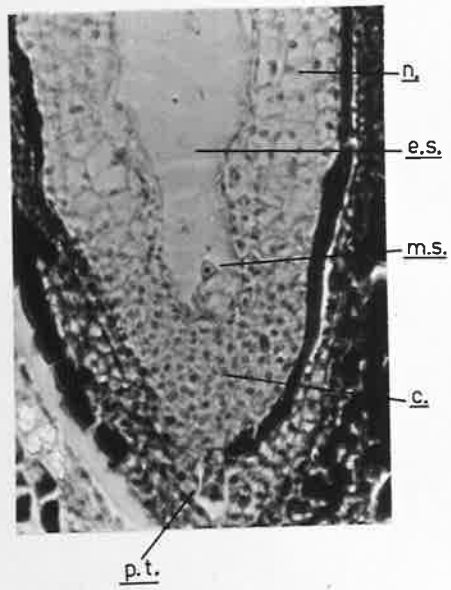


Plate E.8

L.S. of an ovary sampled approximately 2 weeks post-anthesis. Note the lack of further development; c f. plate E.5(phase contrast, x160).

Plate E.9

T.S. of an ovary sampled approximately 2 weeks after anthesis, demonstrating the expansion of the nucellus without a concomitant development of the integuments(phase contrast, x160).

PLATE E.8

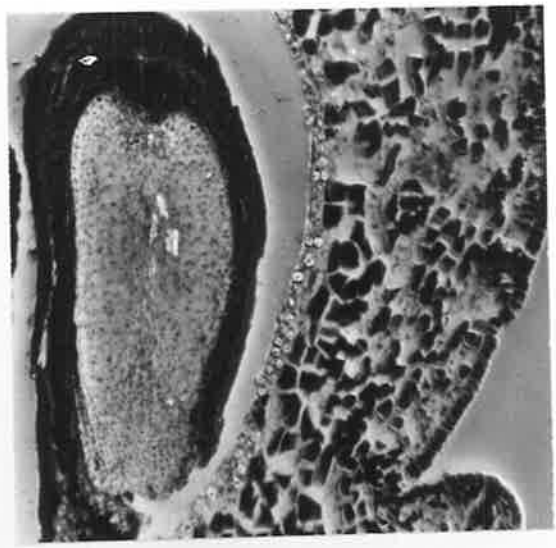
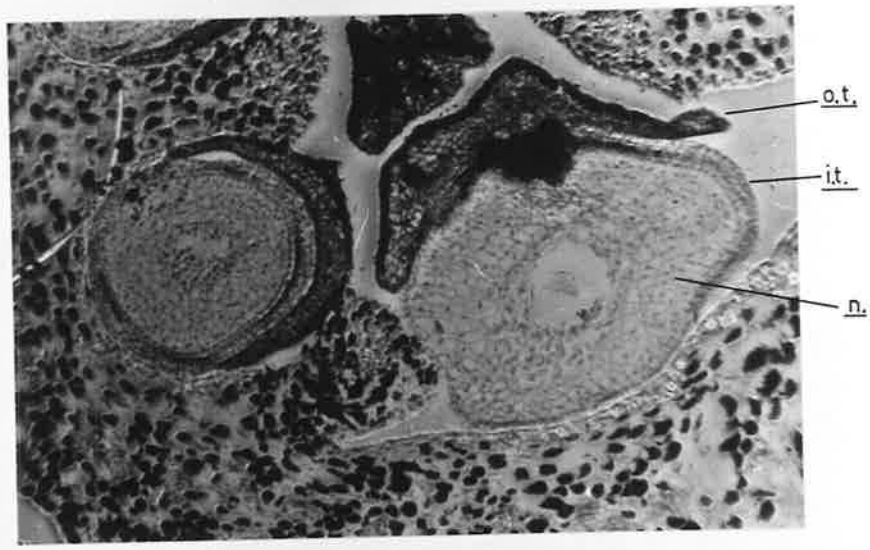


PLATE E.9



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