HORMONAL RELATIONS IN FRUIT GROWTH AND DEVELOPMENT OF *COFFEA ARABICA* L. IN KENYA

BY

W I L S O N R E U B E N O P I L E

A thesis submitted for the Degree of
Doctor of Philosophy in the University
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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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DECLARATION

We hereby declare that this thesis has been submitted for examination with our approval as University Supervisors.

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C O N T E N T S

SUMMARY

The purpose of this study was to examine the role played by hormones in fruit growth and development in order to find out how best they could be utilized to improve crop yield and quality.

1. Endogenous Hormones

Activities of abscisic (ABA)-like substances and gibberellin-like substances (GLS) in the extracts of Arabica coffee fruits, were followed throughout the growth pericd. Levels of the ABA-like substances showed more or less a bimodal type of distribution. The ABA-like substances concentration was substantial in the "pinheads" (0-9 week old fruits), declined during the endosperm formation (expansion stage), rose again when mature fruits were beginning to ripe, and declined again in ripe fruits. Abscisic acid-like substances gave a slightly different retention time from cis-trans ABA on the GLC. The GLS activity was low in the "pinheads", rose rapidly as the fruits expanded and a maximum level was detected in fully expanded fruits. At later stages the GLS levels decreased.

The distribution of the cytokinin-like substances (CLS) also appeared to be bimodal with the first peak appearing when the fruits began to expand rapidly and the second was detectable in ripening fruits. The levels of the CLS

were low in the "pinhead", at the endosperm (seed) formation stage and in mature fruits.

Changes in the levels of endogenous growth hormones during the fruit development appeared to be closely linked to various developmental stages of coffee fruits. This finding could help in making effective use of externally applied hormones.

2. Effects of GA_3 and Kinetin on Fruit Growth, Bean Weight, Bean Quality and Yield

The fruits treated with ethanolic GA_3 at the early stages of fruit growth between 4 and 8 weeks from the time of anthesis had their growth rates increased and eventually they were about 20% bigger than the untreated controls.

Endosperm dry weight was increased by about 25% by the ethanolic GA_3 micro-drops, particularly when applied at the "pinheads" stage. GA_3 at 25, 50 and 100 ppm (a.i.) in water applied two to four times to whole trees bearing 4 to 10 week-old fruits increased bean dry weight by 6-10% and grade-A beans by 13-23%. The overall quality of beans treated with the aqueous sprays of the GA_3 increased slightly.

Kinetin alone had very little effect on the rate of fruit enlargement. However, it was effective in increasing the bean dry weight when mixed with GA_3.

Foliar sprays of GA_3 at 100 ppm applied to mature trees three times during the rainy season between February and April or March and May, 1975, at various altitude sites increased yield of the following year's crop by 12-26%. Size, liquor and appearance of beans were not affected.

It was concluded that growers might benefit from the apparent improvement in bean quality which was probably due to the chemical's ability to increase bean dry weight and/or proportion of grade A beans. However, it has been suggested that more work is needed before GA_3 can be applied commercially. This is to find out very precisely the cause of variation in its response at different ecological zones, and economic viability as efforts to have the desired effect at a low concentration (50 ppm) were not promising.

3. Regulation of Ripening

The effect of (2-chloroethyl) phosphonic acid (CEPA) on ripening varied according to the stage of fruit development at which it was applied.

(2-chloroethyl) phosphonic acid at 1400 ppm a.i. (2.9 ml of the 48% a.i. CEPA/litre of water) appeared to be the optimum concentration needed for the acceleration of ripening. The 75% fruit maturity (about 27 week old) proved to be ideal for CEPA application as this had no adverse effect on the

quality. Used in this way, CEPA brought forward ripening and enabled about 40% or more of the crop to be harvested within 3 weeks. However, if cropping was not uniform and berries of various stages were present on the tree, young expanding fruits always abscised as a result of CEPA application.

(2-chloroethyl)phosphonic acid at 1400 ppm sprayed five days before anthesis made 40% of the expanding flower buds to abscise. Thus CEPA could be used also as a thinning agent in helping to remove expanding flower buds and bring controlled flowering. This could be one way to prevent overbearing of young trees in the first year of cropping and the other by removing part of young expanding berries by spraying CEPA. Internode lengths of primaries (1st order branches) were reduced by CEPA sprays but node production was not affected.

Ripening was significantly correlated with the age of fruits and also with the concentration of chemical used. As the fruit maturity advanced, the percentage of fruits ripened increased while unwanted abscission decreased. The altitude at which trees were grown altered the extent to which ripening was accelerated but not the amount of abscission.

Fruits on the two and three year old trees ripened faster when treated with CEPA at either 700 ppm or

1400 ppm than those on 4-year-old trees. Such a response was probably due to differences in crop foliage ratio on the trees. (2-chloroethyl) phosphonic acid (CEPA) at 1400 ppm, was effective in causing fruit ripening irrespective of volume of water used per tree. Defoliation of the tree did not affect the rate at which CEPA normally accelerated ripening.

(2-chloroethyl)phosphonic acid at 1400 ppm sprayed onto leaves only (fruits covered by paper bags) promoted fruit ripening, indicating that CEPA is mobile in coffee. However, the fruits hit by sprays directly (leaves covered) ripened faster than fruits sprayed together with leaves at the same time, implying that CEPA accumulates on treated fruit/and thus promotes fast ripening. /s

Sprayed at concentrations ranging from 1-200 ppm in December, when the main crop was ripening, Naphthaleneacetic acidø (NAA) reduced the leaf abscission induced by CEPA sprayed at 1400 ppm by 15-20%. After spraying with triiodobenzoic acid (TIBA) at 10 ppm 35% of the ripe cherries on trees could be shaken off compared with 28% for trees sprayed with CEPA at 1400 ppm. The number of cherries falling was increased to 50% when concentration was increased to 50 ppm. In this case many of the fallen fruits had fully developed abscission layers. It has been suggested that with more work it may be possible to employ these chemicals to assist in mechanical

harvesting.

C H A P T E R I

GENERAL INTRODUCTION

1.1 Background

In Kenya, Arabica coffee (Coffea arabica L.) is the major cash crop for export. The endospermous seeds(also known as bean) obtained from the fruits are the ones marketed. Coffee contributes about 25% of the farm revenue and about 30% of the total export revenue. There are about 120,000 hectares of land under coffee in Kenya and this takes up a considerable proportion of land between 1450 to 1900 m altitude with over 90 cm annual rainfall, where most of the people live on subsistence farming.

One important criterion of good quality coffee is the bean size. Thus 'mild' Arabica coffee beans, large enough to be retained by 6.75 mm sieve (Wallis, 1967), produced in East Africa, Colombia and some parts of Central America command a high price in the European markets. In Kenya bean size and quality vary from year to year apart from the quantity produced each year, and depend on the environmental conditions under which trees have been growing. Thus farmers have little control over most of the conditions that determine fruit growth resulting in a particular bean size. As a result, growers are usually unable to predict their income and sometimes incur losses. It would therefore be of a great value if a way is found to enable coffee growers to

regulate fruit growth and development, in order to achieve the desired size of beans.

The environmental and cultural practices that influence the cropping level and quality of Arabica coffee have been described (Wormer and Gituanja,1970; Cannell, 1971a; 1973, 1974) and some work employing hormones for changing the fruiting pattern (Cannell, 1971 b) and regulating ripening (Browning and Cannell, 1970) has been done. More information, however, is needed on hormonal factors that regulate fruit growth and ripening.

Fruit growth and bean size are greatly influenced by irrigation, mulching, pruning and probably by fertilizer application. For any Arabica coffee genotype, as is the case in cocoa (Glendinning, 1963), there appears to be an upper limit to seed size determined physically by the amount of expansion that can be made by the ovaries. Fruit growth involves rapid cell expansion before ovule reaches its maximum size (Mendes, 1941) and at this stage the fruit is sensitive to water stress.

In a place like Ruiru ($1^{o}08'S.$, $36^{o}56'E.$ altitude 1508 m; annual rainfall 90-120 cm) the largest differences in bean size recorded in field trials were associated with differences in rainfall, and presumably the tree water status, while the fruits expanded (Cannell, 1974). For this reason "early crop" is reported to have quality which is not so favourable (Wormer,

1966; Wooton and Wormer, 1967) apparently because "early crop" develops during the hot dry season when net assimilation rate of the tree is small (Cannell, 1971 a).

Irrigation and water conserving mulch for example at Ruiru, are the most important field treatments which increase bean size (Cannell, 1974). Thus the only means that a grower has to ensure that a large proportion of beans reach their genetically determined maximum size is, therefore to mulch and irrigage on time. Other factors which tend to increase bean size include treatments which decrease yield per tree such as shading and thinning (Cannell, 1971 a).

Irrigation may be involved in the onset of ripening in coffee. In Robusta coffee (Coffea canephora Pierre) ripening was reported to be affected by irrigation, which induced earlier ripening (Awatramani et al., 1973).

Removal of unwanted branches and leaves is also one of the management practices that influence fruit yield. The main reasons for pruning and how to prune coffee have been outlined (Anonymous, 1976). The principal aim of pruning is to control crop level by maintaining a reasonable leaf/crop ratio from one season of growth to the other, so that there is enough carbohydrate supply for various activities in all parts of the tree. On the average about 20 cm^2 of leaf area per fruit (that is about two fruits per leaf) is required on

a bearing tree (Cannell, 1971 a). The risk of having excessive crop on the tree ('overbearing') is less when the trees are pruned as soon as possible after picking, giving the trees the longest time to remake reserves, new leaf and absorbing root surface before the next crop begins to develop.

Yield responses of Arabica coffee to various forms of fertilizer, particularly those used in Kenya, have been outlined in several papers mentioned in a recent review on the subject by Oruko (1977). Fruiting coffee trees are reported to take most of the major minerals such as nitrogen, phosphorus, potassium, calcium, sulphur and magnesium (Cannell and Kimeu, 1971). Economic yield responses have been obtained consistently from nitrogen fertilizers. Yield responses to N can be increased by up to 50% when coffee is irrigated (Mitchell, 1969), possibly because more P is then taken up (Robinson, 1969). On the other hand, there have been no consistent yield responses to soil applied P, K or Mg, but it is known that a large amount of K is absorbed by coffee, much of which goes into the fruiting pericarp (Arzolla et al., 1965), and leaves.

Fertilizers seem to control also the onset of ripening. For example the application of a compound fertilizer containing N, P and K in the ratio of 13:13:13 at increased rates to both shaded and unshaded coffee was reported to result in progressive delay in fruit ripening (Anonymous, 1964), and the effect was more marked in the unshaded crop.

1.2 Other related Physiological problems

Several physiological problems affect fruit growth and development indirectly. The problem which still remains unresolved, is the control of cropping. Several approaches have been made in an attempt to regulate cropping by trying to control shoot growth and its periodicity (Browning, 1975 b) flower initiation (Cannell, 1971 b), flower bud dormancy and opening (Mes, 1957; Alvim, 1960 ; Browning, 1973 a) with little success.

On the East of the Great Rift Valley, Kenya, shoot growth becomes more vigorous (flushes) during the two annual rainy seasons (Rayner, 1946) that usually occur between February to May and October to December. It is also true that coffee trees growing in other countries behave in the same way during wet seasons of the year (Gopal and Vasudeva, 1973).

It appears that growth 'flushes' control growth periodicity of shoots, nodes and fruits (Cannel, 1971 a). The main problem is that growers have not been able to control 'flushes' by using management practices such as pruning, fertilizer application, crop thinning, shading or irrigation. At Ruiru, for example, water stress is severe during hot dry period between December and February (Wormer, 1965). The 'flush' that follows this dry period is normally between March and April. This 'flush' is more intense than the one which occurs between October and December following a

relatively mild stress that is experienced during the cold dry period between June and August (Rayner, 1946).

The main cause of flushing is not very clear, but it is likely that water stress plays a determinant role in at least the intensity of flushing. Kumar (1979) has attempted to give the reasons why there is growth flushing during the rains and also why the growth rate which is present at the time of flushing cannot be maintained throughout the growth period. It appears that plants, besides growth, keep on performing other functions such as photosynthesis (Kumar and Tieszen, 1976) and absorbtion of minerals (Tesha and Kumar, 1975) during the dry weather. The result is that there is enough food material accumulated in the trunk during the hot months as has been shown by Wormer(1963). This has also been observed by Cannell (1972 a). When conditions for growth become favourable after the rainfall and the resulting rehydration of tissues, trees then exhibit fast growth (flushing) because of the presence of enough carbohydrates and other nutrients in the trunk. It is also possible that nitrogen fertilizer applied during the previous season prior to the onset of rain can accumulate as metabolites in the plants and induce higher flush intensity (Tesha, 1976).

On the other hand, lowered growth rate during the cool dry months may presumably be due to the weather conditions. For instance low light intensity due to generally heavy overcast and lower temperatures results in lower stomatal conductance and lower photosynthetic rates (Kumar and Tieszen,

1976). In addition, there is also a heavy sink created due to coffee fruits being in the expanding stage at this time. Cannell (1971 a) showed that besides the first two pairs of leaves all the carbohydrates synthesized move towards the developing fruits of coffee.

Water stress also plays some role in coffee growth. For example, Browning and Fisher (1975) postulated that water stress seems to stimulate trees into physiological compensatory growth when stress is eventually relieved by rain; and that stress might do so possibly by decreasing root resistance to water uptake. Nutman's (1941) suggestion that roots of Arabica coffee have exceptionally high root resistance to water uptake was confirmed by Tesha and Kumar (1975).

Closely associated with shoot growth is the rate at which nodes (leaves) are produced per unit-time. Nodes are the major yield component in coffee production. This is because flower initials are formed in the leaf axils at the nodes, and therefore the more the nodes a tree has, the more centres become available on the tree shoots for fruit formation. The trees produce nodes in succession of growth 'flushes' (Browning, 1975 b). The rate at which nodes are produced can be accelerated by irrigation and nitrogen fertilizers (Cannell, 1973) and seemingly by the application of gibberellic acid (Browning, 1975 b). The main problem

which still remains to be solved is how to control flower initiation.

There are many factors that affect flowering in tree crops (Luckwill and Cutting, 1970). The factors include nitrogen fertilizers, length of the day, light intensity, temperature cessation of shoot growth, leaf age, presence of fruits, adequate carbohydrate resources, gibberellin and cytokinin balance in the xylem sap, and growth inhibitors. Studies conducted in coffee indicate that day length (Piringer and Borthwick, 1955), rapid fall in temperature (Rees, 1964), water stress (Alvim, 1960 a) and possibly seasonal variation of climatic and edaphic factors around the plants (Gopal and Vasudeva, 1973) are involved in one way or the other in the regulation of flowering.

Coffee flowers are initiated in nature when the day length is short. Thus Arabica coffee behaves like 'short day plants with 13 h critical and 8 h optimum photoperiod (Piringer and Borthwick, 1955). In regions close to the equator, like Kenya, where the day length remains practically the same throughout the year and shorter than 13 h , flower bud initiation apparently takes place throughout the year. Cannell (1972 b), however, concluded that mature trees are photoperiodically less sensitive to day length compared to young trees.

Following initiation, coffee flower buds develop fully but remain dormant after attaining at least a length of 4 mm (Mes, 1957). Water stress is required for dormancy (Alvim, 1960 a; 1960 b). When water stress is relieved by rain or irrigation the dormancy breaks, the buds then resume fast growth and blossoming occurs (Piringer and Borthwick, 1955; Mes, 1957). It has been reported that rapid fall in temperature occurs during the day when it rains and the fall in temperature breaks dormancy (Rees,1964; Browning,1975 a) possibly by reducing the evaporative demand of the atmosphere around the coffee plants. Browning (1973 a) suggested that resumption of active growth leading to blossoming may be regulated by the liberation of free gibberellin from bound form in the buds. He also reported that activity of cytokinin increased in the xylem sap and flower buds when dormancy was broken by rain or irrigation and suggested that cytokinin may be needed for development of buds to anthesis (Browning,1973 b).

Although the flowering and cropping pattern of Arabica coffee in Kenya has been described (Wormer and Gituanja, 1970), main factors controlling flower bud initiation are not very well known. It is not therefore possible to control cropping pattern via the control of flower initiation. That is why efforts to regulate flowering with plant growth regulators (Cannell, 1971 b;Browning 1975 b) have apparently failed to produce a practical solution to coffee growers in Kenya.

Sometimes fruits fail to set and this has been found to vary between 40 and 100% (Huxley and Ismail,1969). The variation is thought to be probably due to failure of abnormal flowers which vary in degree, to set fruit.

Following anthesis and fruit set, the fruits remain in a quiescent state known as "pinhead". The "pinheads",however respire normally (Cannell, 1971 c), active cell division goes on in them (Sybenga, 1960) and they are active sinks for assimilates (Cannell and Huxley, 1969). The reason why the fruits remain without showing any visible sign of growth at the "pinhead" stage is not clear. It is therefore not possible at the moment to regulate cropping either by prolonging or hastening the fruit development at the "pinhead" stage.

Apart from the failure of fruits to set at times, coffee fruits sometimes abort between 8 and 12 weeks after anthesis, that is, during the first month of fruit expansion (Montaya and Sylvain,1962; Huxley and Ismail, 1969). The reason for the loss is not clear. However, when the supply of carbohydrates is exceptionally low, fruits can also shed during the later stages of fruit expansion (Cannell, 1971 a), and fruit loss then partially offsets loss of leaf so that the growth of the remaining fruits is not so much affected.

It is apparent that at increased light intensities the flowering of coffee is enhanced because it has been

reported that unshaded trees can produce and retain an excessive number of fruits, which results in 'overbearing' (Castillo and Lopez, 1966). Such fruits maintain a very high sink strength compared to the vegetative points (Cannell, 1971 c). The main Kenyan varieties tend to bear biennially as a result of 'overbearing' and the shoots as well as roots of heavily fruiting trees can sometimes die (Nutman, 1933; Beckley, 1935). It is suggested that coffee overbears primarily because the trees can become committed to producing dry matter for a large amount of endospermous tissue (Cannell, 1971 a). In this way the tree lacks a satisfactory mechanism to prevent imbalance between the size of the seeds and the other growing regions acting as dry matter sinks.

Arabica coffee requires only intermediate light intensities to photosynthesize optimally and therefore is more suited for high density plantings(Kumar and Tieszen, 1976). This finding implies that the problem of 'overbearing' may be alleviated when coffee is planted closely apparently because of mutual shading. There will be relatively less intense flowering and fruiting and a better leaf/crop ratio may be maintained. Meanwhile, the phenomenon still remains a problem for coffee growers to contend with since it is not possible to control overbearing' even by pruning.

An individual coffee fruit may take about seven to ten days from the time it starts turning yellow to the time it is red and ready for picking. However, not all mature fruits

ripen together even if they all originated from one particular flowering. Thus the picking periods for fruits originating from any one flowering is usually spread over a period of several weeks. In a place like Ruiru where there are two main flowering seasons in a year, picking season could spread from May to December with a peak in August and October (Cannell, 1973). The problem posed by this type of uncontrolled ripening has been stated earlier.

1.3 Growth of the coffee fruit

The coffee fruit, botanically a drupe, is erroneously referred to as "berry" while green and expanding and as a "cherry" when ripe.

Mendes (1941) described the development of embryo sac, pollination and the formation of the fruit for various coffee species. The ovary has small nucellus and a single thick integument. After fertilization, first the integument, not the endosperm, expands by rapid multiplication of the cells until the fruit reaches its final size. At this time the parchment (which is the inner part of the fruit wall) becomes lignified and hard. The tissue of the integument is gradually suppressed by the growing endosperm which fills the whole locule with the exception of the embryo and the silverskin, the later being all that remains of the integument. Wormer (1966) made a complete study of berry and bean development and described in detail the different bean shapes that occur. However, it is not clear as to when cell division of the integument

and the endosperm ceases. Growth of the fruit of Coffea arabica L. is similar to that of other drupes in that it exhibits a double sigmoid type of growth curve (Fig.16).

Soon after anthesis the young fruit enters a period of slow growth of about six to eight weeks, during which it is referred to as a "pinhead". The "pinhead" expands very rapidly from about 9th to 14th week after anthesis until the locule has reached its maximum size and the green berry approached nearly its final size. The endosperm then grows to fill the locule between 18th and 20th week, during which time the fruit expansion is negligible. From about the 25th to 28th week growth is again rapid, dry matter accumulates into the endosperm and the fruit matures and ripens within about 28 to 34 weeks of anthesis. The whole process may be accelerated or retarded by climatic conditions, weather changes and cultural practices. Morphological development of the fruit is shown in Fig. 1 while that of the bean is depicted in Fig. 16.

1.4 Bean Size and Quality

In Kenya ripe fruits are picked and the outer mesocarp (pulp) is removed mechanically. The beans are then allowed to ferment naturally or by adding a fermenting enzyme to remove the mucilage before washing and sun-drying.

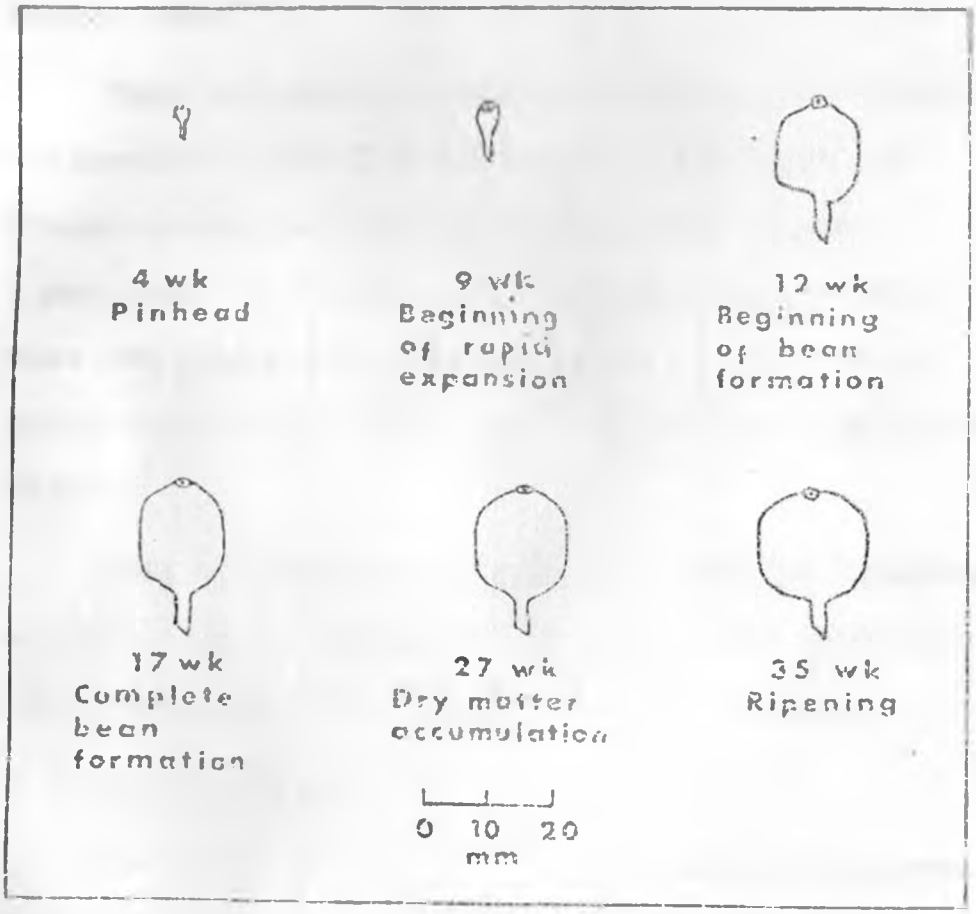
It is preferred that the drying be done evenly until moisture content is about 11%. The dried beans, normally referred to as 'parchment', are then hulled in coffee mill to remove the parchment. The raw green beans obtained after hulling are graded through a series of screens. The beans retained in a 7.2 mm diameter screen and those retained on a 6.75 mm diameter screen are referred to as grade 'AA' and 'A' respectively. Throughout this study the term grade 'A' bean was used to mean all the beans retained on the 6.75 mm diameter screen irrespective of whether or not the 'AA' grade beans are present. Grade 'A' beans have an average weight of between 150 to 180 mg when the bean moisture content is between 7 to 10% (Northmore, 1965).

Although grade 'A' beans fetch higher prices than small beans (Wallis, 1967), there is no significant correlation between average bean weight and overall quality of the bean (Northmore, 1965). Nevertheless, bean size and dry weight is still considered an important attribute because growers prefer having more grade 'A' beans in their coffee for economic reasons mentioned earlier. Therefore, this aspect was also studied in this work.

1.5 Hormonal relations in fruit growth and development

Plant hormones control or influence essentially every facet of plant growth and development. Hormones act and interact in many different ways during fruit growth and development.

Fig. 1 Morphological development of the Coffea arabica L. fruits


4 wk
Pinhead
9 wk
Beginning
of rapid
expansion
12 wk
Beginning
of bean
formation
17 wk
Complete
bean
formation
27 wk
Dry matter
accumulation
35 wk
Ripening
0 10 20
mm

Auxin appears to be important in fruit set. However, fruits of some species of plants fail to set fruit after auxin application; instead, they respond to GA (Crane, 1964) or cytokinins (Crane, 1969). Subsequent development of fruits is also believed to be controlled by hormones. Good correlations have been reported between the hormone content of the seeds with various stages of fruit development in some fruits (Crane, 1964).

There is increasing evidence that hormones also control the movement of substances where certain plant organs and tissues are able to compete preferentially for nutrients. A good example is from the work of Seth and Wareing (1967) where they demonstrated a synergistic effect of IAA, GA and cytokinin on the mobilization of ^{32}P into defruited peduncles of beans.

There has hardly been a report on the hormonal relations to fruit growth and development of Arabica coffee except perhaps those emanating from this work (Opile, 1979).

1.6 Cropping pattern

In Kenya, some coffee growing areas have two main crops while others have only one main crop a year. Branch nodes are usually produced more rapidly during wet seasons than dry ones. The earliest formed flower buds remain dormant and do not open until after irrigation or the beginning of short

rains in October or November. The fruits then develop during hot dry season between January and March of the following year and end up as "early crop" which is harvested between June and September. On the other hand, later formed flower buds are usually initiated between October and December and open at the beginning of "long rains" in February or March. Fruits then develop during the cool and dry period between May and September and are harvested as 'late crop' between October and January of the following year. An example of the coffee growing areas with usually only one main crop is Kianzabe (altitude 1479 m). The flowering time for the main crop at this place is normally between October to December and the crop is harvested from May to September of the following year. There are some areas in between 1600 and 1800 m where "early crop" is quite light for example Kibubuti (1890 m) and this is therefore not very much desired because pickers waste a lot of time looking for only a few isolated fruits and thus growers waste a lot of money. It would therefore be beneficial to suppress "early crop" for such areas.

Another consideration of economic importance is the time one takes in any one picking season to harvest all the ripe fruits. Here in Kenya the nature of flowering and hence the fruiting pattern is such that a farmer is normally unable to predict how much crop he is expecting to harvest in a season and how long each picking will last. As a result he is often late in carrying out other operations such as

pruning, irrigation and fertilization, all of which should be completed as soon as possible after harvesting and before the next flowering takes place. For maintaining the quality of coffee, fruits are to be picked as soon as they ripen. This is, at the moment only possible if coffee fruits are picked by hand, the labour for which is becoming increasingly expensive. Cost of picking becomes high because ripening is not uniform for reasons stated above. There/occasions, /are however, when most of the fruits are ready for picking at the same time. This then leads to shortage of labour, and at the same time factory facilities become inadequate, and as a result farmers incur losses. Coffee farmers would therefore benefit if a way is found to help them to regulate ripening.

1.7 Objectives

Various objectives of this study may therefore be summarized:

(i) to identify the phases of fruit growth at which hormones play some part in determining the bean size and hence the quality. It was hoped that once hormonal requirements of these phases were determined, plant growth regulators might be used to modify the rate of fruit growth to obtain the desired bean size.

(ii) to suppress the "early crop" in areas between altitudes of about 1600 m to 1900 m where such crops are small (negligible) and are not desirable. Cannell (1971 b)

attempted to achieve this objective through gibberellic acid application at relatively high concentration and frequencies. The same was proposed to be done using lower frequencies of application and concentration of gibberellic acid. The effect of this chemical on the crop yield was also investigated.

(iii) to regulate fruit ripening without lowering the quality of beans. An attempt was previously made also to promote ripening of Arabica coffee in Kenya by Browning and Cannell (1970). However, more work is needed to get a complete picture of this particular aspect.

In order to achieve the above objectives, four different approaches were made in this work. One aspect of the study was to use hormones to increase bean size and possibly improvement on the bean quality. This was done by applying hormones to the field trees at different intervals throughout fruit growth. The second aspect of the study has been the measurement of the endogenous hormones within the fruit in order to detect correlative changes associated with different stages of fruit development.

The third aspect of this work was to regulate shoot growth and flower initiation in order to suppress "early crop" in certain coffee growing areas. The study orignally started by Cannell, (1971 a) at Ruiru (altitude 1608 m) was therefore extended to other ecological sites situated between altitudes 1479 m and 1890 m. This study was

conducted using GA_3 foliar sprays only during long rains as suggested by Browning (1975 a).

The fourth approach of the study was on the use of growth regulators on field trees in order to regulate cropping by either spreading or bringing forward harvesting. The work started by Browning and Cannell (1970) was therefore re-examined and extended to other ecological sites using (2-chloroethyl) phosphonic acid (CEPA).

C H A P T E R II

GENERAL MATERIALS AND METHODS

2.1 Plant Material

All the plant materials used in this study were field grown trees of different varieties of Coffea arabica L. They were managed according to the recommended practice, and pests and diseases were controlled in the usual manner (Ombwara, 1968).

2.2 Apparatus,glassware and chemicals

Rotary film evaporators were used for redistilling organic solvents before use and for reducing extracts down to the desired quantities prior to purification.

A Beckman spectrophotometer Model DB was used for optical density measurements. An M S E centrifuge was used for cytokinins work.

All organic solvents used during the extraction procedure were redistilled from laboratory grade reagents prior to use. Other chemicals used were in general of 'Analar' grade.

2.3 Extraction procedure for hormones

Unless otherwise specified, all extractions of hormones from the fruits was done by using 80% aqueous methanol which was prepared by redistilled methanol and glass distilled

water. This was chilled in the refrigerator (4°C) before use. It has been reported (Nitsch, 1956) that methanol is an efficient solvent for extracting growth hormones in tomato fruits and bean seeds. Other workers have also shown that 80% methanol is more suitable for extraction compared to other solvents (Booth, 1958; Phillips,1959). Advantages in using methanol include ease of penetration, precipitation of protein in order to stop most enzymic activities, preventing of browning and general ease of safety of working.

Fruits were normally frozen by placing them in the deep freeze (-15°C) except where immediate extraction was required, in which case liquid nitrogen was used to freeze the fruits. Details of the extraction procedure appears later in the relevant section.

2.4 Chromatography

2.41 Paper

Whatman No. 1 chromatography papers were used. A ml graduated pipette was employed to stripload extract on a line 10 cm from the end of the chromatographic paper. For drying during the course of loading a current of cold air from an industrial blower was used. Marker spots of authentic hormones were sometimes applied on the strips separated from the extract by a slit from the starting line at the bottom of the paper. Chromatograms were developed by a descending method at room temperature (about 22°C)for about 12 h. During

this time the solvent moved 25-30 cm.

Many workers have observed that isopropanol (propan-2-ol) ammonia-water is the most effective solvent for separation of growth substances (Bennet-Clark, et al., 1952; Bennet-Clark and Kefford, 1953; Stone and Thimann, 1954). A mixture of 25% ammonia hydroxide 0.90 sp. gr. and water (10:1:1 v/v/v) was therefore used for most of the work described here.

When the chromatograms were completely dry, they were then cut into ten equal parts. Only the portion between the loading line and the solvent front was included for the purpose. These ten equal parts were corresponding to R_Fs 0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9 and 0.9-1.0.

For water control and hormone control assays, strips of equal width to that of R_Fs were cut from the paper above the starting line of the same chromatograms used for separating the hormones from the extract. As these had been immersed in the chromatographic solvents used, they accounted for any possible effect of the solvents.

2.42 Thin layer chromatography (TLC)

Thin layer chromatography has various advantages over paper chromatography. These two chromatographic techniques involve two important processes namely, absorption and partition. However the two processes are superimposed. The behaviour

of a compound is dependent upon both absorbant and solvent. With paper, one can only alter the solvent but in thin layer chromatography one can alter both to get better separation than with paper. Development of thin layer chromatograms usually requires much less time than paper. Recovery of material is very important, especially in biological work, and can be achieved more efficiently with thin layers. A constant temperature is not so important for TLC as it is for paper chromatography and marked changes in temperature are not encountered during the short development times.

Lastly, the method is highly effective provided the following precautions are taken into account:-

(i) the quality of the layer material must be made to remain the same.

(ii) the activity of the absorbants is determined by the time and temperatur of heating, therefore the plates must have received identical pretreatment during activities.

(iii) layer thickness should remain constant.

(iv) chamber saturation must be maintained for better reproduction of results.

(v) Only one method should be applied for development because of differences in wetting rate from one method to the other.

(vi) development distance and distance of starting point from the surface of the solvent must be kept constant.

(vii) care should be taken against impurities which might change R_F values.

Efforts were made to take the above precautions when using thin layer chromatography.

For the preparation of plates for TLC, the apparatus supplied by Shandon, Ltd., London, was used. Preparation of the TLC plates involved the following:-

(i) cleaning of the plates in a detergent, usually Teepol. Care was taken to remove grease or spots and other material from the plates. The plates were thoroughly rinsed with running water after cleaning and finally with distilled water. The plates were then dried in an oven.

(ii) arranging the plates on a loading table preparing the spreader.

(iii) preparation of suspension of the coating material and filling the spreader.

(iv) spreading the suspension over the glass plates.

Five 20 x 20 cm clean and dry plates were arranged on the aligned tray of the loading platform of approximately 110 cm long. The left and the right hand of the row were

completed with 5 x 20 cm glass plates. The spreader was now placed adjusted to the required size with the key to give the same desired thickness of the coating. A trial run was usually made, to make sure that the end of the plates fitted evenly.

Before coating the plates with the appropriate layer, they were wiped with tissue paper in acetone. Thirty-five grammes of the powder needed for coating was mixed with distilled water in a stoppered bottle and shaken vigourously for 30 seconds. This suspension, after bringing the spreader back to the end plate, was poured into it and was drawn across gently at a constant speed, leaving a coating over them.

The plates were left in position until they were dry. The dry plates were put in an oven fro 2 hours for activation. They were then removed and placed in a desiccator having a bed of silica gel. The plates were ready for use when cool. However, no plates were used which had been prepared more than 10 days before use.

For loading the extract, a glass tube drawn into a small capillary and bent at the end was used. The plate was put on a plastic template. One edge of the template was allowed to coincide with the edge of the thin layer plate while the

other edge of the template was taken as the line for strip loading. When comparison of R_F values with authentic substances was required, the material was spot loaded.

2.43 Column chromatography

Ion exchange resins

Column chromatography using ion exchange resin has been used for many years by people who are interested in the separation of nucleic acid derivatives. Some important properties and functions of resins include the following:-

(i) Strong functional groups i.e. strong acid cationexchangers or strong base exchangers.

(ii) Single functional species e.g. nuclear sulphonic acid devoid of phenolic or other acid groups.

(iii) Chemical stable polystyrene resin as supporting matrix (reducing side reactions of the matrix essentially to zero).

(iv) Ability to produce the exchangers in the form of spherical particles with improved hydrodynamic properties.

(v) Degree of crosslinking which is important for exchange of substances of high molecular weight.

In general the chromatography by use of resins require two steps (1) the absorption of the sample containing the components to be separated and (2) an elution sequence in which

the various compounds are brought off the column separately. The absorption step usually utilizes conditions of high affinity between solutes and exchanger to bring about retention of the sample in the top-most layers of the column. Elution, on the other hand, utilizes conditions in which a larger fraction of the constitution in question is released from the resin, thus setting up a distribution between solvent and exchanger which permits a reasonable degree of movement of the solute down the column with flow of the solvent.

Dowex 1 in chloride form 50-100 mesh, Sigma, USA and Zerolit 225 (formerly Zeo-Karb 225), 100-200 mesh, Bio-Rad United Kingdom were the resins used in this work. The way, the resins were prepared before use, is described under purification of the cytokinin-like substances. The resin consists of a matrix, usually of polymers of vinyl-benzene crosslinked with divinyl-benzene to give three dimentional polyesterine beads of spherical nature. The matrix supports quaternary ammonium which is a strong based anion exchanger (as is the case in Dowex-1) or sulphonic acid which is a strong cation exchanger (as is the case in Zerolit 225). The resin swells in water upto limits determined by cross-linking due to its hydrophilic character.

Other materials used in purification of extracts

The other compounds used for purifying the extracts were silicic acid (SILIC AR CC-4, 100-200 mesh, Mallinkrodt)

and Polyclar AT, an insoluble form of polyvinyl pyrrolidone (PVP), GAF Ltd., Great Britain.

The preparation of the silicic acid before use is described later under the 'gradient elution' of the gibberellin-like substances while that of the PVP is described under the purification of the ABA-like and the gibberellin-like substances in the experimental sections.

2.5 Biological assay methods

2.51 Rumex senescence retardation method

This was based on the assay developed by Whyte and Luckwill (1966). Old leaves which were still green of *Rumex obtusifolus* L. were selected, detached and their petioles immersed in distilled water in a beaker and left standing in the dark-room for 24 h at 25°C. Ten disks, each 7 mm in diameter, were cut from the leaves using a cork borer. The disks were placed on the filter papers that had been moistened with distilled water and placed in 4.5 cm petri-dishes. The dishes which contained either the extract, distilled water (controls) or the GA_3 standards, were run in triplicate and incubated in the dark at 25°C. When a full range of response to standards (0.0001-1.0 µg GA_3/ml) was obtained (usually within four days) each lot of ten disks was extracted in 10 ml 80% methanol for 24 h and the optical density of the solution measured at 665 nm.

2.52 Soyabean callus assay

The nutrient medium containing kinetin (1 µg/ml of medium) was prepared according to Miller's (1963) method for the assay:-

Component	Concentration (mg/litre)
$Ca(No_3)_2$	347.0
$K NO_3$	1000.0
NH_4NO_3	1000.0
KH_2PO_4	300.0
$Mg SO_4$	35.0
K Cl	65.0
Na Fe EDTA	32.0
$Mn SO_4$	4.4
$Zn SO_4$	1.5
H_3BO_3	1.6
K I	0.8
Glycine	2.0
Nicotinic acid	0.5
Thiamin HCl	0.1
Pyridoxine HCl	0.1
Sucrose	30,000.0
Agar	10,000.0

The mixture was heated and mixed well before being adjusted to pH 5.8 with NaOH when cool.

Soyabean seeds were sterilized by soaking them in 0.1% mercuric chloride solution for 15 min. The seeds were rinsed four times with sterile distilled water. Three seeds were planted in each Erlenmeyer flask containing 50 ml of the medium described above.

After seeds had germinated, the cotyledons were removed and cut into about 4 x 4 x 2 mm blocks. One block was placed in each flask on the medium given above to which 0.5 mg/litre of kinetin had been added. After about three weeks, the wound callus was subcultured (under sterile conditions using Laminar Flow cabinet), placing the sub-cultures on the same kind of media. The subculturing was continued until a sufficient quantity of stock culture had been accumulated to initiate tests.

Soyabean callus tissue previously grown on a nutrient medium containing kinetin was transferred to a medium without kinetin. Extracts from fruits tissues to be tested for cytokinin-like substances were added and the resultant increase in weight of the tissue as a result of cell division was used as a measure of activity of the extracts tested.

2.53 Wheat coleoptile assay

This was based on the assay developed by Nitsch and Nitsch (1956). Wheat Triticum aestivum (Local variety) was used in assays for the investigation of inhibitors. The seeds were soaked in water in the dark for two hours at 24°C

and planted on three layers of blotting paper in plastic boxes or shallowly planted in moist vermiculite. Where the seeds were planted in the plastic boxes the boxes were covered with light lids to maintain a humid atmosphere in the boxes in the dark room for 72 h at 24°C.

The coleoptiles, 18 to 22 mm in length, were selected and 10 mm sections were excised below the apex using a coleoptile cutter. The cut sections were floated for one hour to leach out endogenous hormones before placing the sections in the test solutions which were placed either in vials or petri-dishes.

Small petri-dishes, 4.5 cm diameter, or vials 2 x 5 cm were used for the bioassays of the 10 R_Fs. The eluting fluid in case of the bioassays for inhibitors was a mixture of buffer sucrose and water, and was composed of the following (Nitsch and Nitsch,1956):

K_2HPO_4 - 1.794 g)
Citric acid monohydrate - 1.0199) Buffer
Sucrose 20 g)

Made up to 1000 ml with glass distilled water.

The method of Browning (1973 a) was used to elute the test inhibitor extracts from silica gel GF_{254} and paper chromatograms. One ml of the 2% (w/v) sucrose solution (pH 5.0) composed

of the dipotassium phosphate buffer and citric acid monohydrate salts described above was used to elute the extracts in the petri-dishes or vials for about 12 h at 4°C, prior to placing ten selected 10 cm sections in each dish or vial. The cutting operation was performed in physiologically inactive green dim light. The bioassay vials were closed with plastic stoppers each provided with a small hole for aeration and placed in a 'clinostat' with a revolution speed of about two revolutions per minute.. The sections were incubated with the test solutions in the dark at 24°C for 22 h. Each test included a set of authentic ABA standards ranging from 0.01 to 10 µg ABA/ml of the buffered mixture. The length of each section was determined after blotting and the results were expressed, to the nearest mm, as a final mean length per treatment in the form of a separate histogram for each chromatogram.

2.54 Lettuce hypocotyl test

This test for gibberellins was devised by Frankland and Wareing (1960) and utilizes the growth in length of the hypocotyl in response to gibberellin in light. However, the method used in this study was a modified version given by Browning (1973 a). Lettuce seeds, cv Grate Lakes (Simpson and Whitelaw, Nairobi) were placed in moist petri-dish containing 9 cm filter paper and were left to germinate in the dark at 24°C. After about 36 h seedling with radicles approximately 3 mm long were selected; at this stage the

elongation of the hypocotyl had not commenced. The seeds were transferred into 4.5 cm petri-dishes containing chromatogram strips or filter paper-disks with extract previously dried onto them before the seeds were added to the petri dishes. The extracts were eluted overnight at 4°C with 1 ml distilled water. The filter paper disks or strips had previously been impregnated with 0.5 µg zeatin (Browning, 1973 a) because the ABA inhibited promotion of hypocotyl growth by GA is overcome by cytokinin (Sankhla and Sankhla, 1968). Ten selected germinated seeds were transferred to each petri dish carefully with the help of a pair of forceps. The dishes were then transferred into a transparent glass tray containing moist blotting paper and a transparent glass cover to maintain a humid atmosphere. The trays containing the petri-dishes were placed in a growth room illuminated with fluorescent light tubes and maintained at 24°C for 72 h (Browning, 1973 a). The growth room also had a 200 watt incandiscent light bulb which provided some red light. Each bioassay included a part from the test for R_Fs dishes containing known amounts of GA_3 ranging from 0.001 to 1.0 ug. The length of the hypocotyls were estimated by direct measurements and each assay was run at least twice.

2.6 Techniques of dissolving hormones

Stock solutions of gibberellic acid and abscisic acid were prepared by dissolving the appropriate amount of the solid in the smallest possible volume of re-distilled thanol and

then storing the solution at 4°C (in the refrigerator) until required for use. Fresh solutions were prepared every two weeks. Extracted materials were stored in a deep freeze (-15°C).

The GA_3 used for foliar sprays in the field was in the form of powders and the spray solutions were made by dissolving the appropriate amounts in tap water (pH about 6.0). (2-chloroethyl)phosphonic acid sprays solution was prepared by using the appropriate amount of 'Ethrel' (48% a.i. CEPA w/v) which was in liquid form, and then making up the required volume with tap water. The tri- iodobenzoic acid (TIBA) solution was prepared by first mixing the appropriate amount of TIBA with the desired amount of 'Agral 90' wetting agent required per litre of spray solution before dissolving the mixture in tap water. Naphthylacetic acid (NAA) was prepared by dissolving the required solid in tap water. Kinetin used in the field for fruit development studies was first dissolved in small amounts of 0.1 HCl. The solution was then adjusted to pH 6.0 using tap water and 0.1 N NaoH.

2.7 Radioactive techniques

The only radioactive material used in this study was 6-^{14}C benzyl aminopurine (BA) sulphate for estimating the percentage recovery of the cytokinin-like substance in the fruit extracts. The ^{14}C BA was supplied in solid form by Radiochemical Centre, Amersham, Buckinghamshire. The radio-active BA was dissolved in ethanol and stored at 4°C until

required and had a specific activity of 57 μCi/mg.

The advantage of working with a material where carbon was labelled is that the long half life (5700 years) period of ^{14}C precludes the necessity for decay correction. However, the relatively low energy beta emission of ^{14}C makes it necessary to use a correction factor for self absorption, but this could be avoided by using liquid scintillation counting.

For ^{14}C counting, 10 μg of the ^{14}C BA (3.796×10^4 counts per minute/μg) was added into a macerator containing 100 g fresh weight of fruit samples in 400 ml 70% aqueous ethanol prior to meceration. Thereafter the procedure followed for extraction and purification was the same as for other fruit samples. In cases where the cytokinin-like substances were expected they were extracted in 2 ml 80% aqueous methanol (chilled) and were left at $4^{\circ}C$ until required for counting. The extract was usually evaporated down to 0.5 ml using dry nitrogen gas. N.E. 220 was the appropriate scintillator fluid used to mix with the extract. Two millilitres of the scintillator liquid was added and counting was done using a manually operated liquid scintillation counter (Model N E 5503 attached to a Philips Counter/Ratemeter Pw 4251).

The effect of the 'background' was taken into consideration during counting. The atmosphere always contains some amount

of radioactivity. Thus the total count rate of radioactive samples also includes the atmosphere or background radiation. Therefore counting bottles with appropriate amount of blank solvent and a scintillation liquid, as in the other bottles with samples, was added and counted along with the bottles containing the extracts. The time count rate of the samples was obtained by subtracting the 'background' count from the total count rate.

2.8 Statistical analysis of the results

The method of statistical analysis of bioassays presented in this work was used as described by Kumar (1966). This method does not necessitate the use of standard errors to compute a difference between sample means but instead range is used as a measure of variability. Thus the method consists of the following steps:

(a) Sum the ranges of the recorded values in each disk or vial

(b) Multiply the sum of ranges by a critical factor obtained from the table of critical factors (Appendix 1). The value of critical factor is obtained by looking up 'number in group' (coleoptiles or other sections per dish) against number of groups (dishes or vials).

The least significant difference (LSD) between means was estimated at the 1% level of probability.

To summarize in equation form:

$$LSD=\frac{C \leq r}{n}$$

Where r = range; C = critical factor; n= number of sections per treatment.

Difference between sample means of more than the obtained value are considered significant at the 1% level of probability. Means for each R_F are compared with the control mean and the statistical information is incorporated into a histogram by drawing lines above and below the control line.

The appropriate methods of the analysis of variance outlined in Steel and Torrie (1960) were used to analyse data for each design used in the field studies. The computer programmer model Olivetti 602 and the relevant programmed magnetic cards were used to facilitate the analysis.

2.9 Spot tests and UV spectra/cytokinins /for

Bromophenol blue and silver nitrate spray was used to test for the presence of cytokinins. This is a reagent with 2% silver nitrate and 4% bromophenol blue in distilled water, which when sprayed, gives blue coloured spots in the region where adenine and adenine derivatives are present.

Purified extracts after column and thin layer chromatography, were used in determining U.V. absorption spectra. This provides a very useful means for determining

the purity of substances and also the nature of substances to some extent. This was done with the help of the Beckman spectrophotometer. However, the spectra always depended upon the pH of the solution so they were determined in standard 0.1 N HCl (pH 2) and 0.1 N NaoH (pH 12) solution. From the absorption readings characteristics such as maxima and minima were determined between 210 and 320 nm for comparison with authentic nucleic acid derivatives. 'Blanks' were used as reference.

CHAPTER III

ENDOGENOUS HORMONES IN FRUITS

Generally developmental processes in plants are regulated by a pattern of several interacting hormonal substances (Osborne, 1965). The role of hormones in fruit growth and development is well recognized (Leopold, 1964; Wittwer, 1971). However, this information is lacking for Arabica coffee. Therefore, it may be important to find the level of endogenous hormones in the developing coffee fruits in order to determine the stages at which externally applied hormones can be useful.

For the purpose of this study, it is pertinent to confine to only four classes of hormones viz. abscisic acid (ABA), gibberellins (GA), cytokinins and ethylene. Auxins were not considered. Their role is to promote fruit set (Crane, 1964) and decrease mature fruit-drop (Leopold, 1964) and these are not major problems in Arabica coffee.

Abscisic acid is found in young fruits (Dörffling, 1970; Milborrow, 1967; 1974), leaves (Cornforth et al., 1965; Milborrow, 1967; Garb and Guttridge, 1968), and coffee flower buds (Browning et al., 1970). The role of ABA is established in fruit abscission. Thus application of ABA to mature peach, olive, citrus and apple fruits has been reported to accelerate abscission of the fruits and the effect was also marked on young grape berries (Milborrow, 1974). ABA seems to play an important regulatory role in fruit development because the changes in the endogenous ABA levels are closely connected with fruit enlargement and maturation (Dörffling,

1970; Davis and Addicot, 1972; Coombe, 1973). There is now considereable amount of evidence to suggest that ABA is among the growth substances that regulate fruit ripening apparently by increasing the levels of endogenous ethylene (Cracker and Abeles, 1969). Coombe (1973) showed that the application of ABA to young fruits accelerated ripening.

Gibberellins are synthesized in the apical zones of stems and translocated downwards (Phillips, 1971; Crausland, 1972) and in the root tips of several species from where they are exported by xylem sap to the shoots (Butcher, 1963; Phillips and Jones, 1964; Carr et al., 1964; Jones and Phillips, 1966; Skene, 1967; Jones and Larcey, 1968). The main role of GAs is to increase cell size (Jones, 1973). Thus marked stimulation of berry enlargement by GAs has been observed in some fruits such as grapes (Weaver and McCune, 1959 a; 1959 b; 1960; Sachs and Weaver, 1968; Bertrand and Weaver, 1972). Another role of gibberellins appears to be the improvement of fruit set in certain species (Crane, 1964). There are some cases where gibberellins have also been shown to retard ripening. For example in studies of Vendrell (1969;1970), and of Wade and Brady (1971) it was indicated that gibberellic acid (GA_3) delayed banana ripening.

Developing fruits have proved to be a rich source of cytokinins (Letham, 1967) particularly the seeds (Letham and

Williams, 1969; Blumenfeld and Gazit, 1970). Cytokinins are also present in the xylem sap of growing shoots (Jones, 1967). The principal role of cytokinins is to regulate cell division (Lethum, 1967; Skoog and Armstrong, 1970). In this way cytokinins have been shown to enlarge size of certain fruits, for example grapes (Weaver and van Overbeek, 1963). It appears that cytokinins, might be involved in delaying ripening. For example, Wade and Brady (1971) showed that pre-treatment with kinetin delayed degreening of banana peel in response to 16 h treatment with ethylene.

Fruit growth normally occurs both by cell division and cell expansion (Nitsch, 1965). Both processes are controlled by hormones. In Coffea arabica L. fruits, there are five different growth phases, namely 'pinhead', rapid expansion, bean formation, dry matter accumulation and ripening (See Fig.16). It is not known how various growth phases are regulated by growth substances. Therefore, it was important to estimate the levels of activity of major growth regulators at different fruit stages in an attempt to establish their role(s) in fruit growth and development.

Abscisic acid (ABA) has been identified in coffee flower buds (Browning et al., 1970). Gibberellin-like substances have also been detected in coffee flower buds (Browning, 1973 a; Gopal et al., 1975). Flower buds and sap of coffee also contain cytokinin-like substances (Browning, 1973 b). Abscisic acid, gibberellins and cytokinins have been reported to be present in

many fruits (See Chapter 1). However, they have not been investigated in the fruits of Arabica coffee. This consideration prompted a study of the three growth substances in the developing Coffea arabica L. fruits. It was hoped that such studies might help in determing the stage of fruit development at which exogenous growth regulators can be applied in order to accelerate fruit growth and development.

3.1 Abscisic acid and gibberellins

3.11 Experimental

Developing fruits were collected from Coffea arabica L. trees cultivars SL 28, SL 34 and French Mission (mixture of unknown cultivars) growing in the field at the Coffee Research Station, Ruiru, Kenya. Unless otherwise indicated, the fruits were collected once every three weeks starting four weeks after anthesis until the fruits were ripe. The fresh samples were weighed, number of fruits per sample recorded and either extracted as soon as they were collected from the field or stored in sealed polythene bags at -30°C in the deep freeze until required for analysis. The same sample was used for both ABA and GA estimation.

A 100 g fruit sample was homogenized in a blender using 400 ml 80% aqueous ice-cold methanol and extracted for about 20 h at 4°C. After filtering the extract over one layer of cheese cloth in a Buchner funnel, the residue

was washed twice with a total of 200 ml 80% methanol. The combined methanolic extracts were reduced to the aqueous phase by vacuum distillation using a rotary film evaporator. After freezing, the extract was thawed and filtered and equal volume of 0.2 M phosphate buffer (pH 8.0) added. The buffered extract was partitioned three times against equal volumes of petroleum ether (B.P 40-60^{o}C) at room temperatue, and petroleum ether fraction was discarded. The aqueous phase was then partitioned five times against half-volumes of di-iso-propyl ether (DIPE). The combined DIPE at pH 8.0 is referred to as the neutral inhibitor fraction. In some samples (six different stages of fruit development) the aqueous extract was adjusted to pH 4.0 with IN HCl and partitioned a further five times against half-volumes of DIPE. The combined DIPE phase at pH 4.0 is referred to as the acidic inhibitor fraction. After partitioning with DIPE the extract was then adjusted to pH 2.5 with IN HCl and partitioned against five half-volumes of ethyl acetate. The combined ethyl acetate phase is referred to as the acidic GA fraction. Each of the neutral (pH 8.0) and the acidic (pH 4.0) inhibitor fractions and the acidic GA fraction was reduced separately to dryness in vacuo at 38^{o}C, dried with dry stream of nitrogen gas and taken in 5-10 ml of 0.2 M phosphate buffer (pH 8.0) for further purification using PVP.

Purification on PVP

Each fraction was purified using 10 x 20 cm columns of PVP prior to the paper or thin layer chromatography. Under the conditions used, the PVP complexes with phenols (Lenton et al., 1971; Glenn et al., 1972). Before using the PVP, the powder was suspended in distilled water and the fines were removed by repeated decantation. The column was then packed under gravity flow and the bed supported by glass wool at the bottom with one layer of Whatman No. 1 filter paper disks in between the PVP and the glass wool. Each of the extracts was loaded onto the column and eluted from the column with 200 ml distilled water (about pH 4.3). The eluted neutral inhibitor extract was then adjusted to pH 8.0 with 1N NaOH, while the acidic inhibitor and acidic GA extracts were adjusted to pH 4.0 and pH 2.5 respectively with 1N HCl. After adjusting the pH each of the appropriate aqueous extracts was then partitioned separately five times against half-volumes of DIPE and ethyl acetate respectively. After reducing the ethyl acetate or DIPE fraction to dryness in vacuo the residues were taken up in 5 ml methanol for chromatography using either paper, thin layer or silicic acid (Silic AR CC-4, 100-200 mesh Mallinkrodt).

Further separation and purification using chromatography

Descending paper chromatography was carried out on 10 cm wide strips of Whatman No. 1 chromatography paper as described under General Materials and Methods. After development the

chromatograms were dried on clips hanging in a fume cupboard with the exhaust fan on for 1 h. They were then put on a clean sheet of paper and divided into ten equal strips, each strip equal to 0.1 R_f value. Two similar portions were cut above the loading line and these were taken to serve as the controls for the bioassay. Each of the strips was placed a 4.5 cm diameter petri-dish for elution and bioassay, described under General Materials and Methods.

Thin layer plates (20 x 20 cm) coated with 400 µm thick Kieselgel GF_{254} (Merck) were used for purifying the inhibitor fraction for bioassay while those coated with 250 µm thick (400 µm thick in some cases) Kieselgel G (Merck) were used for purifying the acidic GA fraction. Plates were activated for 10 min at 80°C before loading the extract. Ten grammes fresh weight equivalents of each of the fractions was line loaded while the authentic ABA or GA was applied as marker spots on the sides by a line cut in between the extract and the authentic sample. The loaded plates were developed using various solvents (see Notes under Figs. 5 and 8). After drying, the adsorbent was scraped from the plates in bands corresponding to each of the ten R_fs and these eluted with 4 ml water-saturated ethyl acetate. Using the method of Browning (1972 a) the eluates were then dried onto 4.25 cm Whatman No. 1 filter paper disks placed in 4.5 cm diameter petri-dishes for elution and bioassay.

In order to detect and identify GA in the extract without going through the bioassay procedure, marker spots of authentic GA_3, GA_4 and GA_7 were spotloaded on the same plate loaded with the extract in the manner described above before developing with the five different solvents indicated in Table 3. The developed plates were then sprayed with 5% sulphuric acid in ethanol followed by heating at 100°C for 10 min (MacMillan and Suter, 1963) and observed under UV (Hanovia, Slough, England).

Co-chromatography of the neutral inhibitor of the extract with authentic ABA and of the acidic GA extract with GA_3 was also checked for the purpose of preliminary identification. Ten grammes fresh weight equivalents of the neutral inhibitor fraction and also of the acidic GA fraction of the 22-weeks old fruit extract were spotted on TLC plates coated with 400 µm thick layer of silica gel GF_{254} and Silica gel G, respectively, and developed with different solvent systems mentioned in Figs. 5 and 8. The plates loaded with the neutral inhibitor were examined under UV after development and the UV absorbing bands were marked before elution and bioassay.

Gradient elution of the GLS on silicic acid column

Silicic acid was used to clean the extracts before GA assay. Gradient elution was done in order to find out if the extract contained various gibberellins that would move into different fractions. Before using the silicic acid the

powder was prepared by packing about 10 g of this material by gravity flow into a 2 cm diameter glass column and irrigating the powder with 100 ml n-hexane in order to remove the fines. After drying, the powder was partially re-hydrated (0.5 ml water per g) using a slightly modified version of Browning (1973 a). Two grammes of the rehydrated acid was mixed with 0.5 ml methanolic extract (i.e. 10 g extract). The mixture was then dried carefully under a stream of dry nitrogen gas and then placed on top of the 8 g hydrated silicic acid packed in 8 x 2 cm column. The column was serially eluted with 50 ml portions of ethyl acetate in n-hexane, starting with pure hexane, then 10% ethyl acetate, and after that 10% steps of the acetate and ending with pure ethyl acetate. The elution was done at the rate of approximately 2 ml per minute. Each fraction of eluate was dried onto 4.25 cm Whatman No.1 filter paper as described under TLC.

Estimation of ABA-like and GA-like activity

R_Fs in which significant growth activity was detected in each extract sample were used to estimate the total amount of ABA-like or GA-like activity. Using the response curves of the GA_3 and ABA standards shown in Fig.12, the activity levels for each of such eluates or R_Fs were estimated separately in order to obtain the total amount of activity for the sample. The data for ABA-like substances

in the DIPE (pH 8.0 and pH 4.0) and the ethyl acetate (pH 2.5) fractions were pooled together as explained under the results.

Gas-liquid chromatography (GLC) of the inhibitor

Samples for GLC determination were purified on TLC plates coated with 400 um thick silica gel GF_{254}. Each plate was loaded with 10 g fresh weight equivalent of the fruit extract and developed in a 50:5:2 v/v mixture of benzene: ethyl acetate: acetic acid (Lenton et al., 1971). Marker spots of authentic ABA were run on the same plates as the extract and viewed under UV light. The zones with UV absorbing bands were eluted with 4 ml acetone soon after developing. Eluates were then taken to dryness using a stream of dry nitrogen gas before methylation.

The TLC purified, N_2 dried extract was dissolved in 2 ml acetone and methylated as described by Schlenk and Gellerman (1960) with diazomethane prepared from Diazald 99% (Adrich Chemical Co., Gillingham, Dorset, England). Samples were then analysed isothermally using a Hewlett Packard (hp) 5750 research chromatograph fitted with 1.83 x 1.67 cm glass column and flame ionization detectors. The column was pre-packed with 3% SE-30 on 80-100 mesh Chromosorb W as stationary phase. Conditions used for separation of the inhibitor and the authentic cis-trans ABA were identical to

those used by Zeevart (1974). The flow rates of gases were: Nitrogen (carrier gas) 55 ml/min, Oxygen 400 ml/min, and Hydrogen (mixed widt 25% N) 40 ml/min. Oven temperature was 175°C, injection port and detector were set at 220°C and 240°C, respectively. For quantitative determination, the peak areas of activity were measured by multiplying peak height with half the value of peak width (Lenton et al., 1971).

3.12 Results and Discussion

Rumex bioassay results are not presented here. This is because the fruit extracts had brown substances, possibly phenols, which masked the leaf disks and inhibited senescence.

Characterization of gibberellins

The results of the examination of the acidic GA fraction by silicic acid column and paper are presented in Figs. 3 and 4 respectively. Significant growth promotion was detected in different fractions at different fruit stages (Table 1). The relative amounts of the GLS estimated for each sample studied using silicic acid column is shown in Fig. 2. Similar results were obtained with paper and silica gel G chromatograms (Table 2).

The 22-week old fruit extract which appeared to have the highest GLS activity after purification on silicic acid solumn was used for identification purposes. Significant activity at this fruit stage was detected in fractions between five and eight following silicic acid column chromatography and bioassy (Fig. 3F).

Maximum activity was detected in fractions six, seven and eight in this system. On the paper chromatograms, significant activity for the same extract (F) was detected in R_F 0.5-0.6 (Fig. 4F). Authentic gibberellic acid (GA_3) in the same system was located in R_F 0.5-0.8 (Fig. 4L).

Results obtained after development of the extract and authentic GA_3 on TLC plates using different solvent systems and bioassay are presented in Fig.5. Extracts in solvent system 1, benzene: butan-1-ol: acetic acid (75:25:5 v/v) resolved into two peaks: R_F 0.1 and between R_Fs 0.5 and 0.7. Solvent system 2, ethyl acetate: chloroform: acetic acid (15:5:1 v/v) also resolved the extracts into two peaks of activity in R_Fs 0.2 and 0.5. The extract was again separated into two zones of activity in between R_Fs 0.2 and 0.3, and R_F 0.5 when solvent system 3, propan-2-ol:25% (0.91) ammonia hydroxide: water (10:1:1 v/v) was employed. It was found that the extract run as a single peak on R_F 0.9-1.0 when solvent system 4, (water of about pH 4.2) was used. The extract co-chromatographed with GA_3 in solvent systems, 1, 3 and 4 (Fig.5). The peaks in solvent system 2 nearly co-chromatographed with GA_3.

Markers of authentic GAs and of all the acidic GA extracts were run on ethyl acetate: chloroform: acetic acid (15:5:1 v/v) on silica gel G plates. All extracts showed fluorescing bands in R_F 0.3 after spraying with 5% sulphuric acid in ethanol.

Fig. 2 Levels of the gibberellin-like substances (GLS) estimated on fresh weight basis after purifying on the silicic acid column. The broken line shows the GLS while the continuous line shows the growth of the fruits.

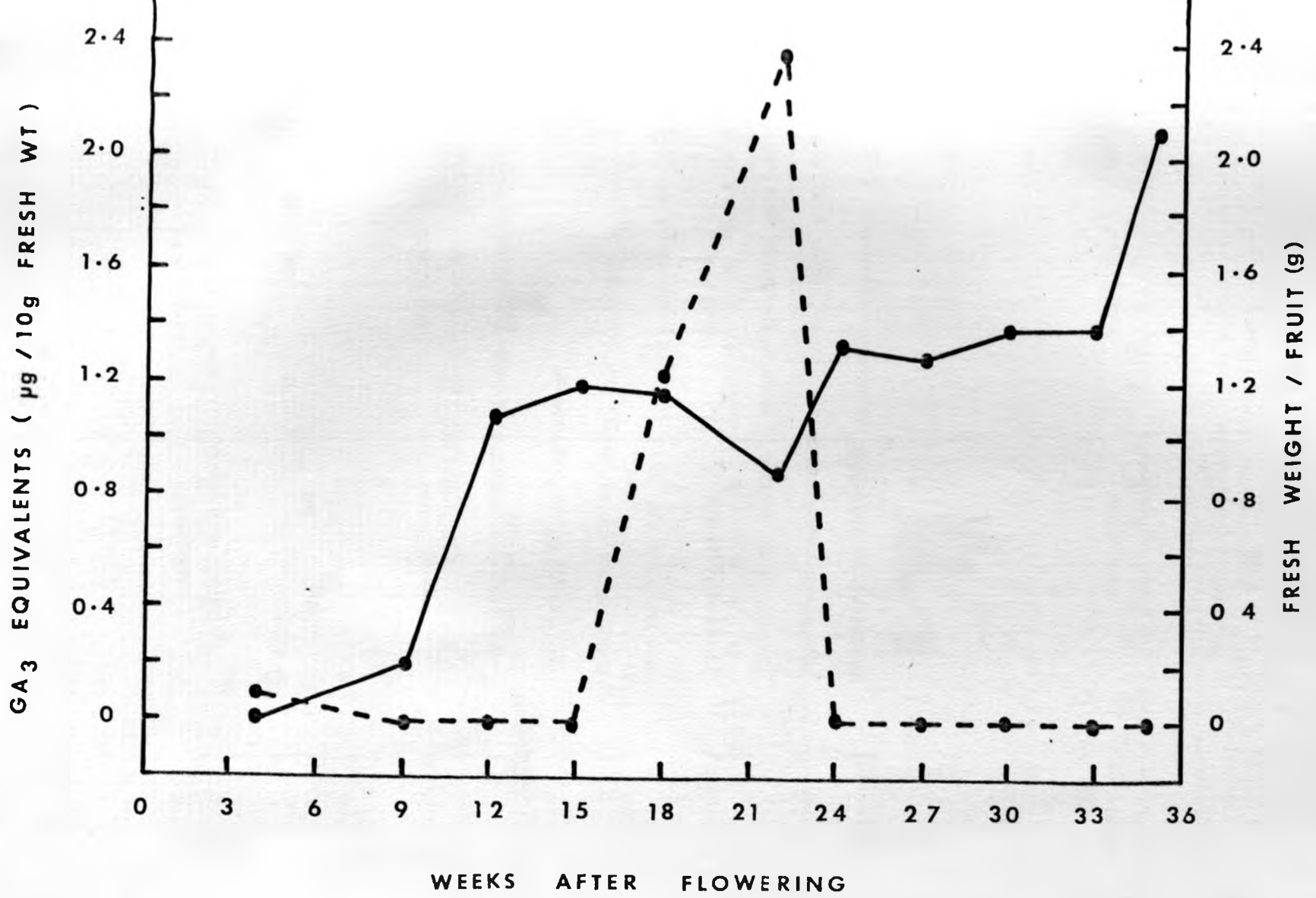

GA3 EQUIVALENTS (µg / 10g FRESH WT)
FRESH WEIGHT / FRUIT (g)
2·4
2·0
1·6
1·2
0·8
0·4
0
0 3 6 9 12 15 18 21 24 27 30 33 36
WEEKS AFTER FLOWERING

Fig.3 Gibberellin bioassay (lettuce hypocotyl test of 10% steps ethyl acetate in n-hexane fractions from silicic acid column. A to K indicate the ages of fruits in weeks:-

A=4 wk, B=9 wk, C=12 wk, D=15 wk, E=18 wk, F=22 wk, G=22 wk, G=24 wk, H=27 wk, I=30 wk, J=33 wk and K=35 wk. L=10 µg GA_3 standard.

The darkened areas indicate significant activity at 1% level of probability.

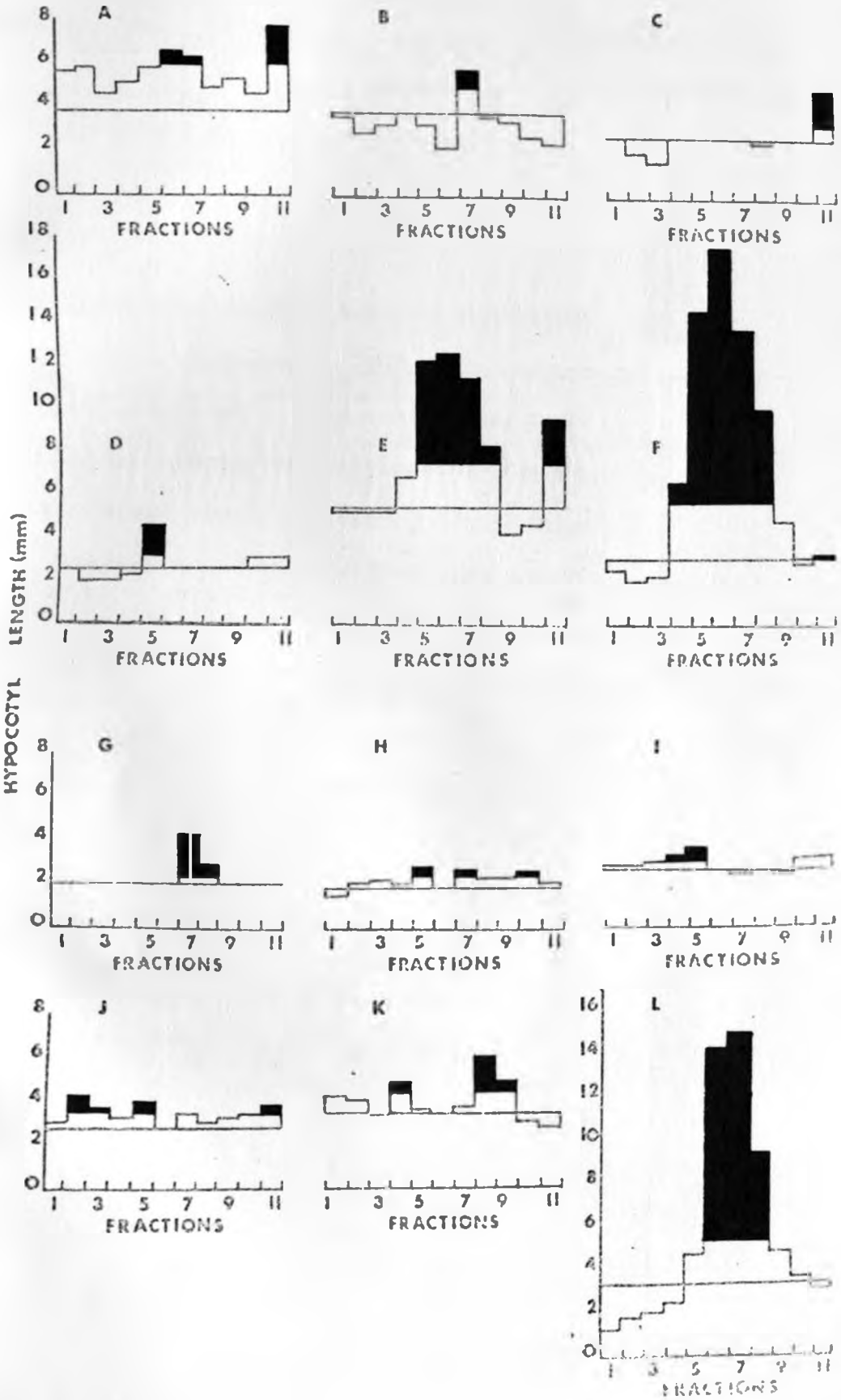

A
B
C
D
E
F
G
H
I
J
K
L
FRACTIONS
HYPOCOTYL LENGTH (mm)

Fig.4 Gibberellin bioassay (Lettuce hypocotyl test) of Whatman No. 1 paper chromatograms loaded with 10 g fresh weight equivalent of the acidic extract. The chromatograms were run in propan-2-ol/water/ammonia solution (10:1:1,v/v/v). A to K indicate the ages of fruits where A=10 days and B to K have ages corresponding to those indicated under Fig.3 while L=10 µg GA_3 standard.

The darkened areas indicate significant activity at 1% level of probability.

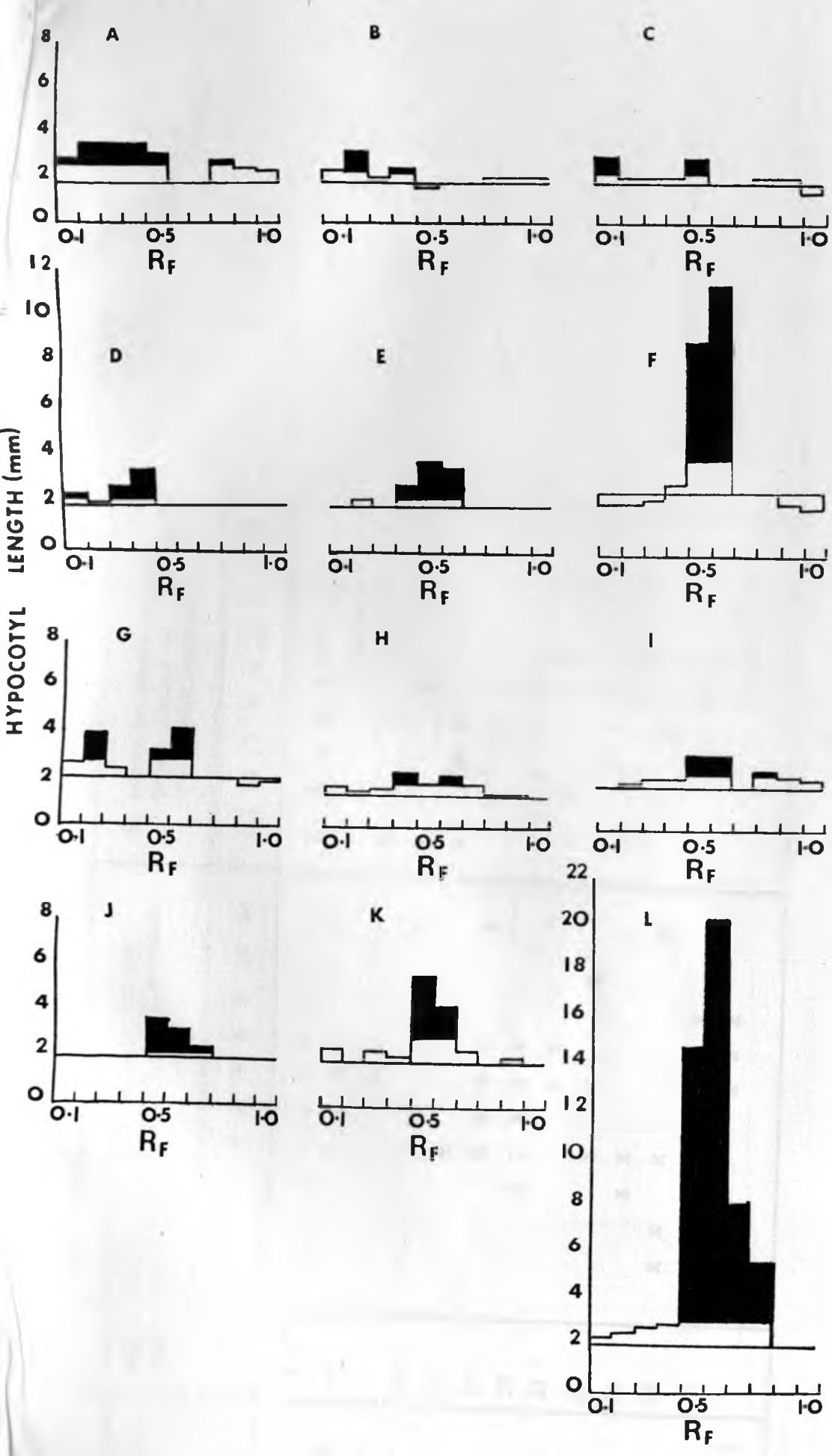

A
B
C
D
E
F
G
H
I
J
K
L
HYPOCOTYL LENGTH (mm)
R_F
0·1
0·5
1·0

Table 1: Zones where significant (P=0.01) growth promotion activity was detected (X) in extracts of Coffea arabica L. fruits of different ages using lettuce hypocotyl assay

Sample No	Fruit Age wks	Fractions from silicic column using 10: steps of ethyl acetate in n-hexane											R_F zones in zones chromagram; 10:1:1 v/v (propan-1-ol:water:ammonia)									R_F zones in silica gel G:15:5:1 v/v (ethyl acetate:chloroform: acetic acid)										
		1	2	3	4	5	6	7	8	9	10	11	0.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0	0.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
A1	1.5	-	-	-	-	-	-	-	-	-	-	-	X	X	X	X	X			X				X								
A2	4						X	X					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B								X					X	X		X										X					
C	12											X	X				X									X					
D	15					X							X		X	X									X	X	X				
E	18					X	X	X	X			X				X	X	X					X		X	X					
F	22				X	X	X	X	X								X	X						X		X				X	
G	24							X	X					X			X	X							X	X					
H	27					X		X			X				X		X									X		X			
I	30				X	X											X	X		X							X				
J	33		X	X		X						X				X	X	X								X	X				
K	35				X				X	X							X	X								X	X				X
GA_3	-							X	X	X							X	X	X	X						X	X				

Table 2: Fruit age, mean weight per fruit at sampling and gibberellin-like substances (GLS) in *Coffea arabica* L. fruit grown in the field at Coffee Research Station, Ruiru (CRS)

Sample No	Location at CRS (Plot No)	Fruit age (weeks)	Number of fruits sampled per 100 g Fresh weight	Fresh weight per fruit (g)	GLS levels as % above control		
					Silicic column	Paper 10:1:1	Silica gel G 15:15:1
A1	3	1.5 (10 days)	12500	0.008	-	25.8	23.3
A2	5	4	8340	0.012	23.7	-	-
B	5	9	500	0.200	14.0	29.6	11.11
C	3	12	92	1.087	47.1	38.6	12.0
D	3	15	84	1.190	39.4	36.9	15.23
E	3	18	86	1.163	54.1	47.8	9.26
F	5	22	107	0.935	130.0	161.5	105.77
G	5	24	75	1.299	48.0	34.48	38.33
H	5	27	75	1.282	11.0	16.7	9.72
I	3	30	70	1.429	8.0	36.0	14.3
J	5	33	71	1.408	3.0	40.9	17.4
K	10	35	46	2.174	18.18	74.1	32.6

Fig.5 Activity of the GLS in the 22 week old fruits extract (10 g fresh weight equivalent) loaded on thin layer plates coated with 400 µm thick silica gel G and run in different solvent systems (1-4):

1. Benzene/butan-1-ol/acetic acid (75:25:5,v/v/v)
2. Ethyl acetate/chloroform/acetic acid (15:15:1/v/v/v)
3. Propan-2-ol/water/ammonia solution (10:1:1,v/v/v)
4. Water (about pH 4.3)

The darkened areas indicate significant activity at 1% level of probability.

HYPOCOTYL LENGTH (mm)
GA3 ZONE
1
2
3
4
0
0·5
1·0
R_F

Table 3: R_F values in five solvent systems on TLC using Kieselgel G for fruit gibberellin. The activities were detected by spraying and viewing the TLC plates under U.V. (MacMillan and Suter,1963).

	R_F values of the active zones			
	Fruit extract	GA_3	GA_4	GA_7
A Benzene:butan-1-ol: acetic acid(75:25:5 v/v/v)	0.5-0.6	0.5-0.6	1.0	1.0
B Water (about pH 4.2)	1.0	1.0	0.9-1.0	0.9-1.0
C Propan-2-ol:water: ammonia 10:1:1(v/v/v)	0.7-0.9	0.7-0.8	0.8	0.8-0.9
D Di-isoprophyl ether: acetic acid (95:5 v/v)	0.0-0.1	0.3-0.4	0.4-0.5	0.4-0.5
E Ethyl acetate:chloroform acetic acid (15:5:1 v/v/v)	0.1-0.4	0.5	0.7-0.8	0.6

Bioassay using gibberellic acid (GA_3) standard showed activity in R_F 0.3-0.4 (Fig.5). Fluorescing bands due to GA-like activity (MacMillan and Suter, 1963) were also possible to detect on the plates when other four solvent systems were used. This was to check whether the acidic GA extract co-chromatographed with authentic GAs (GA_3, GA_4 and GA_7 (Table 3). Gibberellin-like substances activity in the extract coincided with that of all the authentic GAs used in between R_Fs 0.7 and 0.9 when propan-2-ol:25% ammonia hydroxide (0.90):water (10:1:1 v/v/v) was employed. All the three authentic GAs moved to more or less the same R_Fs as the extract between 0.9 /when water was /and 1.0 used as a solvent.

Figure 2 shows the changes in the total amount of the acidic GLS activity in the fruits. Upto 15 weeks from anthesis little GLS activity was detected in the extracts. Subsequently, however, there was a very rapid build up of GLS and highest concentration was recorded in the 22nd week. Earlier work by Wormer (1966) has shown that this is the stage at which the seed (endosperm) in the fruit locule attains the maximum size. After the 22 weeks the amount of GLS fell. Mature fruits contained low amounts of GLS.

Detection and identification of ABA-like substances

Results which demonstrate the presence of ABA-like inhibitors in the neutral fraction (pH 8.0) in extracts of fruits of 11 different ages are depicted in Fig. 7. The extracts were found by wheat coleoptile assay to contain

inhibitors in the neutral fraction (pH 8.0) in extracts of fruits of 11 different ages are depicted in Fig. 7. The extracts were found by wheat coleoptile assay to contain inhibitor usually running at R_Fs between 0.6 and 0.8 on silica gel GF_{254} plates developed in propan-2-ol: water 25% ammonia hydroxide (80:19:96:0. 05 v/v). Authentic ABA was detected in R_F 0.6 in the same solvent system (Fig. 7L). The plates were viewed under UV lamp after development and each of the extracts examined showed quenching band on the TLC plates. The band was visible in R_F values 0.6 which co-chromatographed with authentic ABA marker spots.

The neutral inhibitor fraction of a 22 week old fruit extract was used for co-chromatography studies on TLC plates coated with silica gel GF_{254} and the results obtained after developing the plates in three other solvent systems are depicted in Fig. 8. The extract co-chromatographed with authentic ABA in all the three solvent systems Viz. (i) benzene:butan-1-ol:acetic acid (75.25:5 v/v): (ii) benzene: ethyl acetate: acetic acid (50:5:2 v/v and (iii) water at pH 4.3 where they moved to R_Fs 0.9-1.0, 0.1 and 0.9 respectively.

A typical peak obtained in the samples run in the GLC is shown in Fig. 9. The coffee fruit inhibitor showed slightly different retention time from cis-trans ABA.

Figure 10 shows activity of the ABA-like inhibitors

soluble in acidic (1) and neutral (2) DIPE fractions. Significant ABA-like substances were present at the R_F value to which normally authentic ABA moves (R_F 0.6 in propan-2-ol water water: ammonia hydroxide (80:19:95:0.05 v/v). Some inhibitory activities were also detectable at R_F values outside the ABA zone (also see Fig. 7).

ABA-like inhibitor levels at various fruit stages

The relationship between the levels of inhibitors and fruit age, on fresh weight basis is presented in Fig. 6. The total activity of the inhibitors was worked out in the manner described for the GA assays after running 10 g fresh weight equivalent of the DIPE-soluble neutral (pH 8.0) and acidic (pH 4.0) inhibitor extracts on silica gel GF_{254} plates in propan-2-ol:water:25% ammonia (80:19.95:0.05 v/v). The total amount of the DIPE-soluble inhibitors detected in the neutral fraction (pH 8.0) and in the ethyl acetate-soluble acidc fraction (pH 2.5) are presented in Fig. 6.

The distribution of the inhibitor activity appeared to be bimodal during fruit growth. Levels of the inhibitors increased as the fruits continued to expand rapidly. The inhibitor level started to decline when the fruits were 12 weeks old, which is about the time when the endosperm would be expected to begin forming in the fruits. The minimum level of activity of the inhibitors was detected in the 18 to 22-week

old fruits which were fully expanded, but thereafter the levels again rose progressively as the fruits continued to be in the dry matter accumulation stage until a maximum amount was detected in the 33-week old fruits which had started ripening. Thereafter the inhibitors activity level dropped sharply and was low in fully ripe fruits.

The use of paper and column chromatography facilitated detection of GA-like activity at the various stages of fruit development. The GLS levels increase from about the time the endosperm locule begins to grow until the maximum is reached at about 22 weeks from anthesis. At this stage the locule is normally fully grown and a second period of the fruit growth begins when dry matter accumulation process starts in the endosperm (Wormer, 1966). During later stage from 24 to 27 weeks very little GLS was detected including fully mature fruits which were over 27 weeks old.

The pattern of the GLS activity in relation to fruit growth found in this study is similar to that observed by Luckwill et al., 1969) in apples. The pattern is also similar to that reported for other fruits such as tomatoes where the endogenous GLS showed more or less bimodal distribution (EL Beltagy et al., 1976) but differs from that of citrus where GLS are said to be abundant in mature fruits (Goren and Goldchmidt, 1970). It is noteworthy that the levels of GA activity in this study are low in mature fruits However, this is in agreement with well established

Fig. 6 Changes in ABA-like inhibitors in the developing fruits of Coffea arabica L. The solid line shows the growth of the fruits. The broken line shows the inhibitors in the DIPE soluble pH 8 fraction (closed circles) and the Ethyl acetate soluble pH 2.5 fraction (open circles).

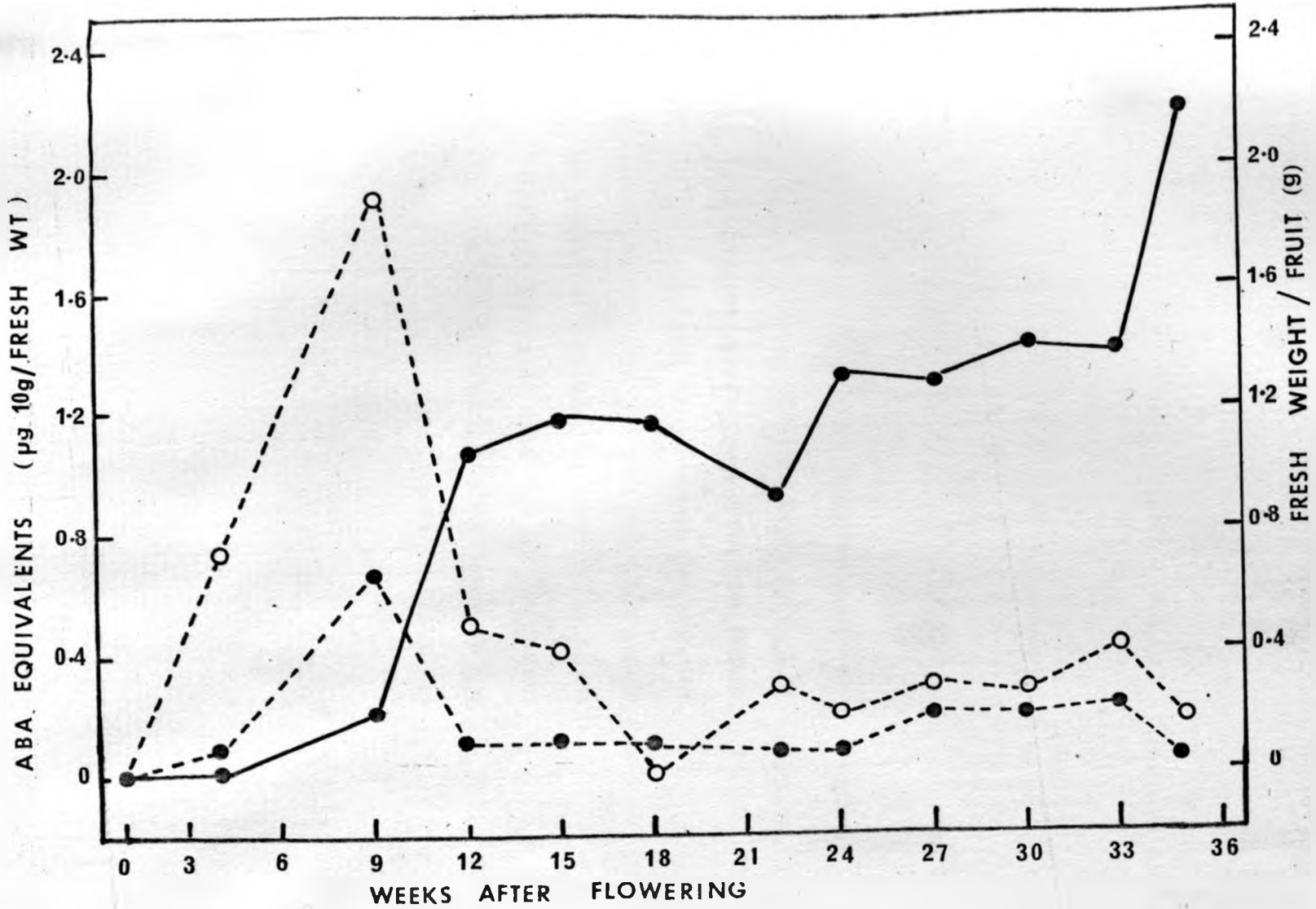

ABA EQUIVALENTS (µg 10g/FRESH WT)
FRESH WEIGHT / FRUIT (g)
WEEKS AFTER FLOWERING
0
0·4
0·8
1·2
1·6
2·0
2·4
0
3
6
9
12
15
18
21
24
27
30
33
36

Fig. 7 ABA bioassay (wheat coleoptile test) of silica gel GF_{254} thin layer chromatograms. The plates were loaded with 10 g fresh weight equivalent of the Diisoprophyl ether (DIPE) (pH 8.0). The chromatograms were run in propan-2-ol/water/ammonia solution 80:19.95:05, v/v/v). The ages of fruits and symbols correspond to the ones indicated in Fig.3. The darkened areas indicate significant activity at 1% level of probability.

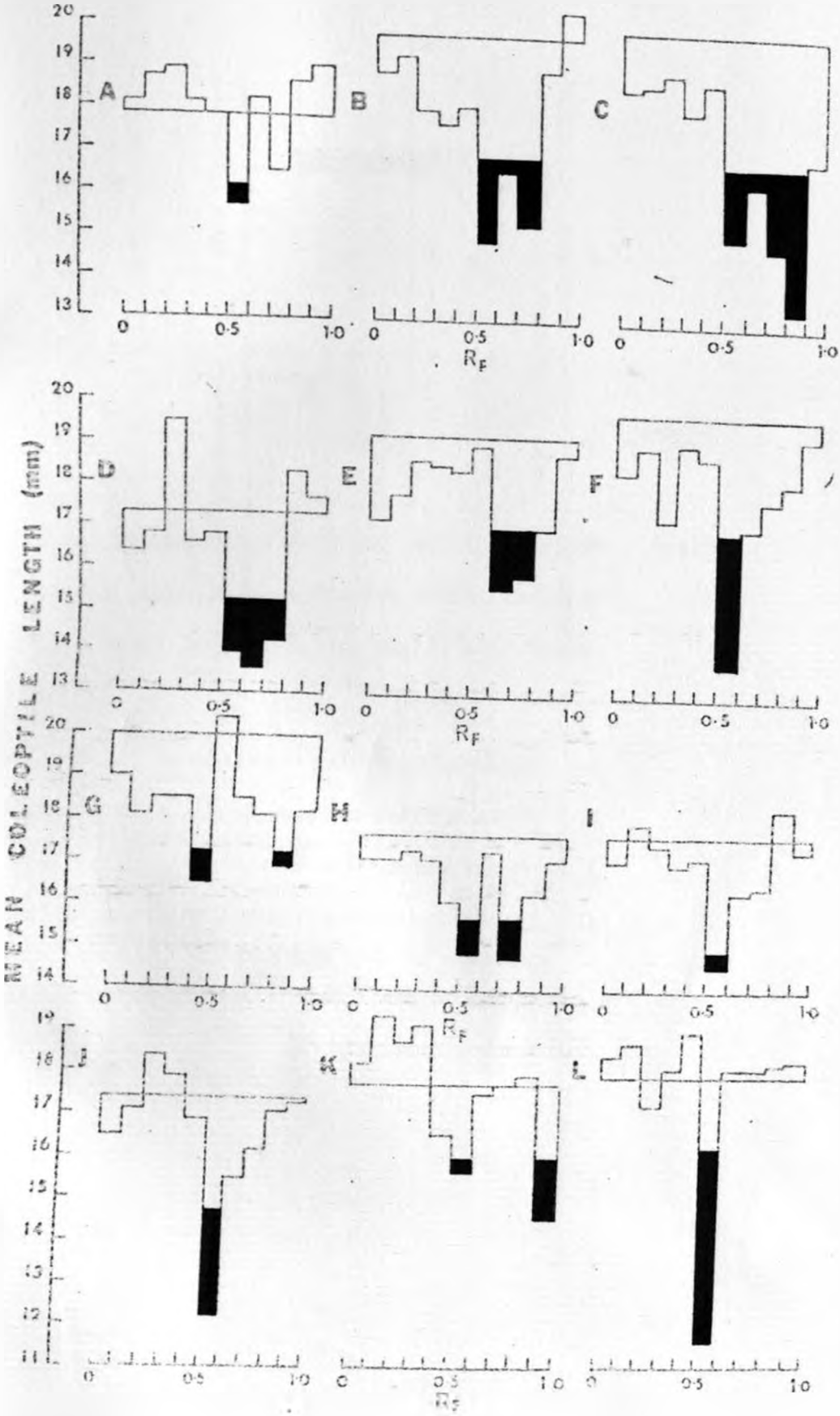

MEAN COLEOPTILE LENGTH (mm)
A
B
C
D
E
F
G
H
I
J
K
L
R_F
0
0·5
1·0

Fig.8 Activity of the ABA-like substances of the 22 week old fruits extract on thin layer plates coated with silica gel GF_{254} and run in different solvent systems:

1. Benzene/butan-1-ol/acetic acid (75:25:5. v/v)
2. Benzene/Ethyl acetate/acetic acid (50:50:2. v/v)
3. Water (about pH 4.3)
4. Propan-2-ol/water/ammonia solution (80:19.95:05, v/v/v).

The darkened areas indicate significant activity at 1% level of probability.

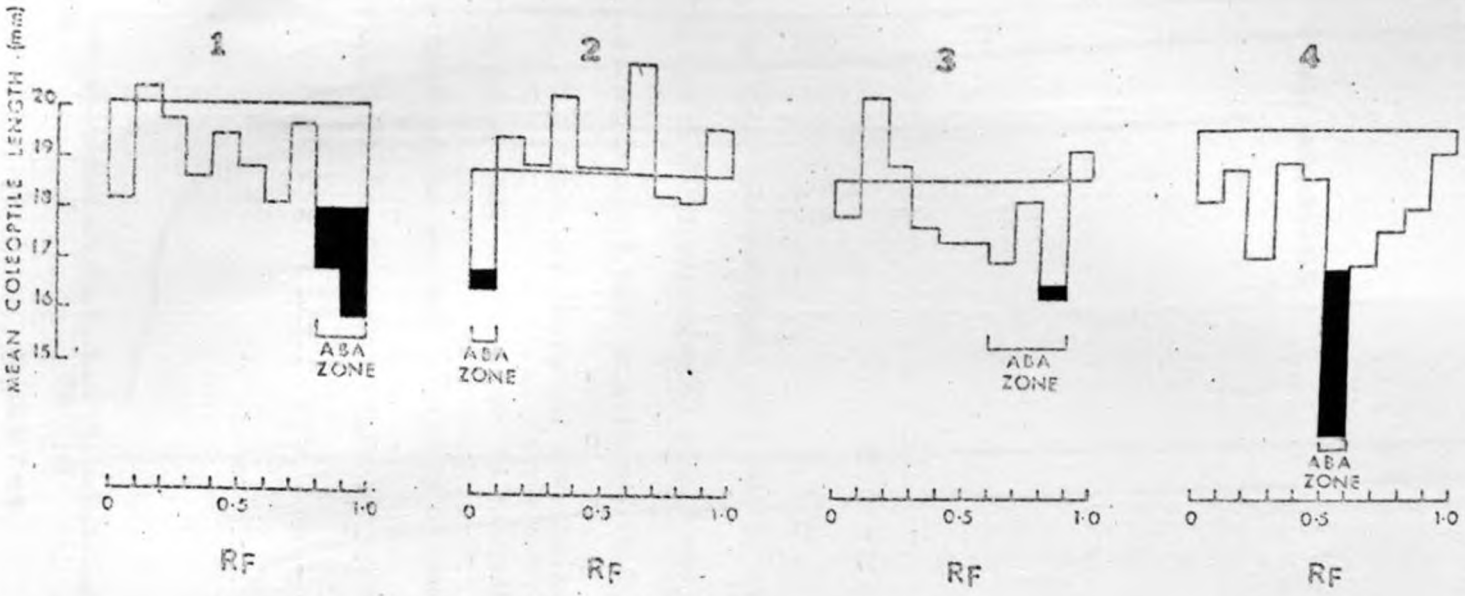

MEAN COLEOPTILE LENGTH (mm)
20
19
18
17
16
15
1
2
3
4
ABA ZONE
ABA ZONE
ABA ZONE
ABA ZONE
0
0·5
1·0
RF

Fig.9 Peaks of ABA-like inhibitor detected by the Gas Liquid Chromatography from the DIPE (pH 8.0) fraction of the fruit extracts. The 25 µl acetone injected contained 0.25 g fresh weight equivalent of the fruits. The values of the authentic cis-trans ABA are on the vertical axis on the right hand side of the Figure.

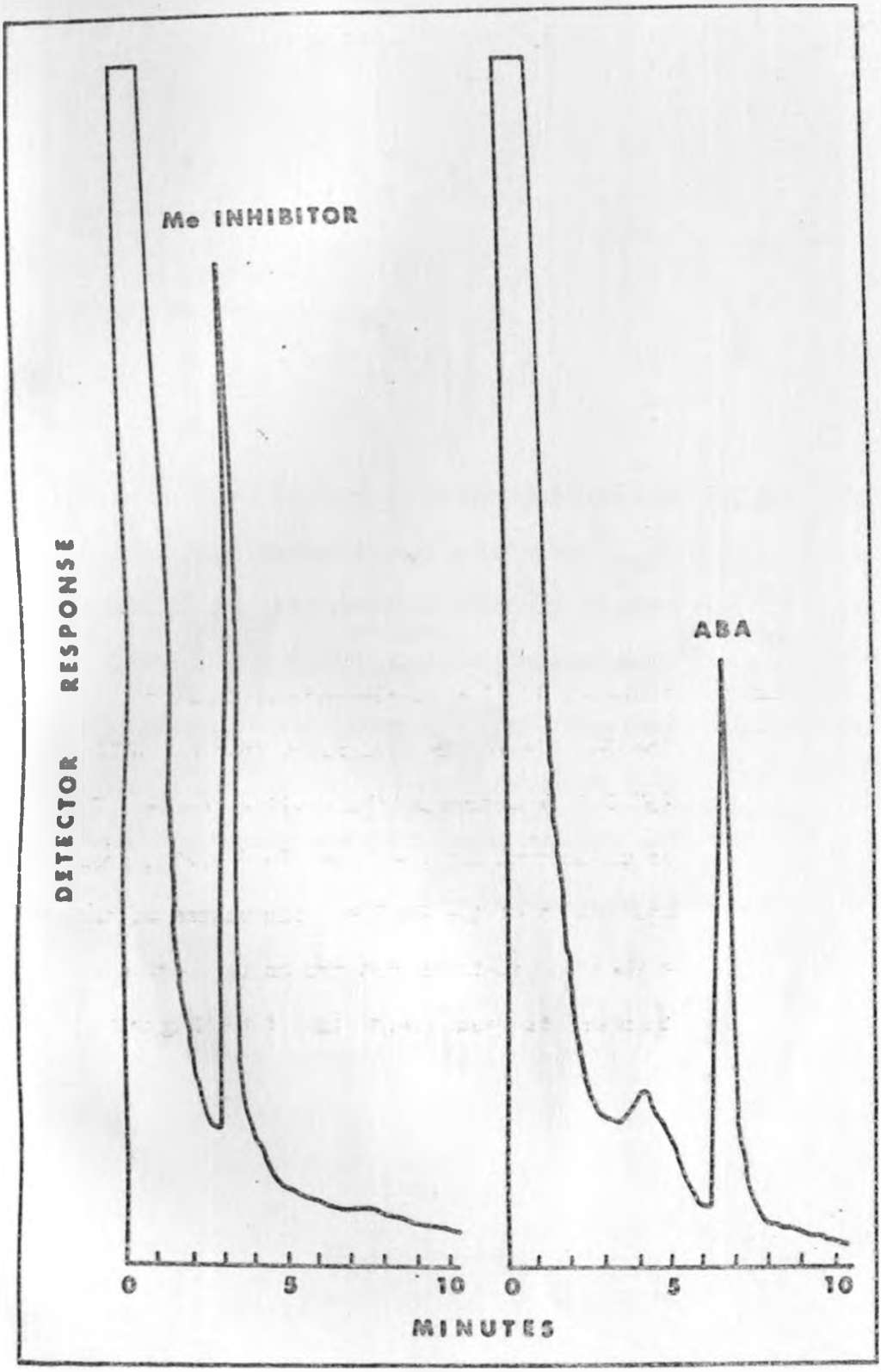
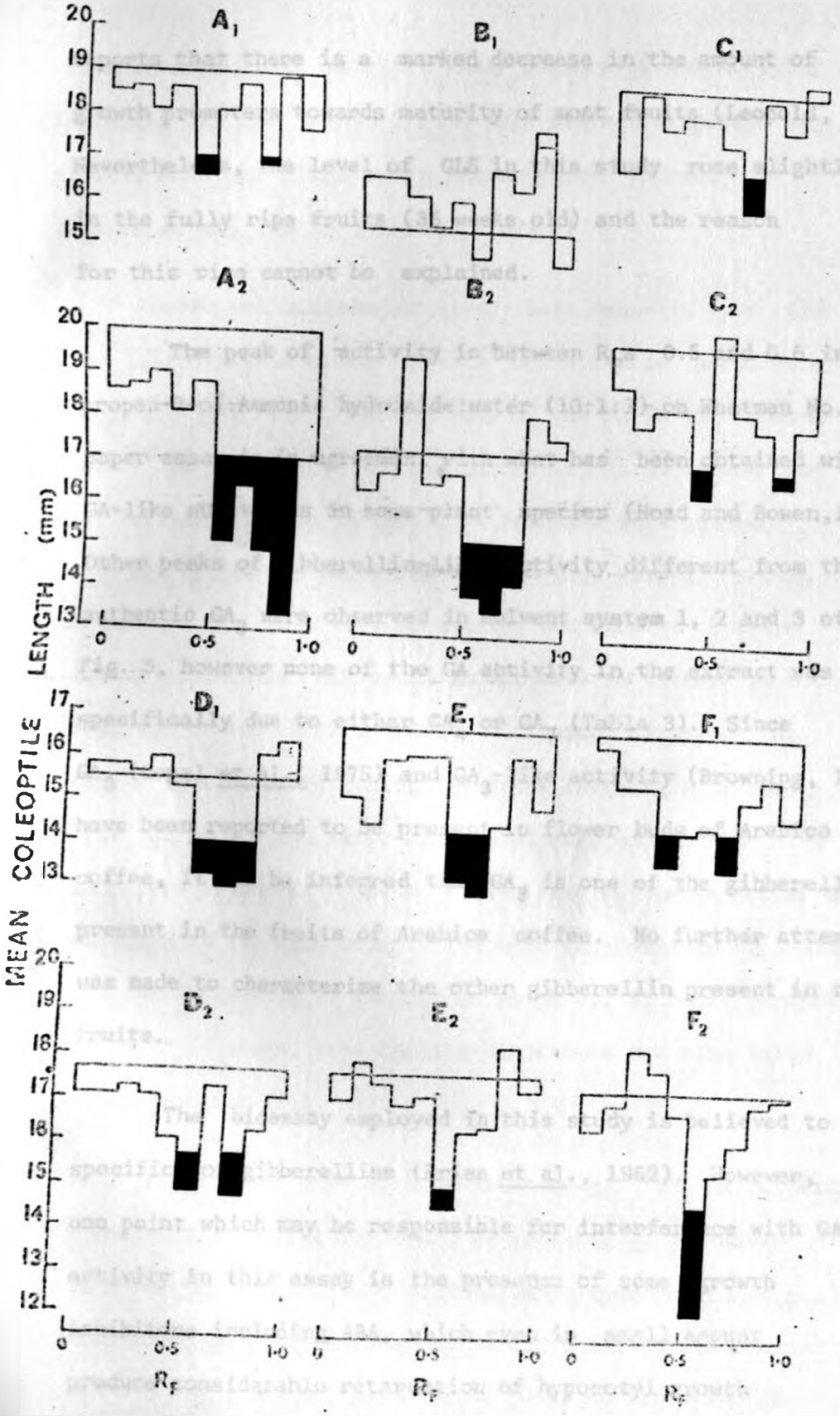

Me INHIBITOR
ABA
DETECTOR RESPONSE
0
5
10
0
5
10
MINUTES

Fig.10 ABA bioassay (wheat coleoptile test) of silica gel GF_{254} thin layer chromatograms. The acid (1) and neutral (2) fruit extracts were run in propan-2-ol/water/ammonia solution (80:19.95:0.5,v/v/v).

A=12 wk, B=15 wk, C=24 wk, D=27 wk, E=30 wk, and F=33 2k old fruits.

The darkened areas indicate significant activity at 1% level of probability.


A1
B1
C1
A2
B2
C2
D1
E1
F1
D2
E2
F2
LENGTH (mm)
MEAN COLEOPTILE
Rf

reports that there is a marked decrease in the amount of growth promoters towards maturity of most fruits (Leopold, 1964). Nevertheless, the level of GLS in this study rose slightly in the fully ripe fruits (35 weeks old) and the reason for this rise cannot be explained.

The peak of activity in between R_Fs 0.5 and 0.6 in propan-2-ol:Ammonia hydroxide:water (10:1:1) on Whatman No. 1 paper assay is in agreement with what has been obtained with GA-like substances in some plant species (Hoad and Bowen,1968). Other peaks of gibberellin-like activity different from that of authentic GA_3 were observed in solvent system 1, 2 and 3 of Fig. 5, however none of the GA activity in the extract was specifically due to either GA_4 or GA_7 (Table 3). Since GA_3 (Gopal et al., 1975) and GA_3-like activity (Browning, 1973 a) have been reported to be present in flower buds of Arabica coffee, it can be inferred that GA_3 is one of the gibberellins present in the fruits of Arabica coffee. No further attempt was made to characterize the other gibberellin present in the fruits.

The bioassay employed in this study is believed to be specific for gibberellins (Brian et al., 1962). However, one point which may be responsible for interference with GAs' activity in this assay is the presence of some growth inhibitors including ABA, which even in small amount produce considerable retardation of hypocotyl growth (Wareing et al., 1968). It was for this reason that further

steps were taken to obtain a clear measure of the activity by including 0.5 ppm zeatin in the test media of fraction of silicic acid and silica gel G chromatograms assayed with lettuce as was done by Browning (1973 a).

Goren and Goldschmidt (1970) have reported that ABA-like inhibitors in citrus fruits which can be partitioned at pH 6.0 into DIPE, leaving most of the GA in the aqueous phase. In this study the preliminary trials indicated that GA_3 activity was low in the DIPE fraction when aqueous solution of GA_3 was partitioned at pH 4.0. But it should be pointed out here that no attempt was made to confirm whether authentic ABA would go entirely to either pH 8.0 or pH 4.0 of the DIPE fraction. It cannot therefore be assumed that by partitioning the fruit extract in DIPE, first at pH 8.0 and later at pH 4.0, most of the ABA- like inhibitors would be removed leaving most of the GA behind as was intended for this study. The main disadvantage of using DIPE for extraction of inhibitors at pH 4.0 is that, depending on the fruit stage, some GA-like substances are also taken in pH 4.0 fraction (Fig. 11).

It is clear that at some fruit stages the GA activity in the acidic GA fraction could be detected by bioassays after either the silicic acid column, paper or silica gel G chromatography, apparently by partitioning at the desired pH. But at certain stages, for example prior to endosperm growth stage and also between 24 and 27 week old stages, it proved

difficult to separate the acidic growth promoters convincingly from inhibitory activity associated with ABA. It may therefore be argued that the absence of more than one peak of GA activity from the chromatograms illustrated in solvent system 4 of Fig.5 (water) reflected interference from inhibitory materials which appeared in the same R_F zone. It is also possible that the two gibberellins in this particular solvent system run at the same R_F.

Like the GLS, the inhibitor activity was detected at all fruit stages during fruit growth. However, the distribution appeared to be bimodal with one peak of activity appearing at the beginning of endosperm growth and the other at the start of ripening (Fig. 6). The inhibitor levels were found to rise progressively as the fruits continued to grow and the activity became minimal when the fruits were already fully expanded before rising again to even higher levels when the fruits were ripening. It is possible that the two peaks of ABA like inhibitor may be correlated with the various fruit stages mentioned above.

The data presented here are more or less similar to those obtained in studies with other fruits. For example, in developing cotton fruits (Davis and Addicot, 1972), the first rapid rise in ABA levels is correlated with the period of young fruit abscission and a second peak at fruit maturation which includes fruit wall senescence. In grapes, for instance,

maximum ABA accumulates at fruit maturation during ripening (Coombe and Hale, 1973). In tomato it has been reported that ABA-like activity increased gradually during fruit growth and development, reached a peak at the green mature stage (Dörffling, 1970; Abdel-Rahman et al., 1975) and decreased during ripening (Dörffling, 1970). However, the pattern of changes in the inhibitor levels found in this study differs from that reported for the ABA-like substances in some other crops. In avocado for example, the amount of ABA in the mesocarp was found to be constant during fruit growth (Gazit and Blumenfeld, 1970). Also in wheat (McWha, 1975) and soyabean (Quebedeaux et al., 1976), maximum ABA accumulates in developing seeds during the most active growth period. It therefore appears that the stage of fruit at which maximum ABA or ABA-like inhibitors occur, vary from one species to the other.

It is clear from Fig. 7F, for instance, that the activity of the inhibitor in the neutral fraction co-chromatographed with the authentic ABA when propan-2-ol:water:ammonia hydroxide (80:19.95:0.05 v/v/v) was used to develop the extract on silica gel GF_{254}. Corroborative evidence was obtained from running aliquots of the extract on silica gel GF_{254} plates in three other solvents (Fig.8). As indicated in Fig.8 the inhibitor co-chromatographed with authentic ABA in all the three solvent systems.

Fig. 11 Gibberellin bioassay (Lettuce hypocotyl test) of Whatman No. 1 paper chromatograms run in propan-2-ol/water/ammonia solution (10:1:1, v/v/v). Extracts were either soluble in DIPE (1) at pH 4.0 or in ethyl acetate (2) at pH 2.5. Each chromatogram was loaded with 10 g fresh weight of the fruit extract. The symbols and fruit ages are as shown under Fig. 10. The darkened areas indicate significant activity at 1% level of probability.

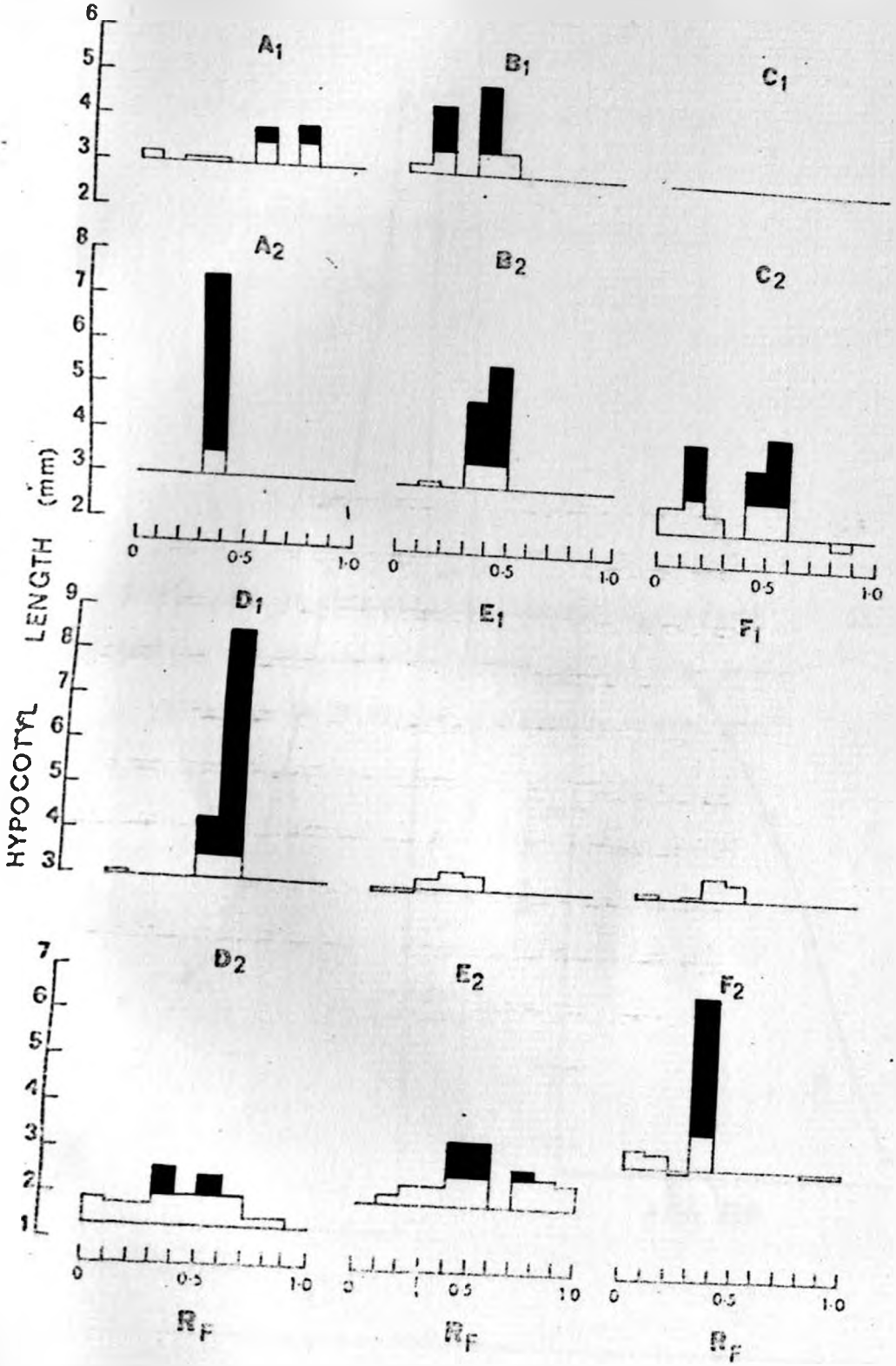
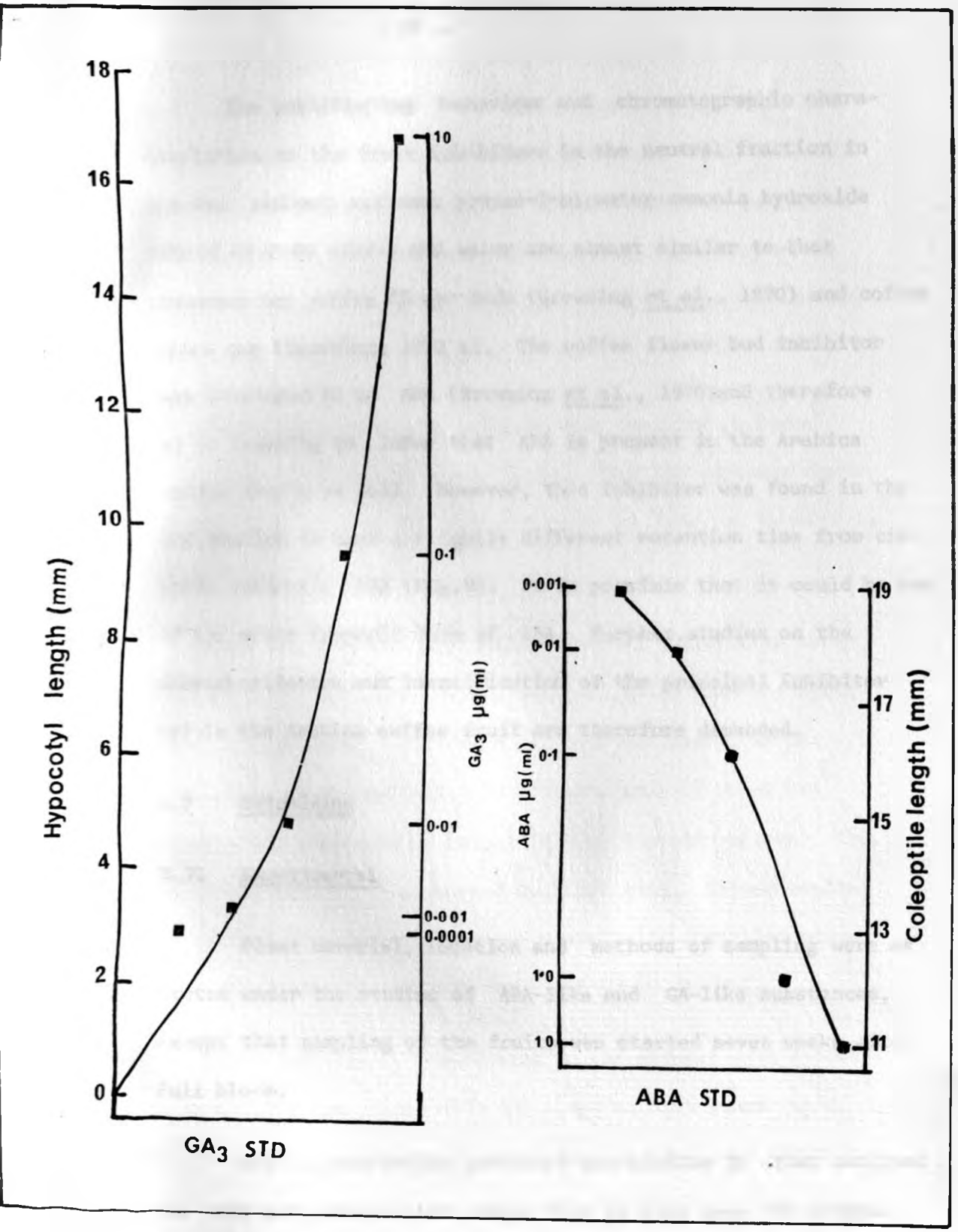

A1
B1
C1
A2
B2
C2
D1
E1
F1
D2
E2
F2
HYPOCOTYL LENGTH (mm)
RF
RF
RF

Fig. 12 Response to the various standards of GA_3 (left) and ABA (right) concentrations used for estimating the levels of activity in the fruit extracts.


Hypocotyl length (mm)
18
16
14
12
10
8
6
4
2
0
10
0·1
0·01
0·001
0·0001
GA3 µg(ml)
GA3 STD
0·001
0·01
0·1
1·0
10
ABA µg(ml)
19
17
15
13
11
Coleoptile length (mm)
ABA STD

The partitioning behaviour and chromatographic characteristics of the fruit inhibitors in the neutral fraction in the two solvent systems; propan-2-ol:water:ammonia hydroxide (80:19.95:0.05 v/v/v) and water are almost similar to that observed for coffee flower buds (Browning et al., 1970) and coffee xylem sap (Browning, 1973 a). The coffee flower bud inhibitor was concluded to be ABA (Browning et al., 1970)and therefore it is tempting to infer that ABA is present in the Arabica coffee fruits as well. However, this inhibitor was found in the GLC studies to have a slightly different retention time from cis-trans authentic ABA (Fig.9). It is possible that it could be one of the other isomeric form of ABA. Further studies on the characterization and identification of the principal inhibitor (s) in the Arabica coffee fruit are therefore demanded.

3.2 Cytokinins

3.21 Experimental

Plant material, location and methods of sampling were as stated under the studies of ABA-like and GA-like substances, except that sampling of the fruits was started seven weeks after full bloom.

Initial extraction procedure was similar to that outlined for ABA and gibberellins except that in this case 70% ethanol was used instead of 90% methanol. The volume of the aqueous

extract was adjusted to 200 ml with distilled water and centrifuged at 8000 g for 1 h. After centrifuging, the supernatant was adjusted to pH 6.5 with 50% ammonia solution and partitioned 4 times against equal volumes of water-saturated butan-1-ol. The pooled butan-1-ol fractions were evaporated *in vacuo* and taken in 15 ml distilled water for loading onto a Dowex column for further purification.

The aqueous butanolic extracts were purified in Dowex 1 (Chloride from, 50-100 mesh, Sigma, USA) ion exchange resin which removed most of the coloured material from the extract (Browning *et al.*, 1970).

Dowex was prepared by washing first with distilled water, followed by IN HCl, then water, followed by IN NaOH and again water in this order. The prepared Dowex was stored in IM formic acid until required. The formate form of Dowex was packed under gravity flow into 15 x 4 cm diameter column. The bed of the column was supported by glass wool. Before loading the extract, the packed Dowex was washed with distilled water until the pH of the effluent was found to be neutral.

After loading, the cytokinins in the extract were recovered by eluting the column with one litre of 0.2% formic acid, and this was then evaporated to dryness *in vacuo* and flashed with a stream of dry N_2 gas to remove the formic. The residue was re-dissolved in 15 ml of 10% aqueous methanol for loading onto 12 x 2 cm diameter 'sintered' column packed with zerolit

225 (formerly Zeo-karb 225), 100-200 mesh, cation exchange resin, chromatograph grade. The packed zerolit was washed with distilled water until the pH was neutral before loading the extract.

After loading, the column was first eluted with 250 ml distilled water to remove yellow substances in the extract and the coloured eluates discarded. Cytokinins were then eluted with 250 ml 0.2 N NH_4OH. The ammonical eluate was reduced to dryness in vacuo, dissolved in 10 ml 80% aqueous ethanol and centrifuged at 600 g for 10 min to remove proteins. The supernatant was reduced to dryness in vacuo, dissolved in a small volume of 80% aequeous methanol, which was used subsequently for characterization studies involving chromatography and bioassays.

Detection of the cytokin activity

Using the method of Browning (1973 b), 10 g fresh weight equivalent of the purified methanolic extract was loaded onto 20 x 20 cm plates coated with 400 µm thick layers of cellulose (Macherey-Negel 300 G/UV 254) and developed with six different solvent systems shown in Table 4. Zeatin, zeatin riboside and 6-benzylaminopurine standards were used for comparison. The adsorbent itself fluoresces in ultraviolet light, and the extract and standards were detected under UV lamp (Hanovia, Slough, England), because of their fluorescence quenching property. The adsorbent was scraped from the plates in bands corresponding to each

of the ten R_Fs and eluted with 80% aqueous ethanol.

Percentage Recovery

Recovery percentage of the cytokinin-like substances was assessed by adding 10 μg ^{14}C 6-benzylamino purine (BA) into some of the 100 g fruit samples ready for maceration in the 400 ml 70% aqueous ethanol. Thereafter, the procedure followed for extraction and purification was the same as for other fruit samples. The radioactivity in the purified extract was determined using the liquid scintillation counter described in the Materials and Methods section.

Estimation of the cytokinin-like substances

Using the response curve of the zeatin standard shown in Fig. 14, the value for each of the R_F with significant activity was estimated separately in order to obtain the total amount of activity for each sample.

3.22 Results and Discussion

Characterization and identification of the CLS activity

The distribution of cytokinin-like substances obtained from fruits at various stages of development is shown in Fig. 14. Cytokinin-like activity was detected at various R_Fs varied according to the fruit age at which it was extracted.

Results obtained when the extract from 13 week old fruits was run in water-saturated methyl-ethyl-ketone and compared with zeatin and zeatin riboside standards, are shown in Fig. 15. A peak of activity which co-chromatographed with zeatin and zeatin riboside as detected in R_Fs 0.5-0.7. On cellulose thin-layer chromatograms developed with five other solvent systems and viewed under UV-light, the cytokinin-like substance in the extract had similar R_F to either zeatin, or zeatin riboside, (Table 4). For example, where water was the solvent, the extract and zeatin-riboside had a similar R_F value of 0.9. On the other hand, the extract, zeatin and zeatin riboside each one of these showed an identical value of R_F 1.0 when water-saturated ethyl-methyl-ketone was used as a solvent. Similarly when propan-1-ol:water:25% ammonia hydroxide (80:19.95:0.05 v/v/v) was used as a solvent, the extract, zeatin as well as zeatin riboside had an indentical R_F value of 1.0. The extract also had R_F value of 0.8-1.0 as was the case with zeatin and zeatin riboside when N-Butanol: 25% ammonia hydroxide (4:1 v/v) was used.

Figure 15 b shows that the absorbance characteristics of the extract was similar to that of kinetin (6-Furfurylaminopurine) and zeatin riboside.

Cytokinin-like substances (CLS) levels of various fruit stages

Figure 13 shows the variation in the total amount of butan-1-ol-soluble CLS activity in the fruits. The activity was

Table 4: R_F values of UV absorption in six solvent systems for extract obtained from 13 week-old Arabica coffee fruits. The extract was chromatographed on 20 x 20 cm TLC plates coated with 400 µm thick cellulose (Macherey-Nagel 300 G/UV 254) or silica gel GF (Merck). Extracts and standards (Zeatin and Zeatin riboside were detected under UV lamp from their fluorescence quenching property.

Solvent system	Extract	Zeatin (10 µg)	Zeatin riboside (10 µg)
Water	0.8-0.9	0.5-0.9	0.7-0.9
Water-saturated ethyl methyl ketone	0.9-1.0	1.0	0.9-1.0
N-Butanol:25% ammonia hydroxide (4:1)	0.8-1.0	0.8-1.0	0.7-1.0
Chloroform:methanol (9:1) (Silica gel GF_{254})	0.7-0.8	0.2-0.3	0.1-0.2
Propan-1-ol: Water: Ammonia hydroxide (80:19.95:0.05)	0.8-1.0	0.8-1.0	0.7-1.0

Fig. 13 Levels of cytokinin-like substances (CLS) in the fruits. The broken line shows the levels of CLS while the continuous line shows the growth of the fruits. The CLS levels in all the three peaks of activity were assessed under the areas of histograms that were significantly (P=0.01) different from the control. Ten grammes fresh weight equivalent of fruit extracts were developed with water on Whatman No.1 paper.

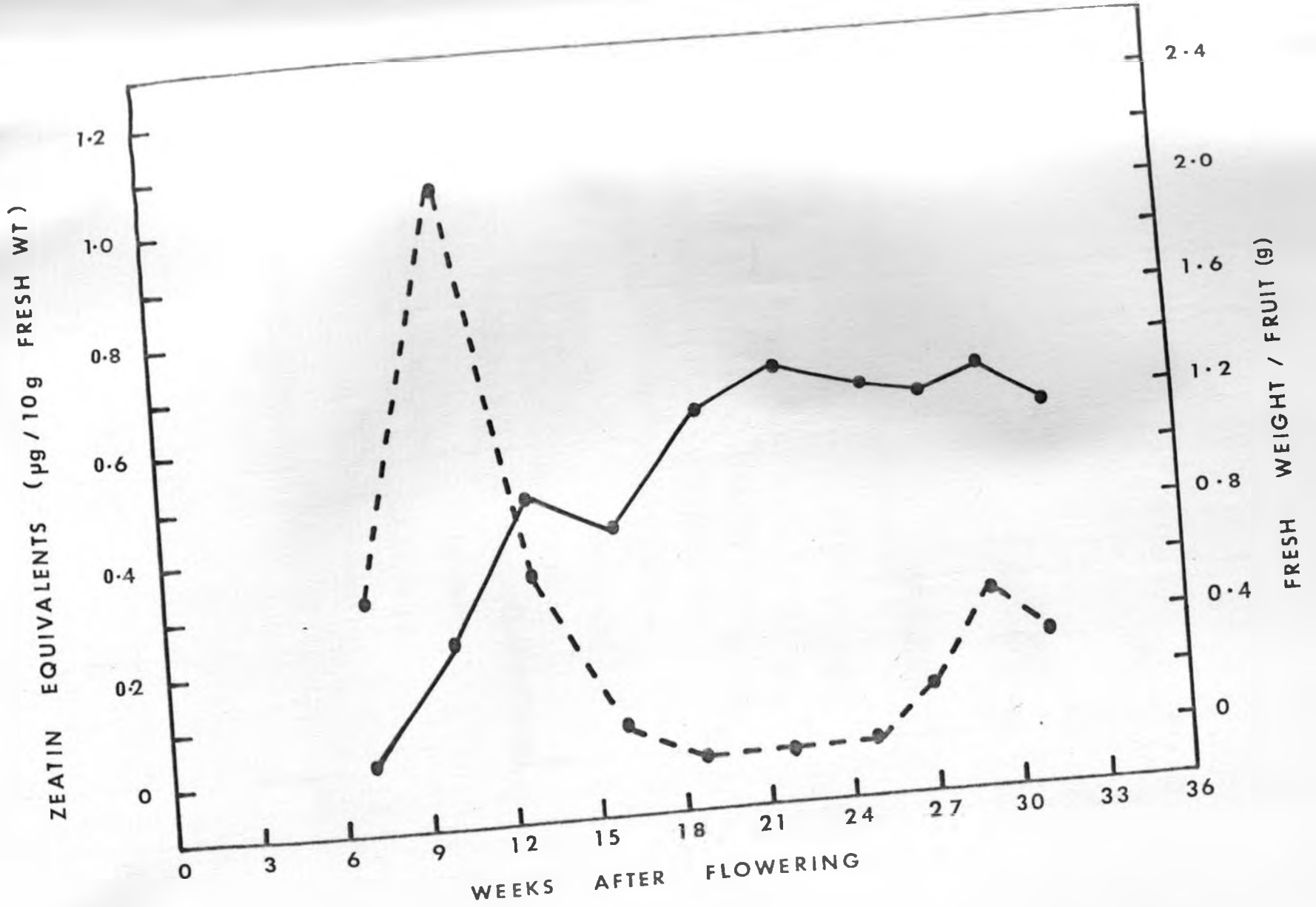

ZEATIN EQUIVALENTS (µg / 10 g FRESH WT)
1·2
1·0
0·8
0·6
0·4
0·2
0
FRESH WEIGHT / FRUIT (g)
2·4
2·0
1·6
1·2
0·8
0·4
0
0 3 6 9 12 15 18 21 24 27 30 33 36
WEEKS AFTER FLOWERING

Fig.14 Cytokinin bioassay (soyabean callus test) of Whatman No. 1 chromatograms in distilled water using 10 g fresh weight equivalent of fruit extracts:

A=7 wk, B=10 wk, C=13 wk, D=16 wk, E=19 wk D=22 wk, G=25 wk, H=27 wk, I=29 wk and J=31 wk old fruits.

The Zeatin standard (10 µl/10 ml of medium) activity under the same bioassay conditions are presented on the right hand side at the top.

The darkened areas indicate significant activity 1% level of probability.

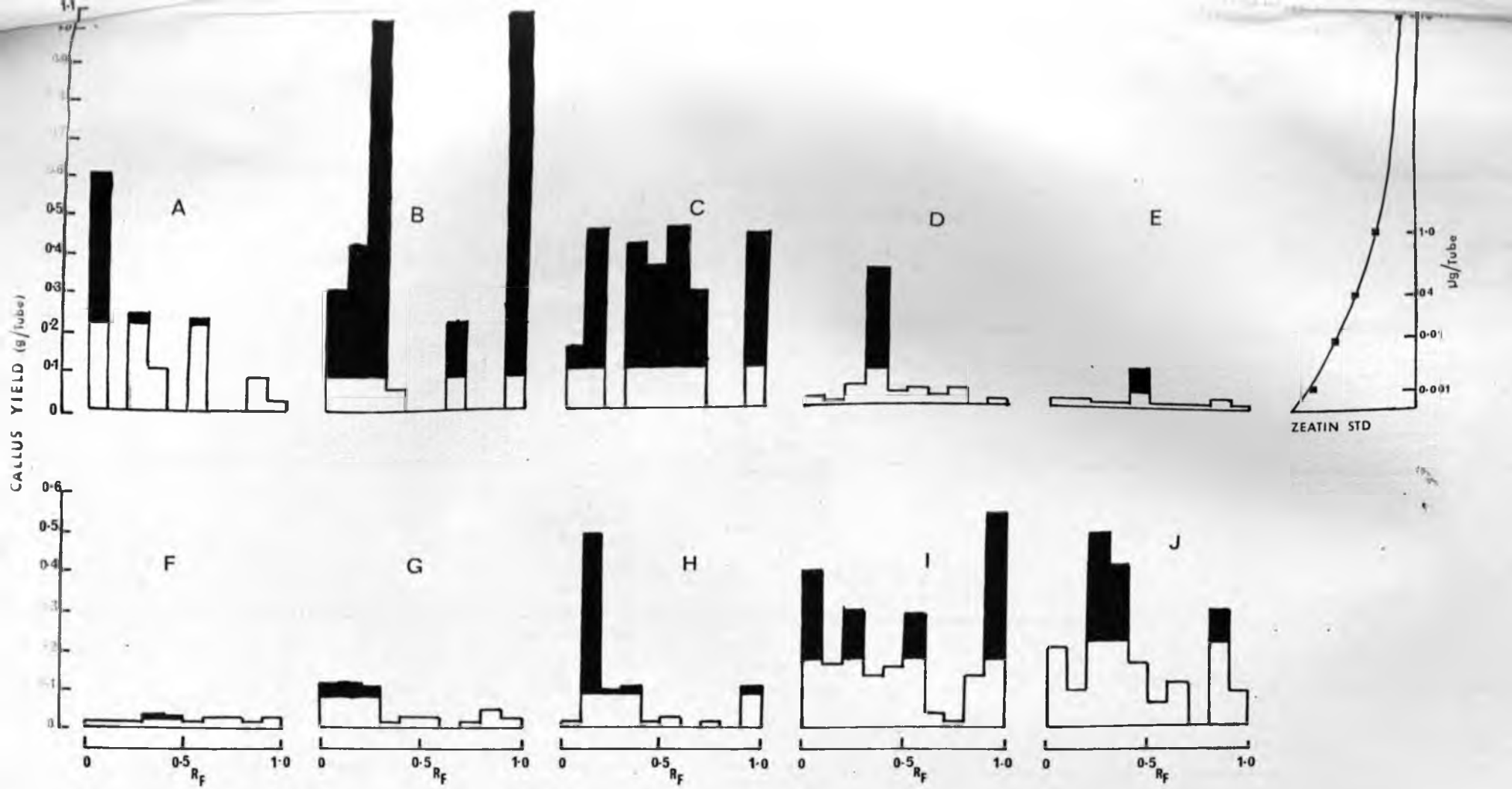

CALLUS YIELD (g/tube)
A
B
C
D
E
F
G
H
I
J
0
0·1
0·2
0·3
0·4
0·5
0·6
1·0
0·4
0·01
0·001
µg/tube
ZEATIN STD
0
0·5
1·0
R_F

Fig. 15 (a) Soyabean bioassay of the extract (10 g fresh weig
equivalent) of the 13 week old fruits. The ext
were run in water-saturated ethyl-methyl-ketone
thin layer plates coated with cellulose 300G/UV
Z=Zeatin, ZR=Zeatin riboside.
The darkened areas indicate significant activity
1% level of probability.

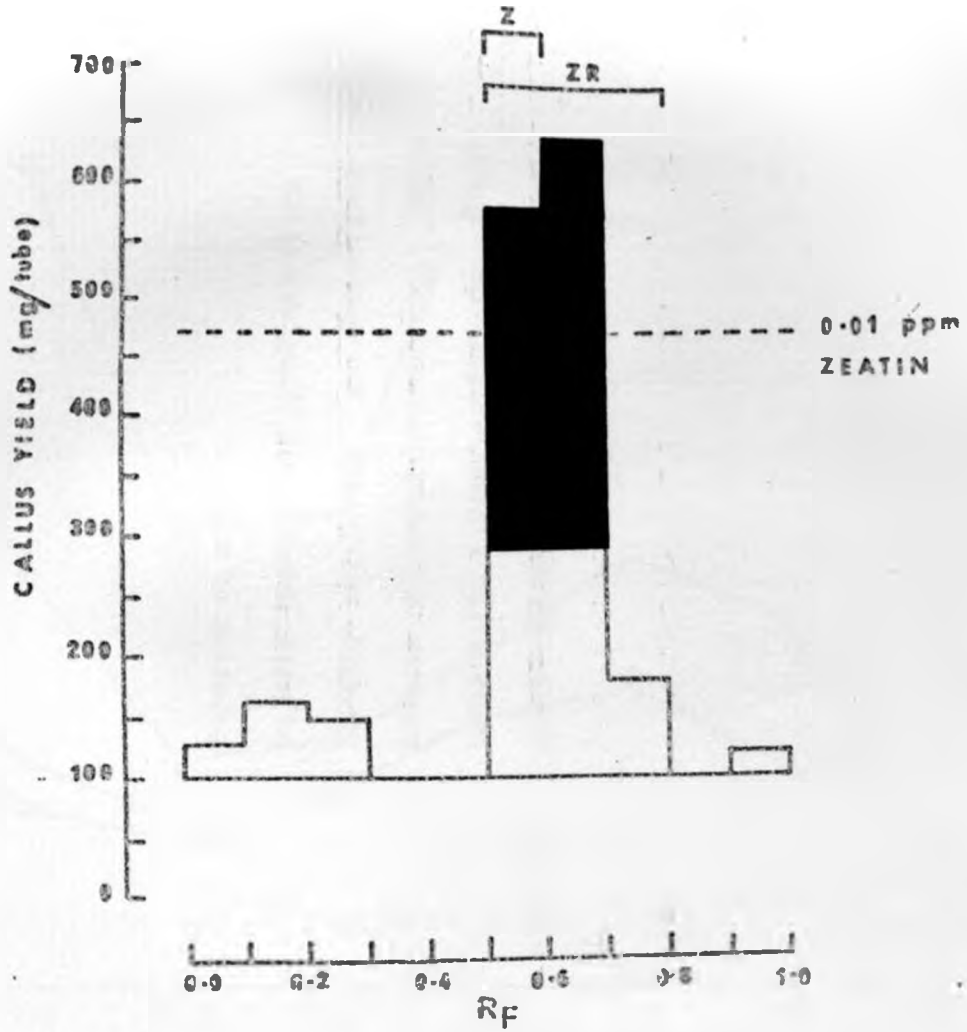
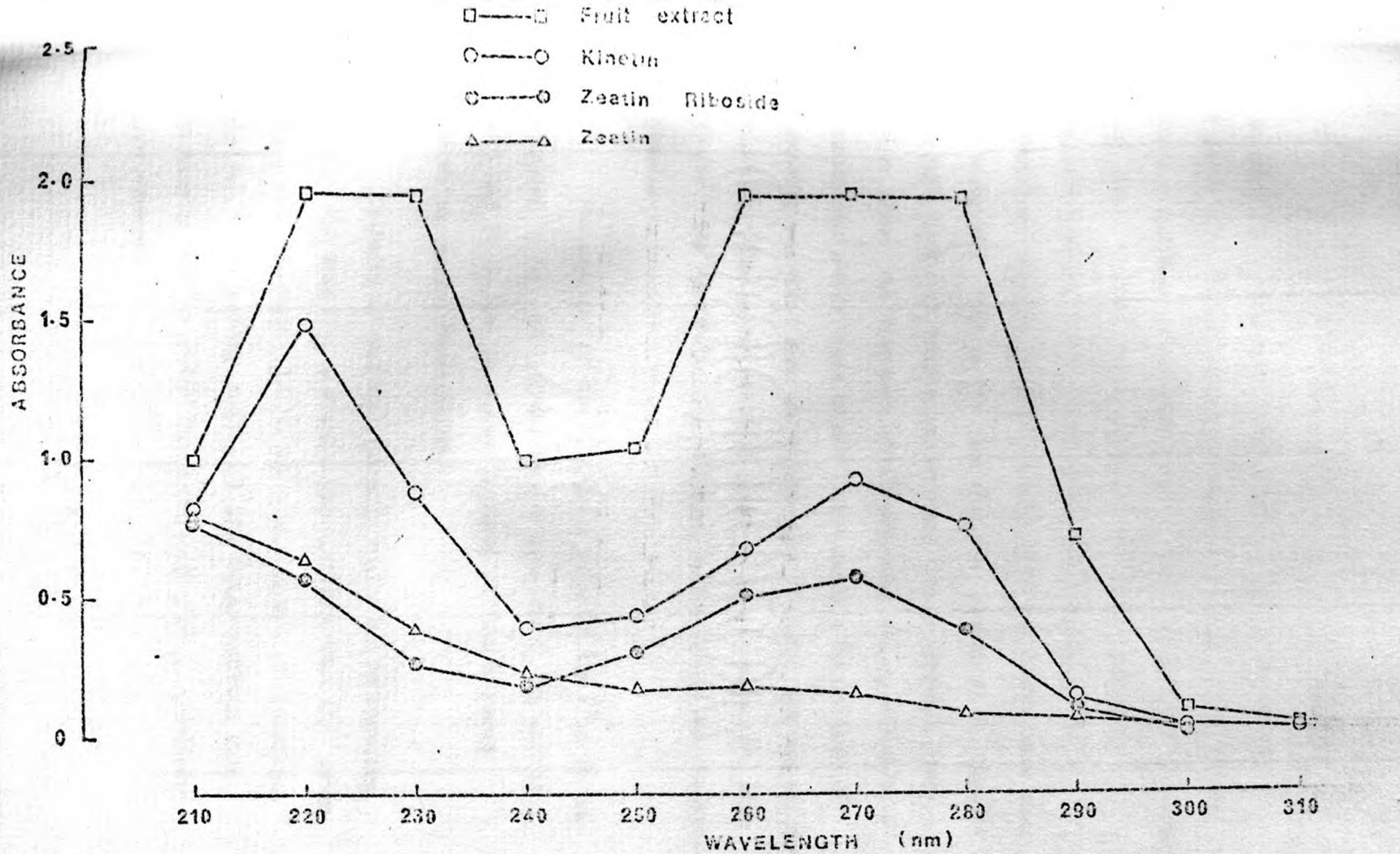

Z
ZR
700
600
500
400
300
200
100
0
CALLUS YIELD (mg/tube)
0·01 ppm
ZEATIN
0·0
0·2
0·4
0·6
0·8
1·0
RF

Fig. 15 b Action spectra of 13 week old fruit extract (10 g fresh weight equivalent), kinetin (6-Furfurylaminopurine), Zeatin riboside and Zeatin. Ten microlitres of each hormone was dissolved in 1N solution of NaOH.


Fruit extract
Kinetin
Zeatin Riboside
Zeatin
ABSORBANCE
2·5
2·0
1·5
1·0
0·5
0
210
220
230
240
250
260
270
280
290
300
310
WAVELENGTH (nm)

low when the fruits were still at the 'pinhead' stage and 7 weeks old. Thereafter the activity increased progressively until a maximum was reached when the fruits were about 10 weeks old, which is also the stage when fruits start expanding rapidly (Wormer, 1964). Thereafter, the cytokinin-like substances began to decline rapidly until the fruits were about 19 weeks old. This is the time when the endosperm is supposed to have completed filling in the fruit locule (Wormer, 1964). From about the 25th week after anthesis, the cytokinin-like substances levels began to rise again until a second peak of activity was noted when the fruits were 29 weeks old and started ripening. The levels declined again in the 31 weeks old ripe fruits. The cytokinin-like activity in the fruits was therefore bimodal.

The results of the experiment in which the ^{14}C BA was included in the 100 g fruit samples from the 7, 10, and 13 week old fruits are presented in Table 5. The recovery percentage ranged from 28-53%.

Extracts of rapidly expanding fruits and of ripening fruits had much more cytokinin-like activity than all other extracts. Developing fruits are a rich source of cytokinins (Letham, 1967) and the results of this study indicate that developing Arabica coffee fruits are also rich in cytokinin-like substances.

Table 5: Effect of the extraction and purification procedure on the recovery of the cytokinin-like substances.

Purified fruit extract from the 100 g fruit sample containing 10 µg 14C BA was taken up in 4 ml methanol and the radio-activity in a 100 µl aliquot ofthe methanolic mixture counted. The activity was counted before loading the extract on paper. Ten microgrammes of the 14C BA was also taken up in 4 ml methanol and a 100 µl aliquot from the standard used for calculating the percentage recovery.

Type of sample	Counts/100 seconds recovered from the 100 µl aliquots	Percent of 14C BA standard
7 week-old fruit	740	53
10 week-old fruit	390	28
13 week-old fruit	424	30
14C BA standard	1398	100

The changes in cytokinin-like substances observed in coffee fruits appears to be correlated in time with changes in the growth rate of the fruits. The cytokinin activity increases as the fruits continue to expand. The maximum level of activity appears to be reached when the fruits are about 10 weeks old, declines thereafter and is minimal on 16-25 week old fruits before rising again when fruits are ripening. The Arabica coffee fruit locule expands fully when fruits are about 16 weeks old and the endosperm develops in the locule between 13-20th week from the time of anthesis (Wormer, 1964). In this study the activity of cytokinin-like substances declined rapidly from 13-19 week (Fig. 13) which is about the time the locules are supposed to be filling in with a endomous material. This particular aspect of the study is therefore more or less in agreement with other studies carried out in fruits of other plant species where it has been reported that developing seeds are the main source of natural cytokinins (Skoog and Armstrong, 1970).

It is clear from Fig.13 that there was very little cytokinin-like activity detected in mature fruits which were 19-25 weeks old, which is about the time when dry matter is supposed to be accumulating into the endosperm (Wormer, 1964). The decline in activity of cytokinins in mature fruits is similar to that reported for mature fruits of tomato (Abdel-Rahman *et al.*, 1975; El Beltagy *et al.*, (1976). However, there was some rise of the cytokinin-like activity on the

onset of ripening (yellow) and in ripe (red) fruits.

It is clear from Table 5 that the recovery percentage of the ^{14}C BA ranged from 28-53% depending upon the fruit stage used for extraction. The loss in percentage recovery in the extracts compared to the standard imply that the long procedure adopted for purification might have been responsible for the low percentage of recovery due to inevitable losses in the process.

It is known that cytokinin-like substances are present in flower buds and xylem sap of Arabica coffee (Browning, 1973 b). The purification procedure used in this study was more or less similar to that adopted by Browning (1973 b) in which the extracts yielded only caticnic cytokinin-like substances in the flower buds and xylem sap which were shown to be similar to zeatin, zeatin riboside and 6YY (diamenthylallyl aminopurine (2-ip). Three peaks of CLS have been observed in most of the extracts (Fig.14). At least one of the cytokinin-like substances in the fruit moved to R_F similar to zeatin and zeatin riboside (Fig.15).

3.3 Conclusion

The studies described in Chapter III were aimed at providing background knowledge of the GA, ABA and cytokinins content of the Coffea arabica L. fruits. Changes in patterns of GLS, inhibitors and CLS activities in fruit extracts were determined in an effort to establish both qualitative

and quantitative differences in the activities of the two growth substances with a view to obtain further information about how such changes occur at different fruit stages. It is shown from this study that GLS and CLS are present in the developing Arabica coffee fruits and that the growth promoters might play an important role in fruit growth and development particularly in view of the fact that much evidence has accumulated that mobilization of metabolites into developing fruits is related to the hormone content of these tissues (Crane, 1964; Leopold, 1964; Seth and Wareing,1967). Also this work supports the view that ABA-like inhibitors and other inhibitors may play some roles in fruit growth and development in addition to their well known roles as general inhibitor of plant growth, or substances involved in dormancy, senescence and water stress (Milborrow, 1967; Wareing and Saunders, 1971).

In interpreting the results presented here, it must be remembered that these figures are for whole fruit and it was difficult to know whether the seed or the pulp or both were responsible for the increase in the activity. It is therefore pertinent to say that further studies on the precise location of GA, ABA, cytokinins as well as other plant growth regulators are needed. It should also be noted that although the actual losses were not recorded, the lengthy purification procedures to which extracts had to be subjected before bioassays might have led to variable and

indeterminate losses of the GLS, CLS and the inhibitor.

CHAPTER IV

EFFECT OF EXOGENOUSLY APPLIED HORMONES

Bean size depends on the number of rainy days during the time when locules are expanding (Cannell, 1974). Rainfall regulates supply of gibberellin-like and cytokinin-like substances from xylem sap of Arabica coffee (Browning, 1973 a, b). It is possible that gibberellic acid and kinetin play some role in bean filling and therefore in bean weight and size. Thus, it must be important to know how they are determined on the tree.

Basic information concerning the use of GA_3 to change the fruiting pattern had already been obtained (Cannell, 1971 b) The trials by Cannell, however, had only been limited to a medium altitude site (Coffee Research Station, Ruiru 1608 m).

Since Arabica coffee in Kenya is grown from 1400-2000 m altitude, it is possible that the effect of GA_3 on the fruiting pattern and yield may vary from one altitude zone to the other.

4.1 Application of gibberellic acid and kinetin directly onto fruits

4.11 Experimental

Experimental details are given in Table 6.

Mature trees of French Mission. SL 28 and SL 34 were used for the experiments described below in 1973, 1974, 1975 and 1976.

All the varieties were unshaded open growing trees. Only berries on two nodes per branch (about 50 fruits) in four branches per tree were used in experiments I, II and III (Table 6). Treated fruits were from a uniform flowering and any fruits of other ages were removed.

Design and Treatment

In Experiments I and II, factorial combinations of GA_3 and kinetin in ethanol at 0.2, 2.0, 20 µg levels and ethanol alone (16 treatments in all) were dispensed in 20 µl portions to each fruit using a micro-applicator (Hamilton Co., Whittier, California, USA). The fruits of Experiment I were treated every two weeks starting ten weeks after anthensis and continued until the fruits on control trees had stopped expanding rapidly. But the treatments in Experiment II were applied only at five selected stages of fruit development indicated in Table 6. In Experiment III GA_3 dissolved in ethanol was used at 0, 20, 40 and 100 µl per fruit. The GA_3 was applied every two weeks between the 4th and 14th week after flowering but only at three different stages of fruit growth (Table 6). In Experiment IV, GA_3 was dissolved in ethanol and the solution was dispensed in 10 µl portions containing 100 µg GA_3 onto each fruit at each application date given in Table 6. In this way, each fruit received a total of either 200 µg or 400 µg GA_3 during the whole experimental period, depending on the stage of treatment (see Table 6). The fruits were either treated at the

Table 6: Experimental details of application of gibberellic acid and kinetin directly onto fruits.

Experiment: Hormone applied and plant material	Date of flowering	Details of treatment application				Harvest period
		Year	Date of treatment	Fruit age at treatment (wk)	Fruit stage at treatment (wk)	
I GA_3 and Kinetin French Mission	26 Oct 1973	1973	12 and 25 Jan 9 and 23 Feb 8 and 24 Mar 5 and 25 April 7 and 17 May	11;13 15;17 19;21 23;26 28;30	1 Rapid expansion 2 Endosperm growth 3 Dry matter accumulation 4 Ripening	25 May to 16 July 1973
II GA_3 and Kinetin French Mission	5 Oct 1973	1973/ 1974	9 November 7 December 3 January 22 February 17 April	5 9 13 20 28	1 Pinhead 2 Beginning of expansion 3 Beginning of bean formation 4 Beginning of dry matter accumulation 5 Ripening	7 May to 2 July 1974
III GA_3 Cvs. SL 28 and SL 34	12 March 1975	1975	8 and 23 April 6 and 23 May 8 and 17 January	4;6 8;10 12;14	1 Pinhead 2 Rapid expansion 3 Endosperm growth	7 Nov to 11 Dec 1975

...../continued

Experimental details of Table 6 continued

Experiment and mode of GA_3 application	Date of flowering (1976)	Details of treatment application			Harvest period
		Date of treatment (1976)	Fruit age at treatment (1976)	Fruit stage at treatment	
IV Ethanolic micro-drops	6 March	29 March and 14 April	4 & 6	1 Pinhead	29 Sept to 17 December 1976
		27 April and 11 May	8 & 10	2 Beginning of expansion	
V Aqueous sprays on whole trees	12 February	11 March and 25 March	4 & 6	1 Pinhead	12 October 1976 to 26 January 1977
		8 April and 22 April	8 & 10	2 Beginning of expansion	

'pinhead' stage (A, 4-6 week old) only or at the stage of fruit expansion (B, 8-10 week old) only. or at both stages (AB, 4-10 week old stage). Two types of control fruits were used for comparison, namely one treated with ethanol alone and those receiving no treatment.

Experiments I and II were set up on 2^4 factorial design, replicated four times in randomized blocks of single tree plots. The trees were grown without supplementary irrigation. Experiment III was of a split-split-plot design with randomized blocks replicated four times on single-tree plots. Trees grown with and without supplementary irrigation formed the two major plots in the design, each of which was divided into seven sub-plots according to stages of application and their factorial combinations (A,B,C,AB,AC,BC and ABC). Each time of application was in turn divided into five sub-sub-plots, representing the four levels of GA_3 and untreated control i.e. 0,20, 40 and 100 µg. One supplementary irrigation treatment was applied on 19 May 1974, following the usual practice based on the recommendation of Pereira (1957).

Experiment IV was laid on a split-plot design with each stage of application (A,B, or AB) forming the main plots. Each of the main plots was subdivided into three sub-treatment plots i.e. untreated, ethanol and GA_3 in ethanol. The treatments were replicated four times on randomised blocks.

Experiment V was conducted for the purpose of assessing the effect of GA_3 on the bean quality. In this experiment aqueous sprays of GA_3, formulation PRO-GIBB 10% w/w a.i., were applied to whole trees at the rate of 0.25,50 and 100 ppm (a.i.). All sprays including water controls contained non-ionic wetter'Agral 90' at 0.05% and were applied during the day to near run-off (about 700 ml/tree) using a knapsack sprayer. These treatments were carried out both for irrigated and un-irrigated blocks of trees. The representative bean samples from each of the irrigated and unirrigated trees were assessed separately for quality standards. Analysis of variance was done using mean values of duplicate samples (one each from irrigated and unirrigated trees) and the stage of fruit treated was regarded as a block. The plant material, date of flowering, stage of treatment and design was as in Experiment IV .

Growth rates of the fruits

In Experiments I, II and III, berry collections were made every two weeks beginning on the day the initial treatment was applied and continued until the first berries turned yellow. At each collection, eight berries from trees that received each hormone treatment were randomly selected and used for fresh weight, volume (by water displacement), and oven dry weight ($30^{\circ}C$) determinations.

Fruit size

Four fruits (one from each replicate) in Experiments

IV were collected from amongst those treated at the 'pinhead' (A) stage only and photographed. This was done when the fruits were 11 weeks old (5 to 7 weeks after treatment), 16 weeks old (10 to 12 weeks after treatment) and 30 weeks old (24 to 26 weeks after treatment). The ages of the fruits selected for photography were made to coincide with the period when the GA_3-treated fruits were either expanding rapidly, had apparently attained their maximum size, or were ripening.

Length to diameter ratios and locule size of the fruits

Length to diameter (L/D) ratios were derived from maximum fruit length and maximum equitorial diameter. Locule size was measured after cutting the fruit transversely and measuring the maximum width and maximum length of the space occupied by integument and which was to be filled later by the developing endosperm when the fruits were mature.

Transverse sections of whole fruits were also photographed using an ordinary 20 roll film ASA (125) when fruits in Experiment IV were 30 weeks old. The fruits had previously been preserved in a mixture of acetic acid, ethanol and water as explained under histological studies below.

Histological studies

An extra sample was collected in the exact manner described

under fruit size section in Experiment IV and preserved in a mixture of 10% formalin, 5% acetic acid, 50% ethanol and 35% distilled water (FAA) (Jensen, 1962). Following the process of dehydration the samples were embeded in paraffin wax, 15-micron thick longitudinal sections were obtained and mounted, and stained in fast green and safranin according to the technique described by Jensen (1962). Photomicrographs were taken with ordinary 35 roll film ASA (125) using a camera mounted on a Vickers photometer J 36 model.

Determination of bean dry weight and quality

In experiments I, II and III, ripe (red) fruits were collected every week during the harvest periods, indicated in Table 6. Fresh weight of the fruits and of beans obtained from the fruits as well as the oven-dry weight of unhulled (parchment) beans and hulled beans were determined.

In Experiment IV, weekly records of number of cherries picked and their fresh weight were made. The cherries were pulped by hand. The beans were then soaked; fermented and washed in the usual manner (Ombwara, 1968) but in beakers in the laboratory. The washed beans were placed on wire-mesh trays to dry normally (Ombwara, 1968), until the moisture content reading (Twin-tester moisture meter, Kongskilde, Moskinfabrik, Denmark) was about 10%. The parchment (hulls) was then removed by hand and dry weight of the parchment and clean beans were recorded. The ratio of the number of clean beans obtained and the parchment beans were also recorded. Samples of

clean beans from the parchment beans were then graded into grade 'A' (beans retained on 6.75 mm sieve) and the rest of the grades.

All ripe cherries on trees used for Experiment V were picked weekly as in experiment IV, but pulping, fermenting, washing, drying and grading was done at the Coffee Research Station Factory in the usual manner (Ombwara, 1968). The hulled sun-dried bean samples were then taken to Mild Coffee Trade Association (MCTA) of Eastern Africa, Nairobi for the assessment of liquor quality.

4.12 Results and Discussion

Growth rates

Figure 16 shows the rate, in terms of dry weight, of untreated fruits growth without supplementary irrigation in Experiment II. The fruits showed a normal double sigmoid type of growth (Wormer, 1964; Cannell, 1971 c).

Kinetin effect on growth rates is shown in Figure 17. Fresh weight and volume increased by about 20% between the stage of dry matter accumulation and ripening, while by the week of ripening, the dry weight of fruits was increased by about 14%. Kinetin + GA_3 was effective in increasing growth rate but not as much as GA_3 alone.

It is clear from Fig. 17 that GA_3 increased growth rate

Fig.16 Coffea arabica L. fruit growth expressed on dry weight basis. The histograms show the weekly rainfall totals. The morphological development of the fruit beans is shown in the upper portion of the Figure.

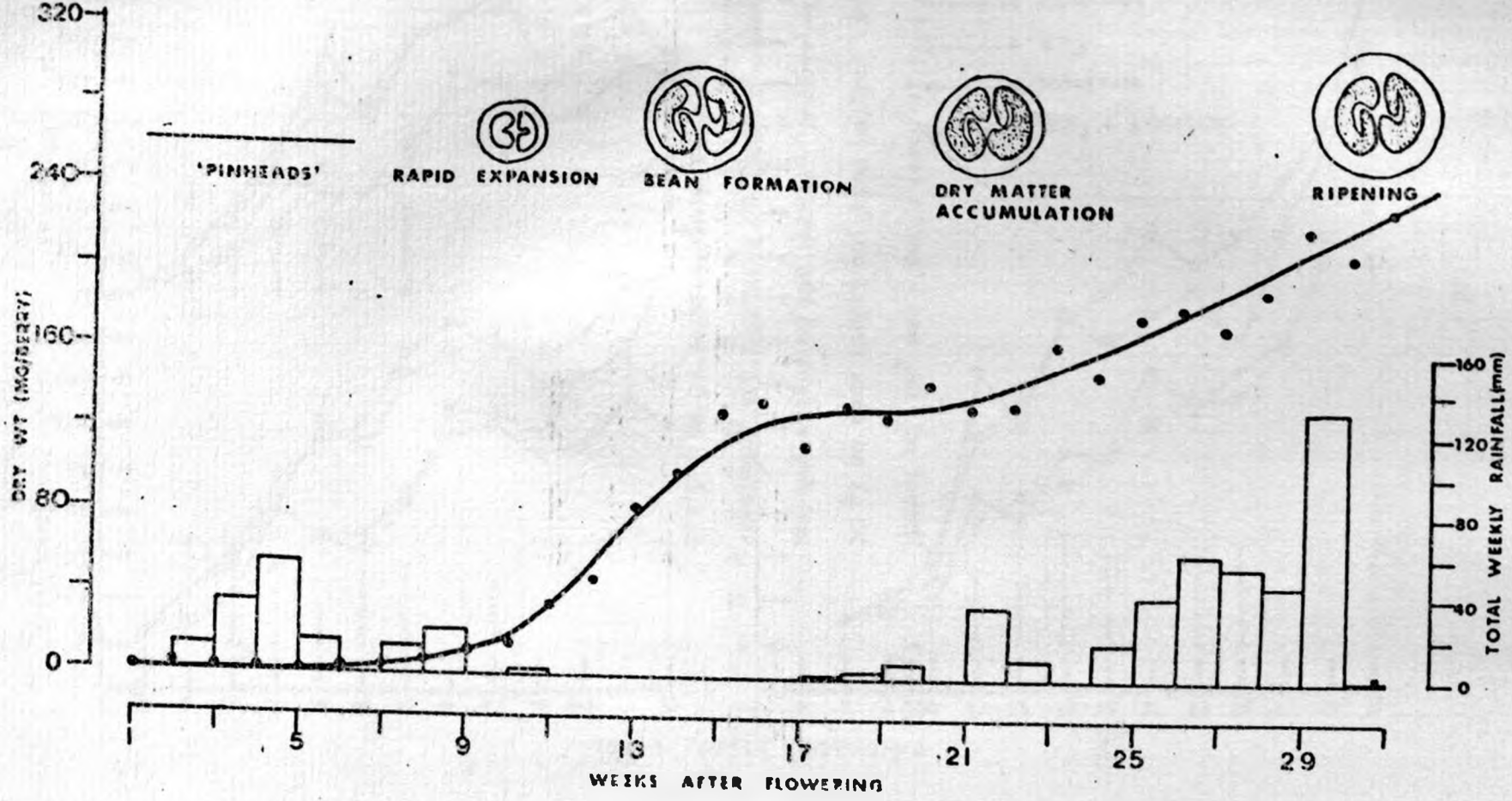
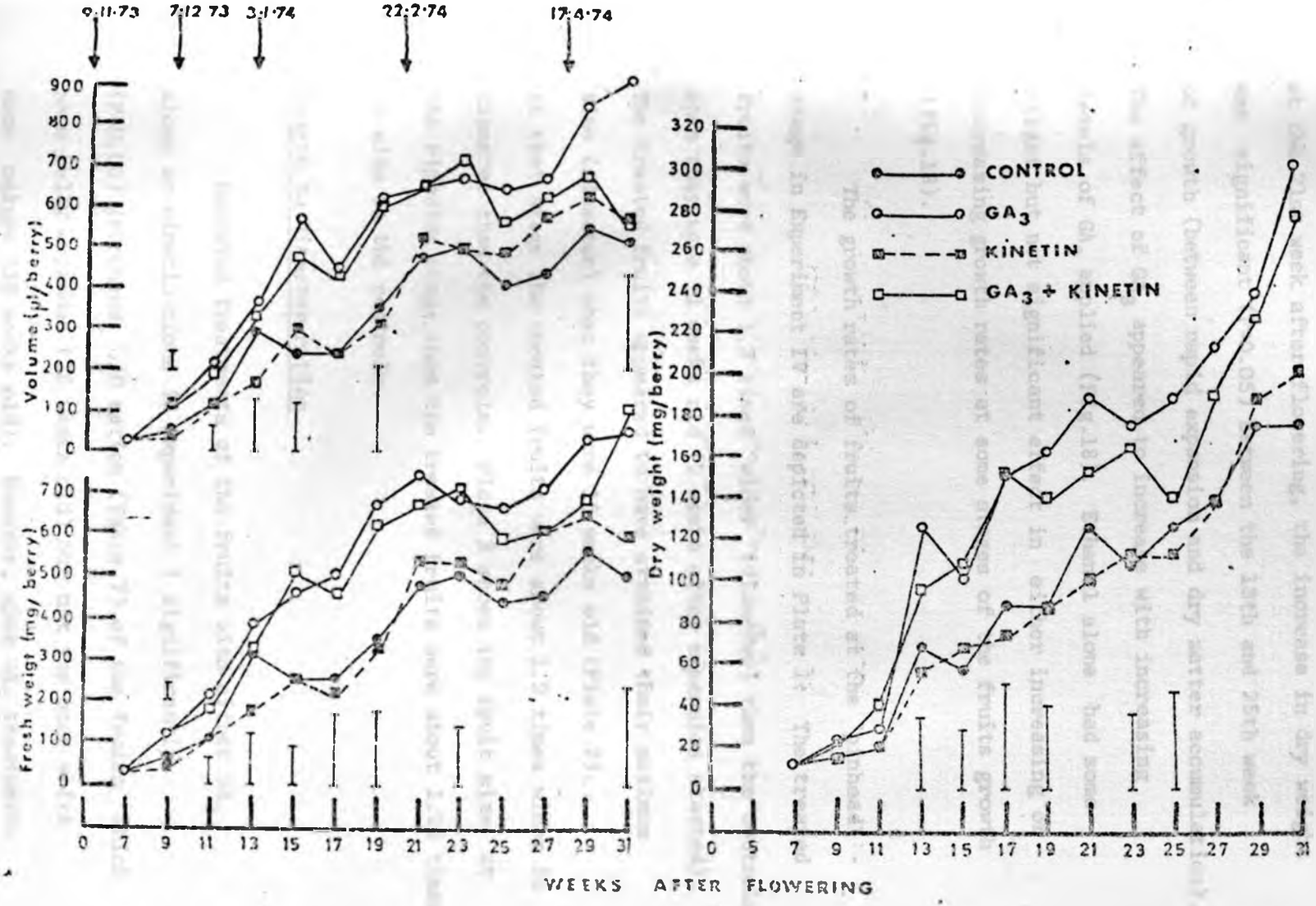

320
240
160
80
0
DRY WT (MG/BERY)
'PINHEADS'
RAPID EXPANSION
BEAN FORMATION
DRY MATTER ACCUMULATION
RIPENING
160
120
80
40
0
TOTAL WEEKLY RAINFALL(mm)
1
5
9
13
17
21
25
29
WEEKS AFTER FLOWERING

Fig.17 Effect of GA_3 and kinetin (each applied at 20 µg/ 20 µl on each fruit) on fresh weight, volumes and dry weight of the fruits. Details of treatment are indicated on Table 6. Bars indicate L.S.D. at 5% level of probability.


9.11.73
7.12.73
3.1.74
22.2.74
17.4.74
Volume (µl/berry)
Fresh weigt (mg/ berry)
Dry weight (mg/berry)
CONTROL
GA3
KINETIN
GA3 + KINETIN
WEEKS AFTER FLOWERING

at all stages of fruit growth by about 20-25%, and except at the 21st week after flowering, the increase in dry weight was significant (P=0.05) between the 13th and 25th week of growth (between rapid expansion and dry matter accumulation). The effect of GA_3 appeared to increase with increasing levels of GA_3 applied (Fig.18). Ethanol alone had some slight but not significant effect in either increasing or decreasing growth rates at some stages of the fruits growth (Fig.18).

The growth rates of fruits treated at the 'pinhead' stage in Experiment IV are depicted in Plate 1. The treated fruits were about 1.3 times wider (diameter) than the controls when they were 11 weeks old (2 weeks after expansion started). The treated fruits appeared to have attained their maximum size (diameter) when they were 16 weeks old (Plate 2). At that stage the treated fruits were about 1.2 times wider in diameter than the controls. Plate 3 shows the fruit size at the ripening stage when the treated fruits were about 1.25 times as wide as the controls.

Length to diameter ratios

Repeated treatments of the fruits with either GA_3 alone or kinetin alone in Experiment I significantly (P=0.01) increased L/D ratios (Table 7) of the fruits which were fully expanded (16 weeks old) but not the ones which were mature (28 weeks old). However, when GA_3 treatments were only applied twice at the pinhead stage in Experiment IV,

Fig. 18 Effect of GA_3 applied at 100 µg per fruit on fresh weight, volume and dry weight of the fruits. Bars indicate LSD at 5% level of probability.

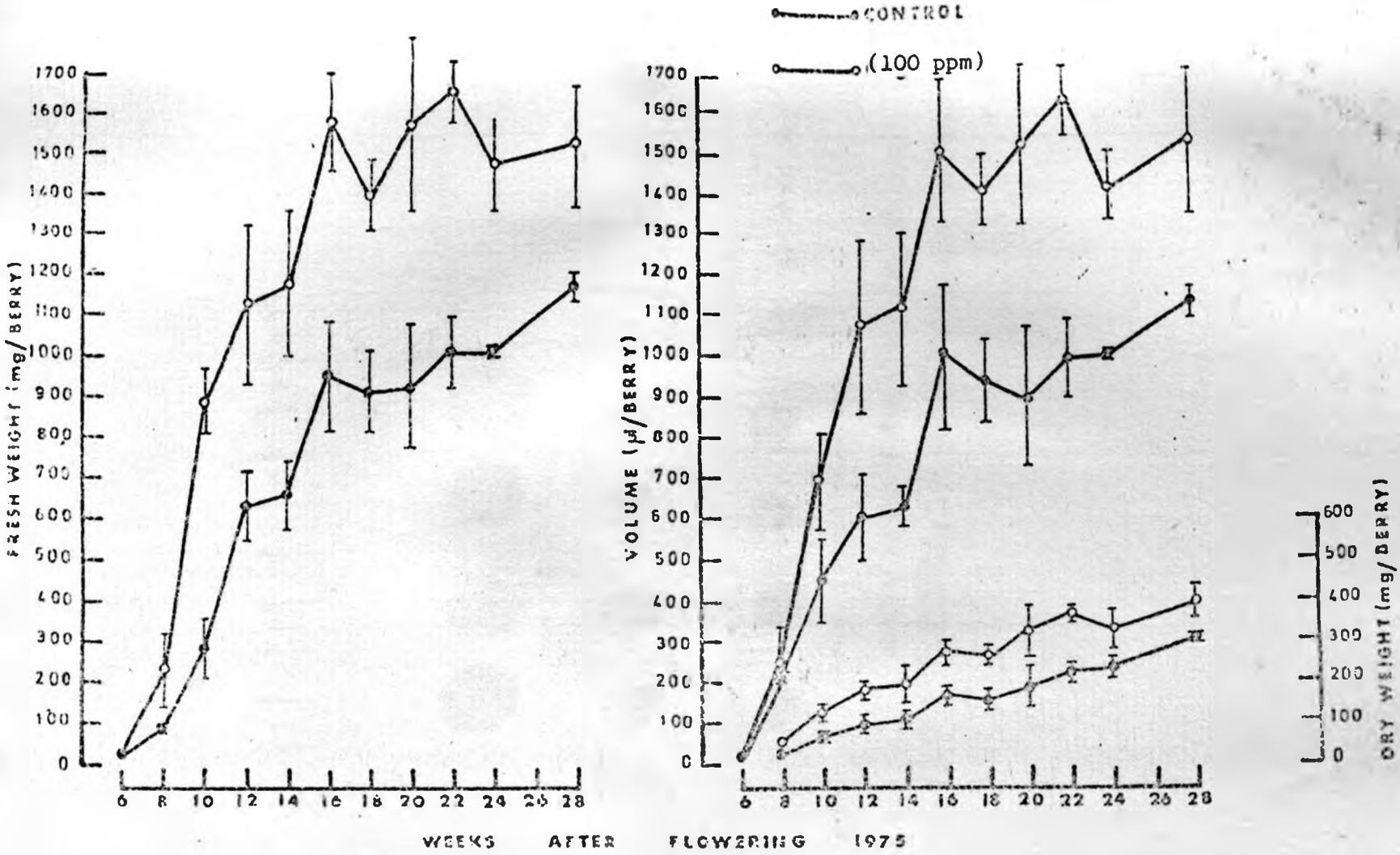

CONTROL
(100 ppm)
FRESH WEIGHT (mg/BERRY)
VOLUME (µ/BERRY)
DRY WEIGHT (mg/BERRY)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
6 8 10 12 14 16 18 20 22 24 26 28
WEEKS AFTER FLOWERING 1975

Plate I Eleven week-old fruits treated with GA_3 in ethanol at 100 µg per fruit when the fruits were 4 and 6 weeks old.

11 WK OLD FRUITS Cv. S.L. 28

UNTREATED

ETHANOL

TREATED

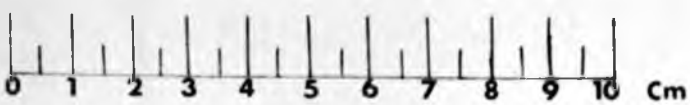


Plate 2 Sixteen week-old fruits treated with GA_3 as explained under Plate 1.

16 WK OLD FRUITS Cv. S.L. 28

UNTREATED

ETHANOL

TREATED

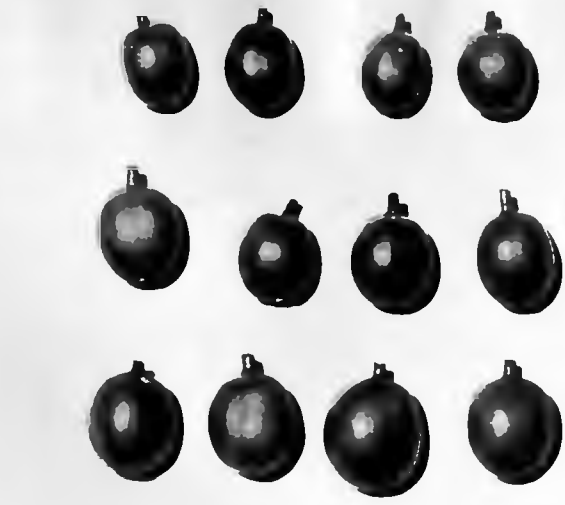


Plate 3 Thirty week-old fruits. The GA_3 was applied as explained under Plate 1. On the left hand side is the cross-section of the fruit which is also shown whole on the right hand side.

30 WK OLD FRUITS Cv. S·L· 28

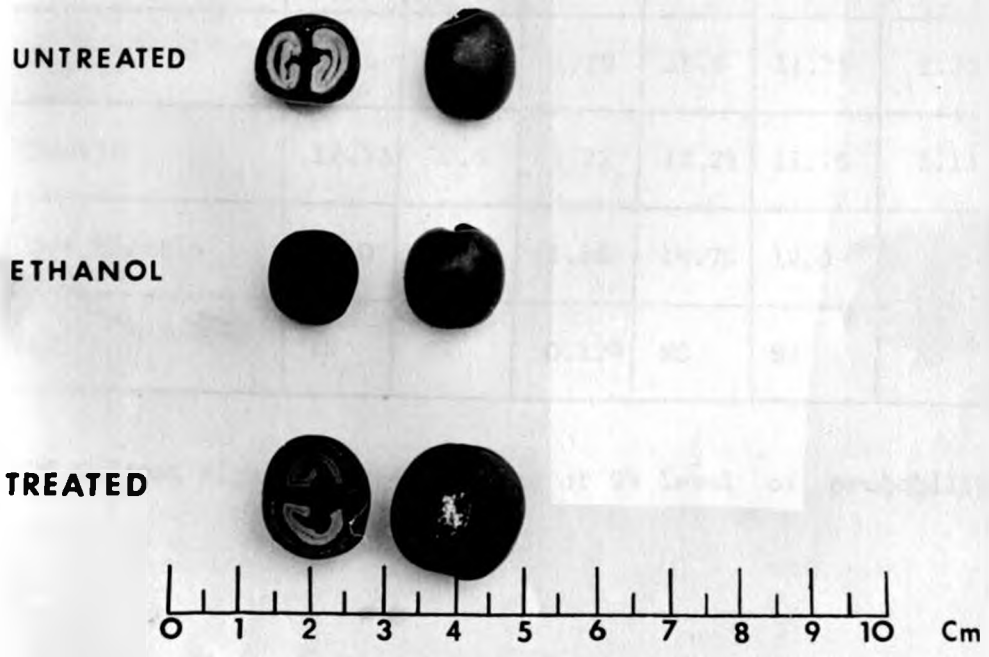


Table 7a: Mean length (L) to diameter (D) and ratios (L/D) of 16-week-old and 28-week-old fruits following treatment with GA and/or Kinetin at 20 µg per fruit as shown in Table 6 (Expt. I).

Treatment	Fruit length (L), diameter (D), and L/D ratio					
	16-week old fruit					
	L mm	D mm	L/D	L mm	D mm	L/D
Ethanol control	12.25	11.25	1.09	13.25	11.5	1.15
GA	14.5	11.25	1.29	15.5	11.75	1.30
Kinetin	12.75	10.5	1.22	13.25	11.75	1.13
GA + Kinetin	13.0	11.25	1.15	14.75	12.0	1.23
LSD	NS	NS	0.12*	NS	NS	NS

* LSD = Least significant difference at 5% level of probability

Table 7: Effect of GA_3 on cell size (mm^*) of *Coffea arabica* L. fruits treated when six weeks old and fixed in FAA when 12 weeks old

Treatment	No of different slides examined (Means of five determinations)				$\bar{X}$	SE	CV
	1	2	3	4			%
Control:							
Length	1.0	0.9	1.5	1.1	1.2	0.3	25
Width	1.1	1.2	1.0	1.1	1.1	0.2	18
GA_3 (100 ppm):							
Length	2.2	2.6	2.3	2.3	2.4	0.3	12
Width	1.0	1.1	1.3	1.0	1.1	0.2	18

* Magnified: 12.5 x - eye piece and 40 x - objective

SE = Standard error of 20 samples

CV = Coefficient of variation

the L/D ratios of the fruits were not affected (Table 6). The treated fruit had greater locule length and wider diameter compared to the control (Table 9).

Treatment effect on fruit tissue

Plate 4 shows a longitudinal section of the whole fruit following treatment with GA_3 in ethanol. Compared to the untreated fruit of the same age in Plate 5, the endosperm tissue of the treated fruit did not fill locule completely and therefore appeared to have been damaged during the sectioning.

The section through the pulp (outer mesocarp) of the treated fruit is shown in Plate 6 for comparsion with a similar section of the untreated fruit of the same age photographed at the identical magnification (Plate 7). It appears that the GA_3 treatment made cells in this region slightly bigger than the cells in the control fruit (Table 7 b).

Delay in ripening

In Experiment I it was observed that GA_3 delayed fruit ripening by about 10 days (Table 10). Also when aqueous sprays of PRO-GIBB (GA_3) were used in Experiment V, the harvest was delayed but concentrated so that about 50% of the fruits were harvested during the peak of harvesting period (Table 11).

Table 8: Mean length (L), diameter (D), and length and diameter (L/D) ratio of the 16 week-old fruits following treatments at pinhead stage in Experiment (See Table 6)

Treatment	L mm	D mm	L/D ratio
Untreated	11.05	5.88	1.87
Ethanol	10.88	5.88	1.85
GA_3 (100 µg/fruit)	12.35	6.65	1.85
SE of Treatment mean	0.7 NS	0.5 NS	0.1 NS

NS = Not significant at 5% level of probability

Table 3: Mean length and diameter of the locule at the early stage of fruit expansion (9 weeks of growth) and at the apparent maximum expansion stage of the treated fruit (10 weeks of growth in Experiment IV of Table 3).

Treatment	Locule of the 9 week-old fruit		Locule of the 10 week-old fruit	
	Length (mm)	Diameter (mm)	Length (mm)	Diameter (mm)
Untreated	4.2	2.8	11.0	5.9
Ethanol	5.6	2.8	10.9	5.6
GA_3 (100 ug/fruit)	7.4	3.7	12.4	6.6
S.E. of treatment Mean	0.49**	0.31 NS	0.78 NS	0.53 NS

** = Significance difference at the 1% level of probability

NS = Not significant at the 5% level of probability

Plate 4 Longitudinal section through the eleven week-old Coffea arabica L. fruits. The fruits were treated as explained under Plate 1.

Plate 5 Longitudinal section of the eleven week-old fruits of C. arabica L. The fruits were not treated.

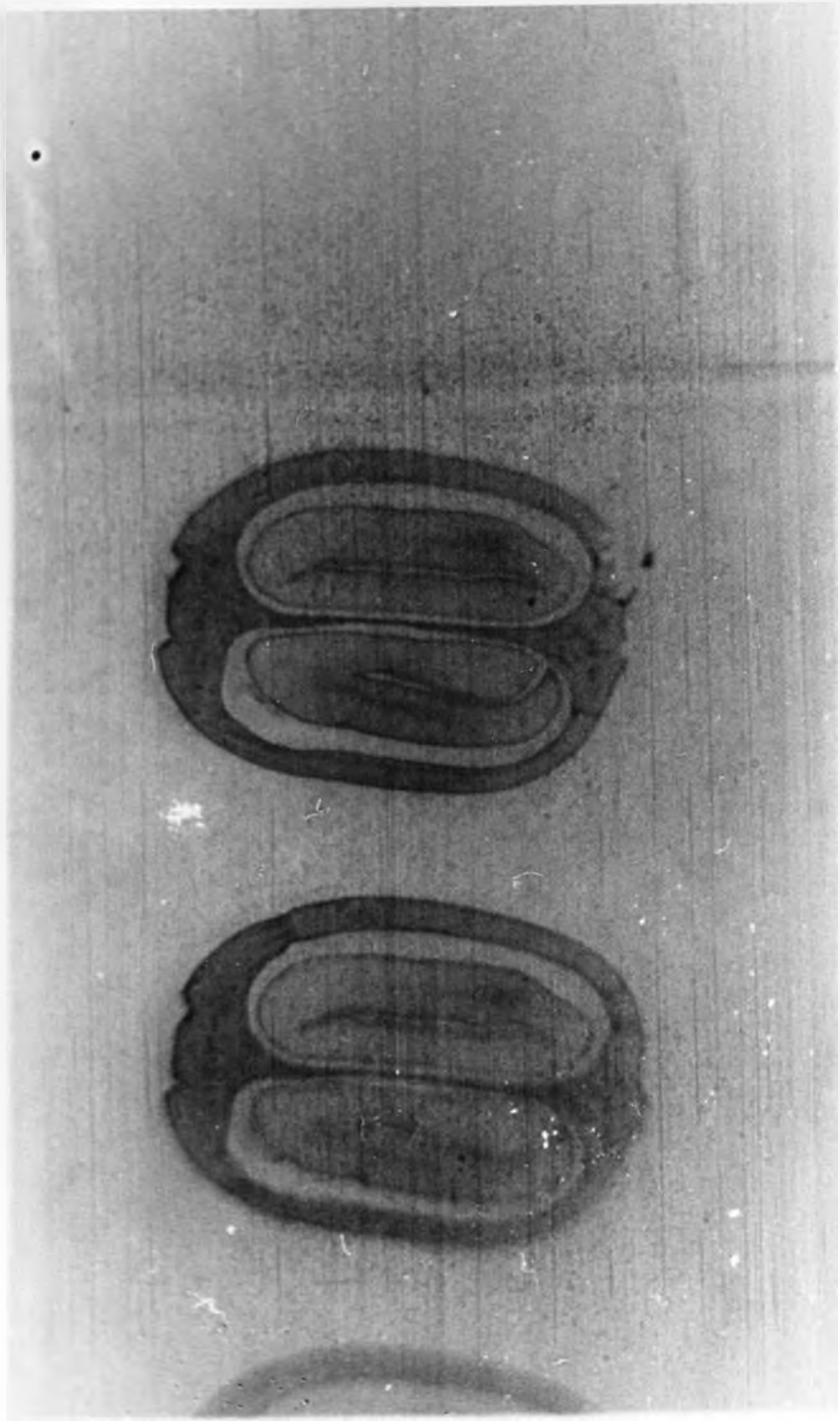


Plate 6 Photomicrograph of the outer mesocarp of an eleven week-old fruit treated with GA_3. The record was made five weeks after treatment.

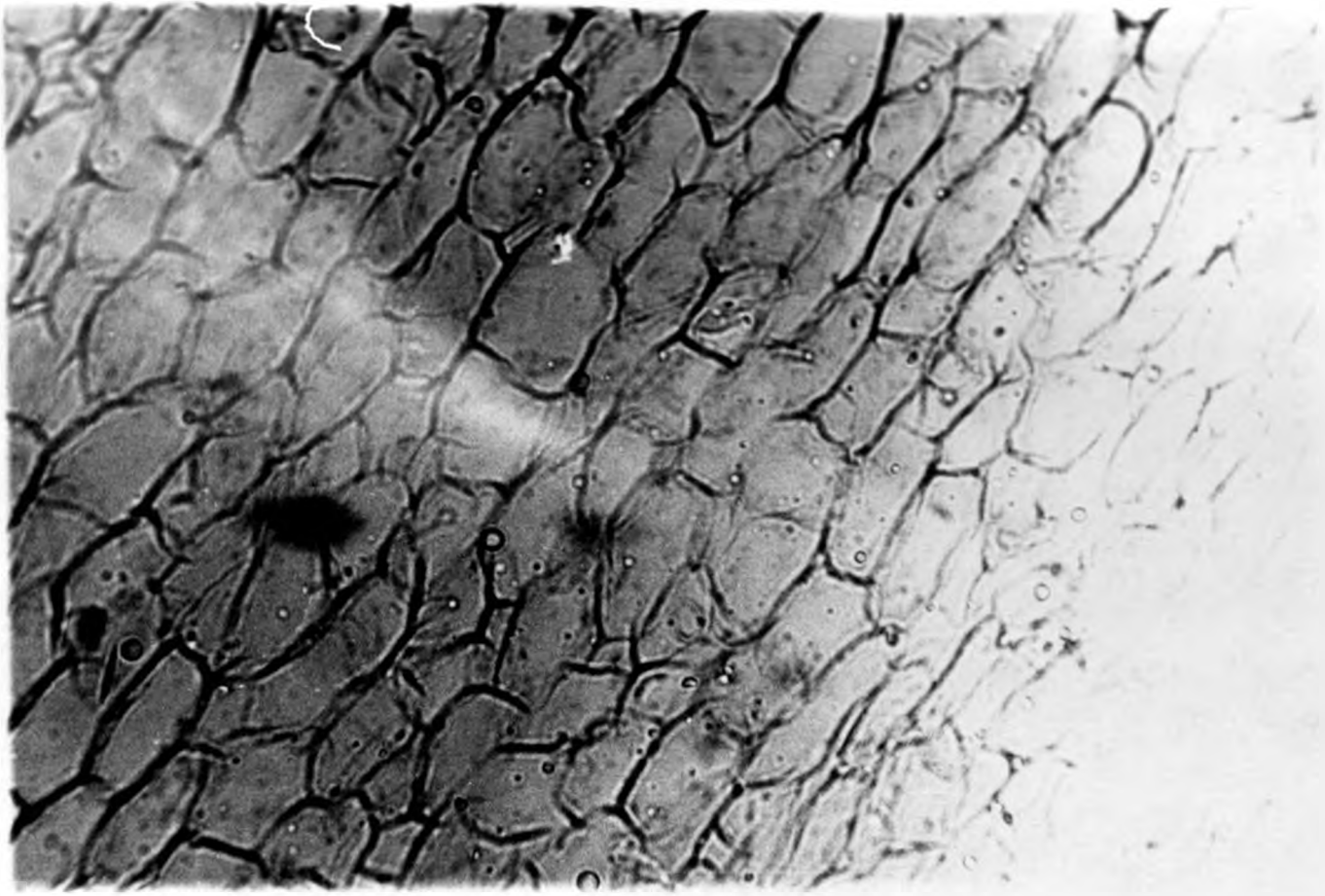


Plate 7 Photomicrograph of the outer mesocarp of an eleven week-old fruit which was not treated.

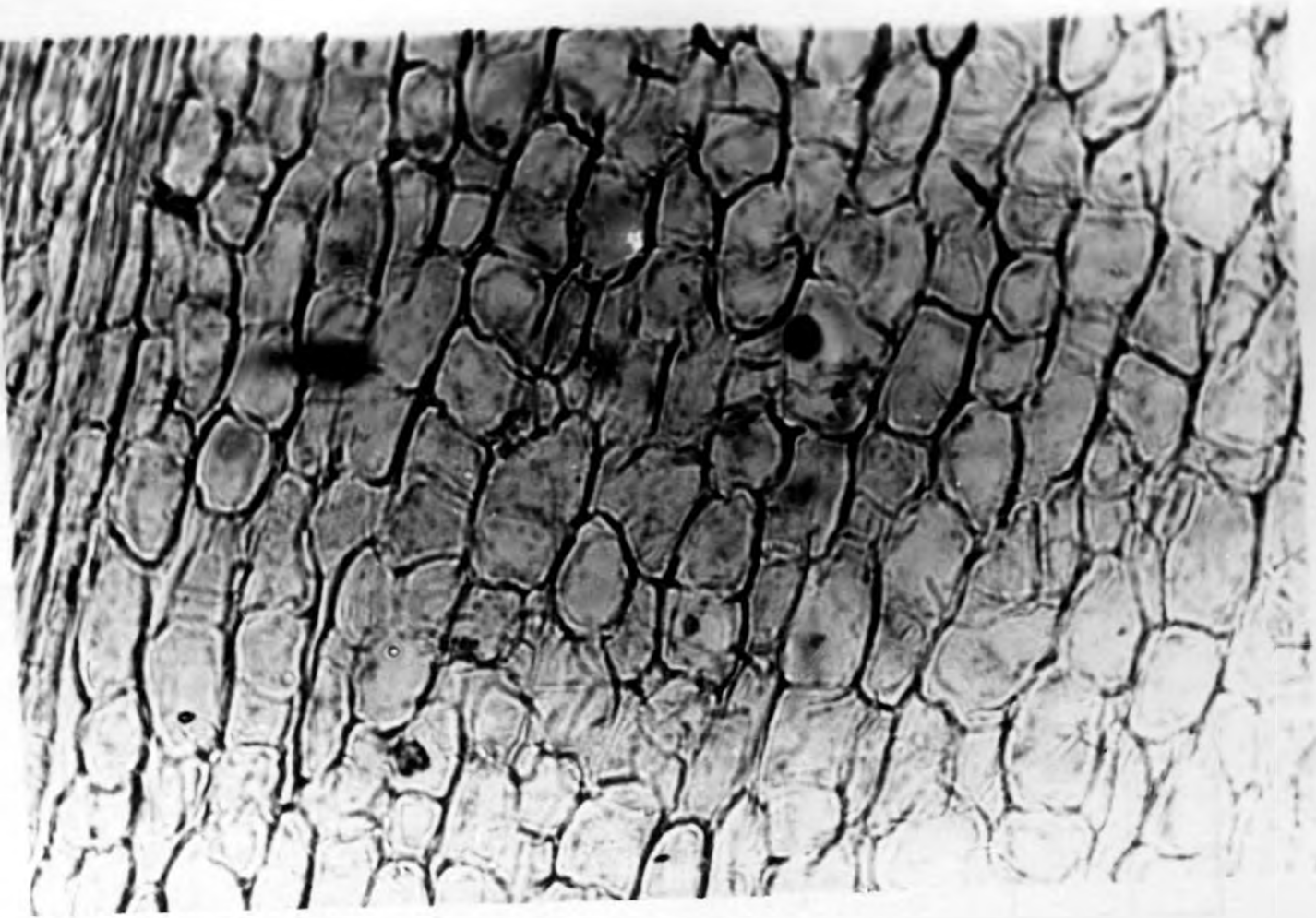


Table 10: Per cent of total crop picked in Experiment I (shown in Table 6) between 25th May and 19th July, 1973.

Number of days from the first date of picking	Crop picked (% of total)	
	Ethanol control	GA 20 µl/fruit
0	3.4	1.5
8	19.6	8.9
14	48.6	43.6
21	20.2	8.2
28	6.2	34.8
35	0	0
43	0	0.8
50	1.0	0
57	1.0	2.2

Table 11: Crop picked (as % of toal available) following aqueous sprays of Pro-Gibb (GA_3) on whole trees when fruits were at pinhead stage and again at the beginning of expansion stage in Experiment II of Table 6.

Date of picking	Crop picked (% of total)	
	Unsprayed	GA_3 (100 ppm)
12.10.76	0.4	0.1
27.10.76	6.0	9.1
4.11.76	20.3	2.5
13.11.76	34.3	53.2
3.12.76	31.8	24.5
15.12.76	5.4	6.7
29.12.76	1.2	2.9
13.1.77	0.4	0.8
26.1.77	0.2	0.1

Bean dry weight

The effect of GA_3 in factorial combination with kinetin at various levels on dry weight of hulled beans is shown in Table 12. In most treatments fruits treated with GA_3 alone had increased in dry weight by about 20-30% over the controls. Fruits treated with kinetin, especially at the 2 and 20 µg levels, appeared to increase in dry weight only when the kinetin was combined with GA_3, and the effect appeared to increase with increasing levels of GA_3. The highest increase of dry weight of the bean was by 50% in the 2 µg kinetin combined with 20 µg GA_3.

Figure 19 summarises the effect of GA_3 at various levels, applied at three different stages, on the dry weight of hulled beans. GA_3 was more effective in increasing bean dry weight when applied at the 'pinhead' stage (A) to unirrigated fruits (by about 35-55% over the controls). Applications at the beginning of endosperm growth stage (C) appeared to be less effective and in some cases the dry weight of beans was below the controls (Fig.19 b). It was observed that the overall response to GA_3 treatment was slightly better when fruits grew without irrigation provided that the drought was not so severe.

It was observed that GA_3 appeared to affect dry weight of the bean and probably the pulp, but not the parchment.

Table 12: Effect of GA_3 and kinetin on dry weight (mg/bean) of Coffea arabica L. beans

Kinetin (µg) applied per fruit at each stage	GA_3 (µg applied per fruit at each stage)				
	0	0.2	2.0	20.0	$\bar{X}$
0	32	42	50	45	42
0.2	38	44	22	31	34
2.0	37	48	48	63	49
20.0	26	26	56	40	37
$\bar{X}$	33	40	44	45	
S E of the treatment difference = 2.8					

Fig. 19 Effect of stage of application and the level of GA_3 applied on the dry weight of hulled beans from unirrigated plots (a) and from irrigated plots (b).

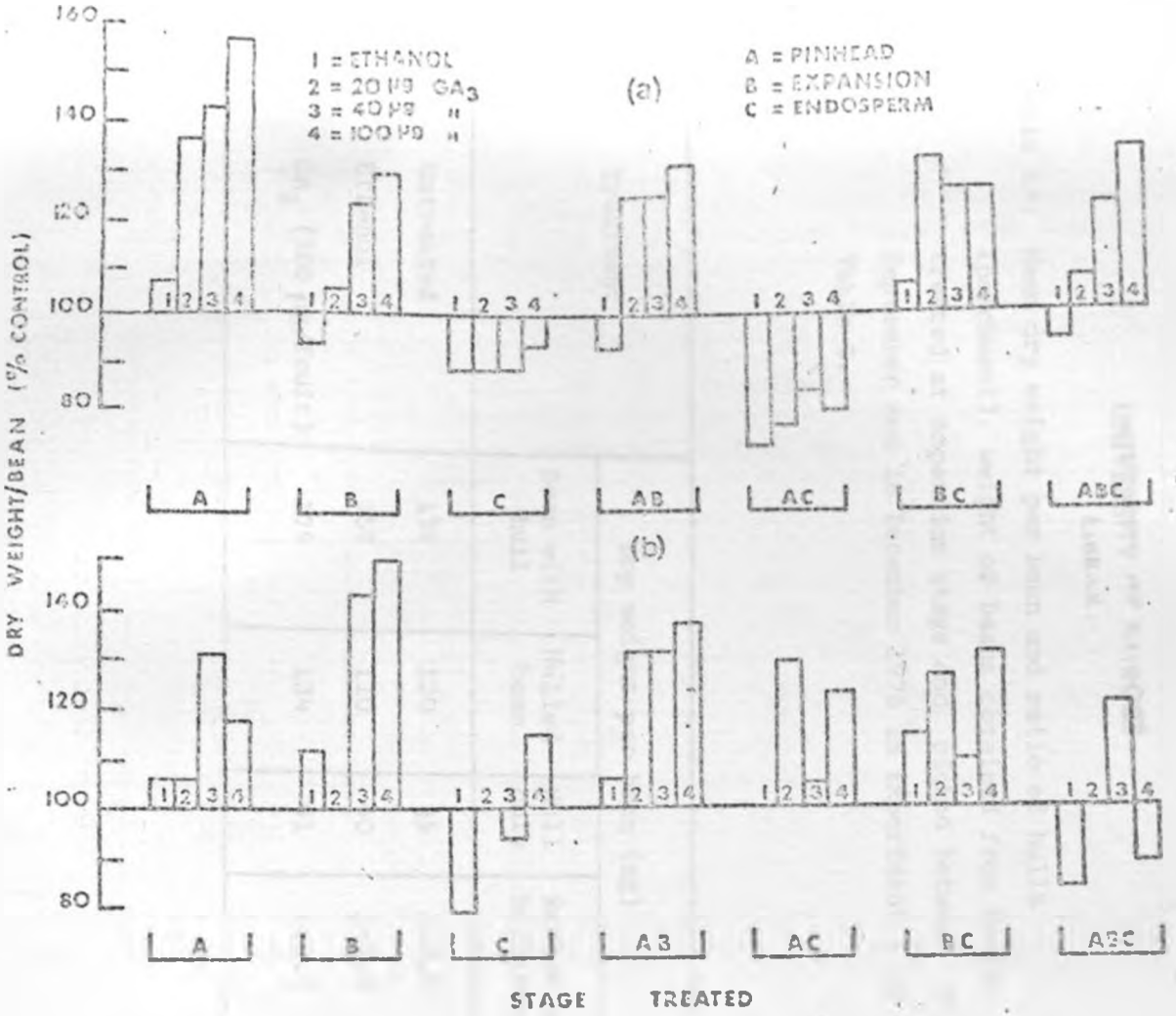

1 = ETHANOL
2 = 20 PPB GA3
3 = 40 PPB "
4 = 100 PPB "
A = PINHEAD
B = EXPANSION
C = ENDOSPERM
(a)
(b)
DRY WEIGHT/BEAN (% CONTROL)
160
140
120
100
80
A
B
C
AB
AC
BC
ABC
STAGE TREATED

Table 13: Mean dry weight per bean and ratio of hulls (parchment): weight of beans obtained from fruits treated at expansion stage and picked between 29 September and 16 December 1976 in Experiment I of Table 6.

Treatment	Dry weight per bean (mg)			
	Bean with hull	Hulled bean	Hull only	Ration of hull:bean
Untreated	174	120	35	1:3.4
Ethanol	159	110	40	1:2.8
GA_3 (100 µg/fruit)	179	134	41	1:3.3

Table 14: Effect of gbberellic acid (PRO-GIBB) on the dry weight, % grade 'A' (6.75 mm sieve) and overall liqur quality standard of beans from fruits treated at eigher "pinhead" (4-10 weeks old) or at both of the two stages. Data are means of the three treatment stages (see text). Overall quality standard was assessed by the Mild Coffee Trade Association, Nairobi.

Treatment Records	Water Control	Treatments			
		Gibberellic acid ppm (a.i.)			
		25	50	100	SE
Bean dry weight (mg/bean) Increase above control	163.3 -	176.7 8%	173.3 6%	180.0 10%	9.2 NS -
Grade 'A' beans (%) Increase above control	61.7 -	72.7 18%	69.7 13%	75.7 23%	6.9 NS -
Overall quality standard (Scores range from 0-7 where 0 = fine and 7 = very poor)	3.3	2.5	2.3	2.7	0.5 NS

For instance, the ratios of the beans:parchment obtained from the treated fruits was the same as that obtained from the control fruits (Table 13).

Quality assessment

It was noticed from the structure of the fruit shown in Plate 3 that the untreated fruits had beans which were harder to cut through with a sharp knife than either of the beans from ethanol or GA_3 treated fruits. This observation led to a suspicion that the bean from the fruit treated with GA_3 dissolved in ethanol may not be of good quality. The beans obtained from fruits treated with aqueous sprays of GA_3 were therefore assessed for overall quality standard.

The overall quality standard and grading of the beans obtained from fruits treated with various GA_3 concentrations are presented in Table 14. The GA_3 treatment tended to improve the overall bean quality standard. The percentage grade 'A' beans was also more in the GA_3-treated samples than in the control samples. The GA_3-treated samples than in the control samples. The GA_3-treatment tended to improve the overall bean quality standard. The percentage grade 'A' beans was also more in the GA_3-treated samples than in the control samples. The GA_3 treatments increased bean weight by 8-10%, percentage of Grade A beans by 13-23% and the overall quality slightly.

Discussion

The GA_3 treatments increased growth rates, possibly by increasing cell division and/or cell size. Although kinetin is better known for its effects in stimulating cell division, GA has also been reported to have a marked effect in stimulating of cell division in the sub-apical meristems of certain plant species, and in presence of auxin,GA is also known to increase cell size (Osborne, 1965). In Vitis vinifera L. fruit, for example, GA has been reported to increase the rate of fruit growth by increasing the cell diameter and hence tissue growth in the region between the locule and the peripheral vascular tissue (Sachs and Weaver, 1968).

It is well known that fruits react in diverse ways to growth substance, depending on the time of application. In grapes, for example, GA applied at or after anthesis promotes pericarp growth mainly through cell enlargement (Coombe, 1973). Gibberellic acid applied at an early stage was also reported to result in larger fruits of Prunus avium L. (Proebsting et al., 1973). In this study it was observed that the response of growth rates to GA treatment was increased more when GA was applied during early stages of fruit growth, irrespective of irrigation treatment.

Coombe (1973) suggested that the hormonal control of the first phase of rapid growth in some fruit resides in the

interactions between auxins, cytokinins, and ABA, and the relative importance of each hormone changes as development progresses from cell division to cell enlargement. The varying response to GA and kinetin treatment in this study might therefore mean that there was low level of endogenous GA where the response was best, and that of the bean (endosperm) formation stage where the response was poorest, fruits might have had high levels of endogenous gibberellin as has been observed in other fruits (Iwahori et al., 1968). In this study the GLS appeared to be low at the 'pinhead' stage and to be high at the endosperm formation stage (Fig.2). The poor response to exogenously applied kinetin may have been due to the fact that the pH of the solution may not have been very well adjusted. The endogenous levels of the CLS appear to be low in the 'pinhead' but high in the rapidly expanding fruits (Fig. 12). This means that the fruits should have at least responded to the exogenous kinetin applied at the 'pinhead' stage if the pH of the solution was well adjusted.

The effect of GA_3 and kinetin on the length, diameter and L/D ratios of the fruits was not very consistent in this study. The increase in L/D ratios by the two hormones (Table 7) is in agreement with what has been recorded in, for example, grapes where the GA effect observed was the result of the enlargement of the distal rather than of the proximal parenchyma tissues (Weaver and McCune, 1959; Sachs and Weaver, 1968; Webster and Growe, 1969). However, GA_3 failed to increase

L/D ratios when applied at other times (Table 8), which agrees with observations on apples (Stembridge and Morrell, 1972). The failure was probably due to timing of the treatment, which in turn might be related to the rate of cell division and hence the stage of fruits when the hormones were applied. Cytokinins alone or in combination with $GA_{(4+7)}$ have also been observed to increase L/D ratio in some fruits (Williams and Stahly, 1969), as was observed in this study.

Saturating doses of GA_3 made the coffee fruit grow larger than the untreated control. The increase was more noticeable when the treated fruits were expanding rapidly (Plate 1). At early stages of "rapid expansion" (11-14 weeks of fruit growth) the treated fruits had bigger locule size than the untreated fruits (Plates 4 and 5). Later on the untreated fruits seemed to catch up in size with the treated ones; so that at maturity the treated fruits were only about 25% bigger than the controls (Plate 3). It can therefore be concluded from this work that GA_3 application increases growth rate of the fruit by accelerating the rate of locule expansion and hence locule size when the fruits are young.

It normally takes about 19 weeks of growth for the fruits to attain their maximum size (Wormer, 1966). In this study the GA_3 treatment made the fruits to attain their

maximum size in about 16 weeks. In this way the GA_3 brought forward the fruit growth by about 3 weeks. However, the final difference in locule size in the treated fruits was not significantly different from that in the untreated fruits (Table 9). Once the locule has reached its maximum size the endosperm wall becomes lignified and physically restricts further internal expansion (Wormer, 1966). As stated earlier, the inconsistency of the effect of GA_3 on L/D ratios may be due to the application timing. It cannot therefore be concluded without further work that GA_3 affects L/D ratios of the Arabica coffee fruit.

The locule contains the integument tissue which the endosperm uses for development. In this experiment it was found that some of the treated fruits had hollow spaces without the integument tissue in the expanded locule. This apparently led to incompletely developed beans. From this study, it is not clear whether it was the ethanol used as a carrier of GA_3 which was responsible for the incomplete development of the integument cells in some fruit locules or GA_3 *per se*. However, GA_3 appeared to increase the size of the cells in the pulp (Plate 7) as would be expected and these cells were not damaged by the treatment.

Gibberellins have been reported to delay ripening in many fruits (Dostal and Leopold, 1967; Sparks, 1967; Ruso *et al.*, 1968). It has been reported to delay ripening by lowering respiration and diminishing softening, especially when applied

at a later stage of fruit growth (Abdel-Gawad and Romani, 1967), by suppression of anthocyanin development (Proebsting et al., 1973) and by delaying loss of green chlorophyll pigments (Lewis et al., 1964) which has been shown on leaves of Nasturtium and Taraxacum officinale to be associated with concomitant delayed loss of protein and RNA (Beevers, 1966; Fletcher and Osborne,1965). In this study the delay (Table 10) might have been due to lowering of respiration rate as a result of the late application of GA_3, since the effect was observed where application was conducted either at more frequent intervals up to the ripening stage (Experiment I) or when the fruits were 28 weeks old and only 4 weeks away from the onset of natural ripening (Experiment V). Gibberellic acid might have slowed down respiration when applied late during fruit development because during and after fruit ripening the pericarp increases in size and respire more than before (Cannell, 1971 c).

Crane (1964) has suggested that the role of hormones in fruits development may be to mobilize elaborate food material from the source to the sink. This view has been strengthened further in apical dominance studies where it has been shown that metabolites are directed from the the source e.g. buds to the point of hormone application (Davis et al., 1966). Since substrate flow from leaves to fruits is increased when the fruits are rapidly expanding (Hale and Weaver, 1962), including in Arabica coffee fruits (Cannell and Huxley, 1969), the GA_3

treatments may be causing an increase in substrate utilization and storage in the coffee seed and this may be responsible for an extra increase in dry weight (Table 12).

The apparent improvement of bean quality might have been due to the tendency of GA_3 increasing fruit size (Plate 1 and Plate 2) and hence bean size (Plate 3). The tendency might have been responsible for the production of the extra grade A beans in the treated samples. The presence of more grade A beans is likely to lead towards the award of slightly higher quality grade as explained in the Introduction.

The results obtained in this work indicate that gibberellic acid (GA_3) alone or in combination with 6-Furfurylaminopurine (kinetin) when applied onto developing fruits, makes the fruits grow bigger and the beans from the treated fruits heavier than those from the untreated controls. It is possible that GA_3 or GA_3 + kinetin enlarged the fruits and increased the bean weight by directing the nutrients to the point of hormone application as has been observed in the studies of Seth and Wareing (1967).

4.2 Foliar application of Gibberellic acid

4.21 Experimental

Field trees used in this study were located at what would be categorized conveniently as low, medium and

high altitude sites of Donyo Sabuk (Kianzabe, 1479 m), Coffee Research Station (Ruiru and Rukera, 1608 m) and Kiambu (Kibubuti, 1890 m), respectively. The experimental details are in Table 15.

Aqueous sprays of GA_3, formulation PRO-GIBB 10% a.i. w/w) were applied to whole trees of 100 ppm (a.i.). All sprays including water controls, contained non-ionic wetter 'Agral 90' at 0.05% and were applied during the day to run-off (about 700 ml/tree), using a hand-operated knapsack sprayer. The applications were repeated on the same trees on three occasions between March to May 1974 (Table 15) or February to April and March to May, 1975 (Fig. 20). The experiment was on randomized block design and each treatment was replicated three times on 180-tree plots in 1974 and 20 times on single-tree plots in 1975.

All pests and diseases were controlled in the usual manner (Ombwara, 1968). However, at the high altitude sites, rapidly expanding fruits got infected with Colletotrichum coffeanum Noack (Coffee Berry Disease) because protective sprays were inadvertently omitted on 19 May 1974. As a result, over 90% of the "early crop" (June-September harvest) was lost and this factor might have influenced the yield figures in 1975.

Prior to the start of the sprays, four first order

TABLE 13. EXPERIMENTAL DETAILS OF TOLIAR

Site and altitude	Date of spray application	Plant material and type of management
Kianzabe 01 Donyo- Sabuk 1479 m	20.3.74	Cultivar SL 28 planted 1953 Unshaded, capped multiple-stem Ground irrigation 1334 plants/ha
Rukera CRS Ruiru 1608 m	26.3.74 19.4.74 17.5.74	Cultivar SL 9 Planted C 1920 Unshaded, capped Multiple-stem Overhead irrigation 1334 plants/ha
Kibubuti, Kiambu 1340 m	22.3.74 18.4.74 17.5.74	Cultivar SL 28 Planted 1950-60 Shaded, capped multiple-stem No irrigation 1334 plants/ha

Type of crop and the date of flowering	Harvest dates from June 1975 to February 1976
Early crop 4 Nov 1974 flowering	June: 6 and 17 July: 4 and 23 August: 8
Late crop 8 March 1975 Flowering	October: 23 November: 12 December: 29
Early crop 17 Nov 1974 flowering	June: 18 July: 16 August: 6
Late crop 12 Mar 1975 Flowering	Oct: 17 and 31 Nov : 12 and 24 Dec: 9 and 22 Jan : 9 and 28
Early crop 15 Nov 1974 Flowering	July: 18 Aug: 7 and 21 Sept. 4
Late crop 10 April 1975 Flowering	Oct: 2,16 and 30 Nov: 27 Dec: 11 Jan: 5 and 27

branches (primaries) on ten selected trees per treatment plot used in the 1974 sprays and on all trees used in the 1975 sprays, were tagged at three nodes back from the apex for the purpose of recording growth extension and nodes produced between the time rapid growth started in February/March to the end of June/July when growth rates became minimal.

Ripe fruits were picked from all the treated trees of each treatment plot, except at the high altitude site where the sampling of the trees sprayed in 1974, was modified towards the end of picking between 5th January to the end of recording period (Table 15) to cover only ten randomly selected trees per plot instead of picking the fruits from all the 180 trees. Samples of the fruits were "wet processed" and graded for size at the Coffee Research Station Factory. The beans were later taken to the Coffee Board of Kenya, Nairobi, for the quality assessment.

4.22 Results and discussion

Growth extension and node production

Results on growth extension and number of nodes produced between March and July, 1974, are given in Table 16. The GA_3 treatment significantly increased growth extension at the low altitude site but not at the medium and high altitude sites following the 1974 sprays. There was, however, a tendency of increased growth extension and node production even at the medium and high altitude sites. The rate of node production was faster on branches of GA_3 treated trees at the low altitude site.

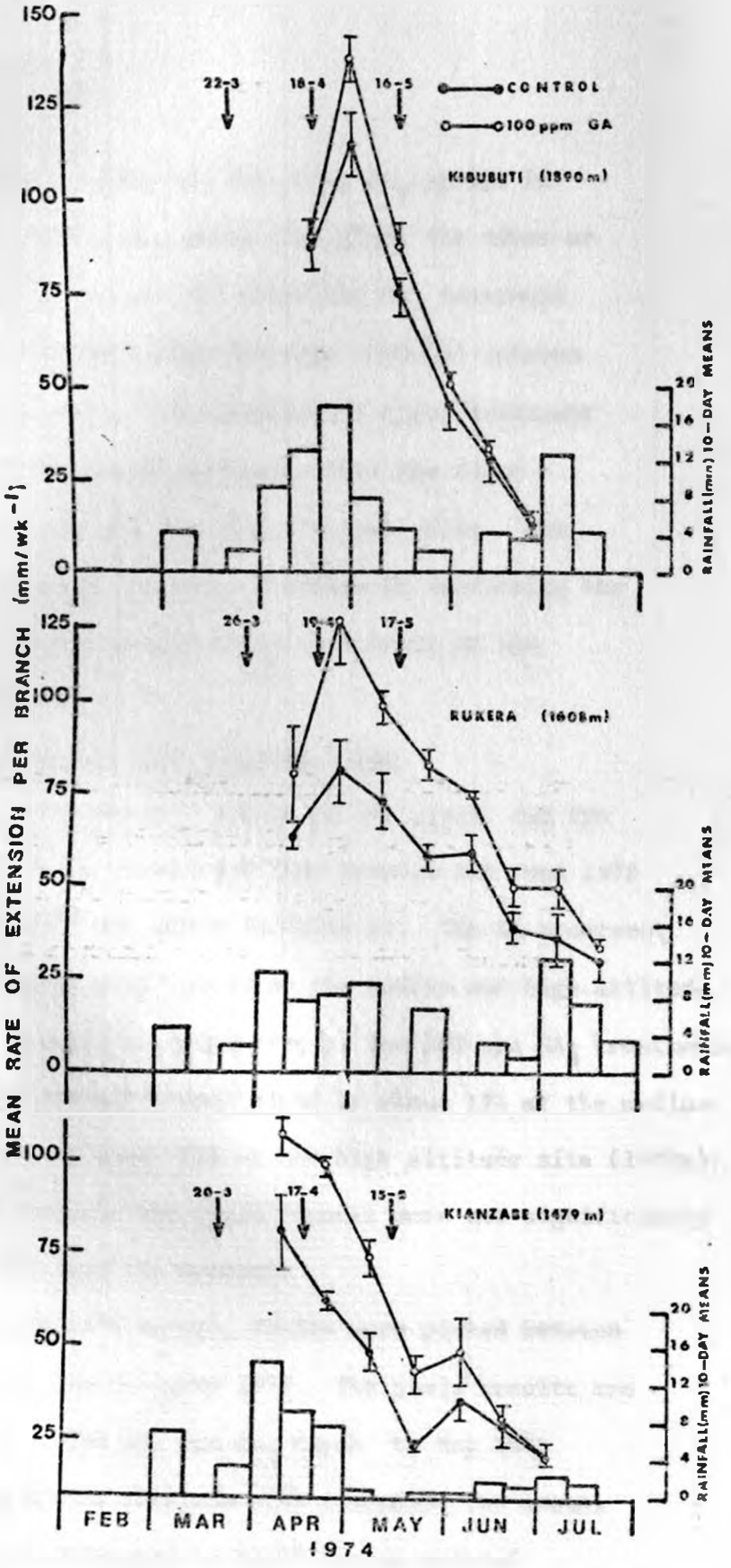
Table 16: Mean increase in length and node number per branch. Records made on 4 branches per tree on 10 trees per treatment replicated three times.

Site and altitude	Growth period 1974	Pro-Gibb (GA_3) (a.i.)	Total length increase cm	Node number (March-August 1974)
Kianzabe (1479 m) (Low)	10 April to 3 July	Unsprayed	6.0	3.3
		100 ppm	8.5	3.7
		SE	0.45*	0.07*
Rukera (1608 m) (Medium)	11 April to 5 July	Unsprayed	8.8	4.1
		100 ppm	11.8	4.4
		SE	1.24 NS	0.21 NS
Kibubuti (1890 m) (High)	18 April to 11 July	Unsprayed	7.6	3.3
		100 ppm	8.6	3.6
		SE	0.92 NS	0.26 NS

* indicates significant at P = 0.05 and
NS indicates not significant

Fig.20a Effect of GA_3 at 100 ppm applied three times between February and April or March and May (indicated by arrows) on the rate of growth increment of primaries from February to June/July at the three altitude sites.
Bars indicate LSD (5x) at P=0.01 .

Fig.20b The response of Coffea arabica L. primary shoots to GA_3 applied foliarly as explained in the text (Chapter IV) during the long rains of 1974 at the three altitude sites.


MEAN RATE OF EXTENSION PER BRANCH (mm/wk⁻¹)
CONTROL
100 ppm GA
KIBUBUTI (1890 m)
22-3
18-4
16-5
RUKERA (1608m)
26-3
17-5
KIANZASE (1479 m)
20-3
17-4
FEB
MAR
APR
MAY
JUN
JUL
1974
RAINFALL(mm) 10-DAY MEANS

Rate of growth extension following GA_3 sprays in 1975 is depicted in Fig.20, which also gives the dates of spray (indicated by arrows) and where the GA_3 treatment promoted growth extension significantly (P=0.01) between February and July, 1975. The February to April treatment had a higher rate of growth extension than the other treatments at the low and the high altitude sites. The March to May treatment was more effective in increasing the rate of growth extension than other treatments at the medium altitude site.

Yield at the medium and high altitude sites

The effects of the 1974 sprays on the yield and the amount of ripe fruits picked per tree between 6th June 1975 and 3rd February 1976 are given in Table 17. The GA treatment suppressed the "early crop" yield at the medium and high altitude sites by about 60 and 12%, respectively. The 100 ppm GA_3 treatments in 1974 increased the overall annual yield by about 12% at the medium altitude site and by about 26% at the high altitude site (1890m). However, on the analysis the yield figures were not significantly different (P=0.05) from the control.

Following the 1975 sprays, fruits were picked between 10th May 1976 and 3rd February 1977. The yield results are shown in Table 18. The 100 ppm GA_3 March to May 1975 treatment at the medium altitude site increased the annual yield by 8% but the February to April sprays reduced the yield by 10%. Only "late crop" was available at the

Table 17: Mean fresh weight of fruits harvested per tree (kg) and total annual yield (kg/ha)

Site and altitude	Pro-Gibb (GA_3 a.i.)	EARLY CROP (JUNE-SEPTEMBER 1975)		LATE CROP (OCT 1975-FEB 1976)		EARLY AND LATE CROP		Kg/ha
		kg/tree	% control	kg/tree	% control	kg/tree	% control	
Kianzabe 1479 m (Low)	Unsprayed	11.803	100%	1.893	100%	13.696	100%	18270
	100 ppm	13.639	116.6%	2.068	109.2%	15.707	114.7%	20953
	SE	0.883*	-	0.456 NS	-	0.420 NS	-	2.542
Rukera 1608 m (Medium)	Unsprayed	0.078	100%	18.103	100%	18.181	100%	24181
	100 ppm	0.031	49.7%	20.327	112.3%	20.358	112%	27158
	SE	0.028 NS	-	0.378**	-	0.358*	-	497*
Kibubuti 1890 m	Unsprayed	0.155	100%	13.510	100%	13.665	100%	18229
	100 ppm	0.140	87.7%	17.097	126.6%	17.237	126%	22988
	SE	0.008 NS	-	1.162 NS	-	1.154 NS	-	1564 NS

* and ** indicate significant at P=0.01 respectively. NS = not significant

high altitude site this time and it was reduced by 6 - 22%.

Yield at the low altitude site

The pattern of cropping at the low altitude site is usually different from that of the medium and high altitude sites. More crop is produced during the "early crop" season than during the "late crop" harvest season. The 100 ppm GA_3 treatment in 1974 increased "early crop" by about 16% and the "late crop" by about 9% giving an overall annual yield increase of about 25% (Table 17). The February to April, 1975 treatment increased yield by 69% which was about 39% more than the March to May treatment of the site (Table 18).

Quality

Bean/fruit ratio, size, appearance and quality of beans produced following the 1974 sprays were not significantly affected (Table 19).

Yield and cropping pattern

The GA_3 affect in reducing the amount of "early crop" is evident at the medium and high altitude sites but cannot be assessed reliably in this study because the "early crop" of these sites were negligible. However, there was an overall increase of "late crop" due to the 100 ppm GA_3 treatment. The percentage increases in "late crop" was much more at the high

Floral initiation is reported to be promoted by leaves through increase in leaf area (Huet, 1973). Net assimilation rate is also reported to be increased by GA in certain species (Austin and Aung, 1973). GA_3 applied at the low site in this study probably increased leaf area and rate of net assimilation. Such a situation might have led to more carbohydrates accumulation in the leaves which were produced relatively faster in the GA_3 treated shoots, than in the untreated controls. The accumulation of carbodydrates might then in turn have led to better flowering initiation (Grochowska, 1973) during subsequent months following spray between March to May and more so between February to April, although GA_3 itself is reported to act as an inhibitor of flower initiation in Arabica coffee (Cannell, 1971 b). In this way the yield of "early crop" might have been increased.

Size, appearance and liquor quality of beans were not adversely affected by GA_3 treatment which confirms Cannell's earlier report (Cannell, 1971 b). In fact the overall liquor quality standard of the beans from the fruits was not affected (Table 19).

It is apparent that GA_3 applied three times at 100 ppm either between February and April, or March and May increases annual yield of Arabica coffee in Kenya. The effect of March to May, 1974 GA_3 application in increasing yield by about 12 to 26% the following year in the large scale trials gives some promise for its commercial application. However, the variability in response from one locality to the other is the main problem.

Table 19: Ratio of clean coffee to fresh weight of fruit harvested, size and quality of beans from three altitude sites

Treatment records	Low altitude site (1479 m) Sampled on 8 August 1975		Medium altitude sire (1608 m) Sampled on 6 August 1975		High altitude site (1890 m) Sampled on 3 February 1976	
	Unsprayed	100 ppm GA	Unsprayed	100 ppm GA	Unsprayed	100 ppm GA
Outturn %	14.8	15.1	14.1	14.4	13.6	13.9
Grade A beans (6.75 mm sieve)	74	78	66	68	54	55
Overall quality standard	3	3	4	4	3	3

Outturn = % clean coffee over total fresh weight

Qaulity standard scores range from 0-7 where 0 = fine and 7 = very poor.

For example, the March to May, 1974 application at the high altitude site increased the yield by about 26% (Table 17) which is not consistent with about 6-22% yield reduction at the same site following the 1975 sprays (Table 18). The environmental conditions during the year of spray, or possibly the spray timing (Table 18) might have influenced slightly the degree of response to GA sprays in this study. But the main reasons for this variation are yet to be ascertained.

It should be noted that there are some areas at the medium and high altitude sites where"early crop" is not desirable. This is because in some years the crop is quite light. The objective of this study was also to inhibit flowering for the "early crop" in these areas, which was partly achieved. There are also definite indications that yield increases may be obtained with the use of gibberellic acid (PRO-GIBB).

CHAPTER V

FRUIT RIPENING

Fruit ripening is a special type of organ senescence during which many processes take place in the fruit. These processes include change in rate of respiration, change in colour, change in carbohydrate constitution (e.g. from starch to sugar) change in pectic composition leading to softening, change in ethylene production, abscission of the fruits from the plants and changes in other processes. All of the above changes are usually related in time to the climacteric rise in respiration and the changes appear to be collectively initiated by ethylene (Pratt and Goeschl, 1969).

Several growth regulators are now known to regulate ripening but ethylene is the growth substance which seems to be directly involved in causing ripening (Pratt and Goeschl, 1969). Ethylene is produced continually throughout the life of the fruits only that its concentration may not rise to physiological effective level until just before the onset of climacteric rise in respiration (Burg and Burg, 1967 ; Lyons and Pratt , 1964; Burg and Burg, 1965). If the fruits have reached the physiological critical state of maturity ethylene production will be activated and will then trigger the ripening processes including further ethylene production (Pratt and Goeschl, 1969).

Amchem Products Inc. of Ambler, Pennsylvania, U.S.A. developed a ripening chemical which is liquid, called (2-chloroethyl) phosphonic acid (CEPA) under the trade name 'Ethrel' and is also known as 'Ethephon'. Several investigators have established that the material is converted to ethylene by degradation (Cooke and Randall, 1968 a; 1968 b; Edgerton and Blanpied, 1968; Warner and Leopold, 1968). The ability of 'Ethrel' to stimulate ripening was first reported by Ruso et al., (1968) when they compared banana ripening of ethylene treated fruit with that treated with 'Ethrel'. Robinson (1969) also reported that field sprays of 'Ethrel' applied two weeks before harvest increased the proportion of ripe fruits of tomato. Since then 'Ethrel' has been found to induce ripening in many plants (Wittwer, 1971) including coffee (Browning and Cannell, 1970; Rodriquez and Molero, 1970; Oyebade, 1971; Upegui and Valencia, 1972; Gonzalez, 1973; Snoeck, 1973).

5.1 Experimental

Use of CEPA and other growth regulators

Experimental details are given in Table 20.

Unshaded, mature trees cultivar SL-28 were used in all the experiments. The fruits on the trees in experiments I, II, IV and V were from major flowerings, on 29 October, 27 November, 8 December and 30 December 1971, and in

Table 70: Experimental

Experiment and date of treatment application	Age of fruits (weeks) approx.	Weeks before natural ripening
I		
24.4.72	20	9
8.5.72	22	7
22.5.72	24	5
II		
27.4.72	26	12
11.5.72	28	10
25.5.72	30	8
2.6.72	32	6
15.6.72	34	4
29.6.72	36	2
III		
18.4.72	28	4
IV		
26.5.72	30	6
16.6.72	33	3

details for studies on the use of CEPA

Site and plant material	Treatments
Gethumbwini, Taika 1525, cv. SL 28 planted 1959	Ethephon, 1400 ppm, whole trees sprayed
Ruiru, 1632 m cv. SL 28 planted 1961	Ethephon 1400 ppm Four branches per tree sprayed
Kianzabe Estate Ol Donyo Sabuk. 1479 m, cv SL 28 planted 1953	Ethephon 500, 750, 1000, 1250 and 1500 ppm Whole trees sprayed
Ruiru, 1632 m cv. SL 28	Ethephon 1400 ppm Whole trees sprayed

Table 20 contd.

Experiment and date of treatment application	Age of fruits (weeks) approx.	Weeks before natural ripening	Site and plant material	Treatments
V 6.12.72	33	3	Ruiru, 1632 m, cv. SL 28	Ethephon at 1400 ppm NAA at 1, 10, 100 200 and 400 ppm Two branches per tree
VI 15.11.71	33	3	Ruiru, 1632 m cv. SL 28	CEPA at 1400 ppm. TIBA at 1, 10, 50, 100 and 1000 ppm. Two branches per tree

Table 20 continued (c)

Layout	Time of recording	Recording
I 120 tree-plots No replication	3,4,5,6,7,9,11,12.13. 14 and 16 weeks after spraying	Cherry numbers weights Quality
II 4 randomized blocks. Four-tree plots	Weekly when the fruits ripened	Cherry numbers weights. Quality.
III 6 randomized blocks. Six tree plots	Weekly from 3rd week after spraying	Cherry numbers weights, Quality
IV 30 randomized blocks. Single-tree plots	Flower abscission: 5 & 7 days after spraying Fort-nightly growth rates. Fruit set after 25 weeks.	Flower-abscission, fruit set, growth, leaf production
V 5 randomized blocks split into plus and minus ethephon. Single-tree plots.	Every two days from spraying for 22 days	Leaf abscission
VI 6 randomized, split into plus and minus ethephon. Single-tree plots.	Every two days from spraying for 24 days	Number of fruits shaken from the branches.

Experiment III from a single major flowering in the first week of October 1972.

Trees or selected cropping primaries were sprayed to near run-off with aqueous solutions of the chemical, and the solutions always contained 0.05% 'Agral 90' non-ionic wetter. In Experiment V, 1-Naphthylacetic acid (NAA) and CEPA were sprayed on the same day, but in Experiment VI 2, 3, 5-triiodobenzoic acid (TIBA) was sprayed 2 days before CEPA.

An attempt was made in Experiment V to prevent leaf abscission induced by CEPA by spraying with NAA. The chemicals were sprayed when fruit ripening had already began and leaf senescence which is marked during fruit ripening was already present. NAA was applied two hours after CEPA was sprayed.

Abscission was recorded by collecting all fallen fruits and leaves after vigorously shaking the trees or treated branches by hand. In Experiment IV flower bud abscission was recorded by counting the numbers of buds lying on the ground below the trees four days after spraying and when the trees flowered the number of flower buds opened and peduncles remaining on the marked branches. At the start of Experiment IV the third node from the apex on each of two primaries per tree was tagged and the distance from the marked node to apex measured every two weeks to provide

estimates of extension growth rates. The same marked nodes were used to estimate node production at the end of the experiment by counting the number of nodes between them and the apex.

Fruit set in Experiment IV was recorded by counting the number of buds flowered at four nodes on each tree and then counting the expanded fruits remaining at these nodes 25 weeks later.

Ripe cherries of Arabica coffee have no natural abscission. Furthermore, it appears that abscission cannot be induced by CEPA, although it eventually has an effect on abscission by making the fruit pericarp softer which can be shaken off the trees (Browning and Cannell, 1970). It was reasoned that the abscission inducing action of CEPA might be increased by applying an antiauxin. Ethylene is probably unable to exert its effect on abscission while sufficient auxin is reaching the abscission zone (Hall, 1952). One substance reported to behave as an antiauxin is 2,3, 5-triiodobenzoic acid (TIBA) (Galston, 1947). An experiment was therefore done to study the effect of TIBA applied alone and in combination with CEPA on the abscission of ripe fruits.

Influence of fruit stage on the use of CEPA

Details of experimental treatments are given in Table 26.

Mature trees of cultivars SL 28 and SL 34 were used for the experiment. The trees were unshaded at low (Kianzabe, 1479 m) and the medium (Ruiru 1608 m) but shaded at the

high (Tinganga, 1829 m) altitude site. Fruits treated at the low altitude site were from a major flowering of 7th November 1974 while those at the medium and the high altitude sites were from the major flowering of 18-20 March 1975. All other fruits from other minor flowerings were removed.

Aqueous sprays of CEPA at 350, 700 and 1400 ppm were applied to whole trees until run-off (about 700 ml/tree). The initial sprays were carried out when the fruits had covered about 36-60% of their normal growth period. Unless otherwise indicated, sprays were continued on other trees for comparison at three weekly intervals until natural ripening commenced on the control trees (fruits turned yellow). The experiment was of a split-plot design in randomized blocks replicated five times on single-tree plots. Fruit stage at the time of treatment application formed the major plots in the design, each of which was divided into four sub-plots according to concentration of CEPA i.e. 0,350, 700 and 1400 ppm.

The number of expanding berries on four selected first order branches (primaries) were recorded when fruits were 56-60% mature and used for abscission and ripening records. Thereafter weekly records of number of abscised and ripe cherries were taken throughout the experimental period.

Percentage of abscised fruits was assessed by adding the number of ripe cherries together with that of the berries remaining on the date when natural ripening started, and then substracting this value from the number of fruits present at the stage when they were 56-60% mature.

Effect of tree size and leaves on fruit ripening response to CEPA

Three experiments were conducted on cultivars SL 28 and SL 34 growing in the field at Coffee Research Station, Ruiru. Sprays were conducted when the majority of fruits were 28 weeks old. All sprays contained non-ionic wetter 'Agrol 90' at 0.05 concentration and were applied with hand operated sprayers.

The trees used in Experiment I were uncapped and grown according to Hawaiian system of double rows spacing of 1.8 x 1.2 x 0.9 m giving 5,980 plants per ha. Under this system, pairs of rows are spaced 1.8 m apart and there is a 0.9 m space between individual rows. There is a 1.2 m space between individual plants and the plants in each row are staggered so that they are not directly opposite each other. The trees on each double row were either 2,3 or 4 years old since the last clean-stumping was done.

CEPA at 1400 ppm was applied to whole trees at the rate of 250, 500, 750 and 1000 ml/tree. The design was a

split-plot, with the age of the trees forming the main treatment and spray volumes of 250-1000 ml/tree forming sub-treatments in each of the three main treatments. The main treatments were on fixed blocks. The sub-treatments were replicated four times on randomized blocks of single tree plots.

Young coffee heads bearing their first crop after stumping were used in Experiment II.

Tree branches were either fully defoliated (0% leaves) half-defoliated by removing one leaf from each node (50% leaves), one quarter-defoliated by removing one leaf per every two nodes (75% leaves) or left with all the leaves intact (100% leaves).

Youngest pair of leaves (not fully expanded) at the tip of the defoliated branches were left intact.

(2-chloroethyl)phosphonic acid at 700 and 1400 ppm (a.i.) was sprayed on to whole trees two days after defoliation. The experiment was on a split-plot design with CEPA at 700, 1400 ppm and controls sprayed with water only forming three main treatments.

The levels of defoliation formed four sub-treatment in each of the three main treatments. The treatments were replicated five times in randomized blocks.

In Experiment III CEPA at 1400 ppm (a.i.) was sprayed only onto ten selected branches per tree in each treatment below. Polythene bags were used to cover either the fruits or the leaves (depending on the nature of the treatment) at the time of spray. The bags were removed soon after applying sprays. The four treatments were on single-tree plots replicated five times on randomized blocks.

In all the three experiments, records of the number of mature fruits on 4-10 selected branches were made soon after the sprays were carried out. The number of fruits ripened each week was recorded.

Processing and quality assessment

After picking, cherry weight and the number of cherries in sub-samples were recorded. The cherries were then processed in the usual manner (Ombwara, 1968) and dried beans graded for size. Bulked samples were liquored for quality assessment by the Mild Coffee Trade Association of Eastern Africa, Nairobi. Some sub-samples of dried hulled beans were viewed under long wave ultra-violent light (Gibson and Butty, 1975) at the Kenya Industrial Research Development Institute, Nairobi in order to detect overfermented coffee beans (stinkers).

5.2 Results and Discussion

5.21 Use of CEPA and other growth regulators

Accelerated ripening and bean quality

In Experiment I, the CEPA sprays brought forward ripening but as natural ripening itself was three weeks later than anticipated, the overall result was a spread of harvest. Although CEPA in Experiment I was sprayed in an attempt to shorten the harvesting period, some crop was still being picked ten weeks later. This clearly implied that relatively immature fruits did not respond to sprays as fast as the others.

The liquoring quality of the treated beans in the above Experiment was not lower than the control (unsprayed). However, the coffee quality was generally poor (Table 21). When fruits with various degrees of maturity are made to ripe together, clearly then there is a risk of lowering the quality by causing some of them to ripen prematurely which is evident from the fact that the sprayed trees yielded 15% fewer grade A beans.

In Experiment II, natural ripening started when the fruits were 38 weeks old. Even the youngest fruits ripened after treatment with CEPA, but early ripening was associated with a marked reduction in the percentage of grade A beans. The correlation between early ripening and bean size was significant at P=0.01 (See Table 21).

The Experiment was designed to determine for one site the earliest stage of fruit development at which ripening can be induced without decreasing the bean quality and size. The results of the experiment are shown in Table 21 and

liquoring quality was not affected. The data suggested that at this site fruits can be made to ripen approximately 5 weeks early without any decline in bean size or quality.

The use of CEPA at 1400 ppm in the above experiment was based on results of experiments at the Coffee Research Station (Browning and Cannell, 1970) where this was the highest concentration which could be used without inducing excessive defoliation and shoot dieback. As the action of CEPA is reported to depend on temperature (Cooper et al., 1968) and as temperature changes with altitude, another experiment was conducted to test the response to CEPA at a low altitude site (1479 m) in Kianzabe (Experiment III). In the low site, CEPA sprayed at 500,750,1000, brought forward ripening by approximately 3 weeks (Fig. 21) where the crop had originated from a single major flowering in October 1972.

In case of CEPA sprayed at concentration above 100 ppm, over 40% of the crop was picked within the first three weeks of spray but the rest was spread to further five weeks. However, in case of unsprayed trees; ripening started from the sixth and was completed about the same time as for the treated trees. In other words CEPA spread the harvesting period (Fig.21). Bean quality and size were not affected by the treatments (Table 21). It is striking to note that all the CEPA treatments made the fruits to ripen at the same time, the percentage increased as the concentration increased. This is in marked constrast to a

Table 21: Effect of early ripening with CEPA at 1400 ppm in experiments I, II, and III on cherry weights, overall bean quality, and percent "A" grade beans

Experiment and origin of sprayed fruits	Time of application. Weeks before natural ripening	Fresh weight per cherry (g)	Percent 'A' grade	Overall quality	Percent moisture at hulling
I					
Multiple flowering, Gethumbwini Estate, Thika	9	1.63	41	6+	11.6
	7	1.62	44	6+	11.6
	5	1.66	33	5-	10.8
	Unsprayed	1.63	57	5-	10.2
II					
Single Flowering Coffee Research Station, Ruiru	*12	2.06	*43	5	10.2
	10	2.10	34	5+	10.5
	8	2.00	35	5	10.5
	6	2.02	55	6	10.4
	4	2.08	70	6+	10.3
	2	2.12	85	5	10.2
	Unsprayed	2.10	74	5	10.2
III					
Single, flowering Kianzabe Estate, Ol Donyo Sabuk	3	2.24	85	4	10.8
	Unsprayed	2.25	80	4	10.8

* the relationship found between percentage grade A (y) and the number of weeks before natural ripening when ripening was induced (x) was $y = 82-4.1x$; $r=0.87$ ($P=0.01$)

Table 22: Effect of ethephon applied 5 days before bud break on flower bud abscission in experiment IV. Percentages transformed to arcsine.

Treatment	Flower abscission. % of total
Ethephon (1400 ppm)	43.6
Unsprayed	17.6
SE = 5.0 (transformed)	Values are means of 10 replications

similar previous experiment (Browning and Cannell, 1970) in which ripening after spraying CEPA depended upon the concentration, but once it started all the fruits ripened at the same time. Whereas in the experiment of Browning and Cannell (1970) CEPA clearly accelerated the processes leading to the onset of ripening, in Experiment III it is likely that in many fruits these processes were already almost completed by endogenous ethylene. It is therefore possible that CEPA triggered the start of ripening itself as a result of being additional to perhaps some rise in the level of endogenous ethylene.

Other responses to CEPA

In Experiment IV CEPA at 1400 ppm was sprayed either 5 days before or 21 days after a major flowering in May 1972, which was 6 or $3\frac{1}{2}$ weeks respectively before the early crop ripened resulted in substantial and significant (P=0.001) abscission of the expanding flower buds within 2 days after the first sprays (Table 22). However, the fruit set from the remaining flowers was not disturbed (Table 23). The same two sprays with CEPA significantly (P=0.05) reduced the extension of primaries for 10 weeks after the first spray was applied, but as node production was not significantly affected, the decrease appeared to be in the internode extension. By the time the fruits ripened on the trees sprayed with CEPA growth extension was still not normal and was significantly (P=0.05) reduced even upto four weeks following the ripening of the fruits.

Fig. 21 Effect of (2-chloroethyl) phosphonic acid (CEPA) at 750,1000 and 1500 ppm applied four weeks before natural ripening, on the percentage of crop picked each week.

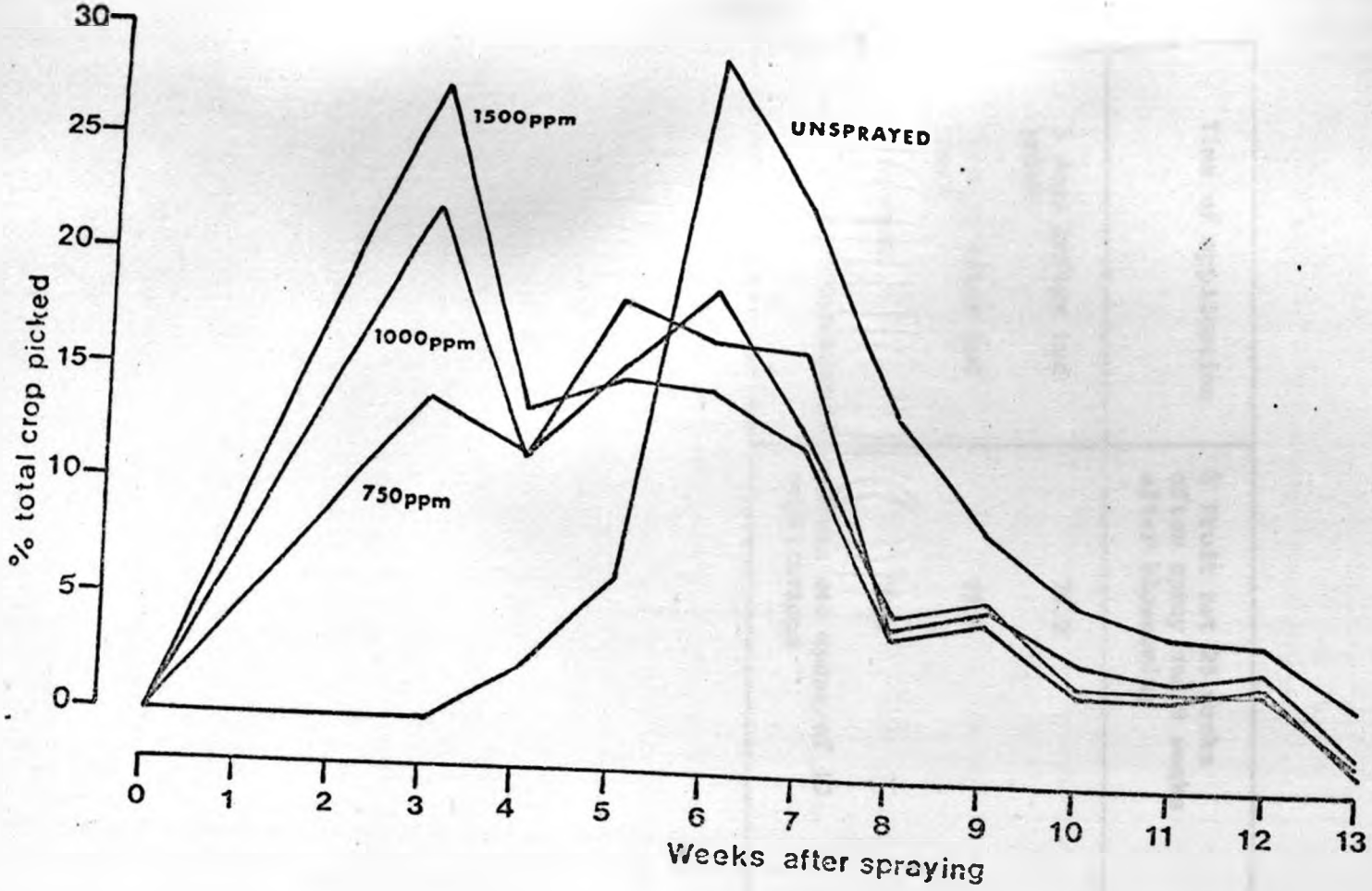

30
25
20
15
10
5
0
% total crop picked
1500ppm
1000ppm
750ppm
UNSPRAYED
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Weeks after spraying

Table 23: Effect of 1400 ppm ethephon on fruit set in experiment IV. Percentage transformed to arcsine.

Time of application	% Fruit set 25 weeks after spray and 9 weeks after blossoming
5 days before bud break	74.2
26 days after bud break	72.4
Unsprayed	72.3
SE= 6.0 (transformed)	values are means of 10 replications

Table 24: Effect of 1-naphthylacetic acid (NAA) on leaf abscission and ethephon-induced leaf abscission in Experiment V. Percentages transformed to arcsine.

	NAA treatment (ppm)					
	0	1	10	100	200	400
NAA alone percent abscission	33.04	24.54	32.62	25.60	34.70	33.94
NAA + ethephon (1400 ppm), percent abscission	63.78	48.28	51.52	45.38	43.42	55.98
	values are means of 5 replications; two recorded branches per replicate. SE = 5.33 (Transformed)					

Effect of NAA on leaf abscission

As can be seen from Table 24, many leaves had senesced sufficiently for approximately, 30% of them could be shaken off the unsprayed control trees. NAA did not reduce the natural leaf abscission. CEPA at 1400 ppm increased the number of leaves which were shaken off the trees to 64%, but this was significantly (P=0.01) reduced if NAA was also sprayed. The maximum reduction of 43% was obtained with NAA at 200 ppm. The response to NAA was not related to the dose used. These data show that once natural ripening starts, CEPA sprays can lead to very substantial leaf abscission which can be significantly reduced(by approximately 60%) with 200 ppm NAA.

Effect of TIBA on abscission of ripe fruits

The results with TIBA are shown in Table 25. TIBA applied alone at concentration ranging from 10 to 1,000 ppm significantly (P=0.001) increased the numbers of ripe cherries which could be shaken off from the trees, with the maximum fruit drop (37%) occurring on the trees sprayed with 1,000 ppm. The response to TIBA did not depend consistently upon the concentration used. CEPA alone sprayed at 1400 ppm stimulated 25% of the cherries to fall, and when TIBA at 50 or 100 ppm was also sprayed the number of fruits falling increased significantly (P=0.001), with approximately 50% of the fruits falling from the trees treated with 50 ppm. Distinct distal abscission layers, with evidence of cell separation, were found on the pedicels of many of the fruits sprayed with CEPA and

TIBA. The cell separation appeared much less advanced in the abscission zones of pedicels from fruits sprayed with TIBA alone.

5.22 Influence of fruit stage on the use of CEPA Ripening

Figure 22 (A-D) summarizes the weekly pattern of ripening of fruits treated at various maturity stages with different concentrations of CEPA. There was a significant difference (P=0.05) in the amount of ripening from one fruit stage to the other. Although at least some fruits ripened at all the fruit stages (Table 26), the best early fruit stage at which to apply CEPA in order to accelerate ripening appreciably appeared to be about 74-77% fruit stage (8-7 weeks before natural ripening is expected to begin). Applications of CEPA at that stage or later brought forward ripening significantly with very little fruit abscission.

There was a significant correlation (r=0.99) between fruit stage treated and the number of fruits ripened (Table 27), and also between the number of fruits ripened at different stages of fruit development and the concentration of CEPA applied (r=0.95). Such correlations arise because the exogenous ethylene released from CEPA acts in concert with endogenously produced ethylene, the level of which may increase as the fruits age as it was observed in tomatoes (Lyons and Pratt, 1964).

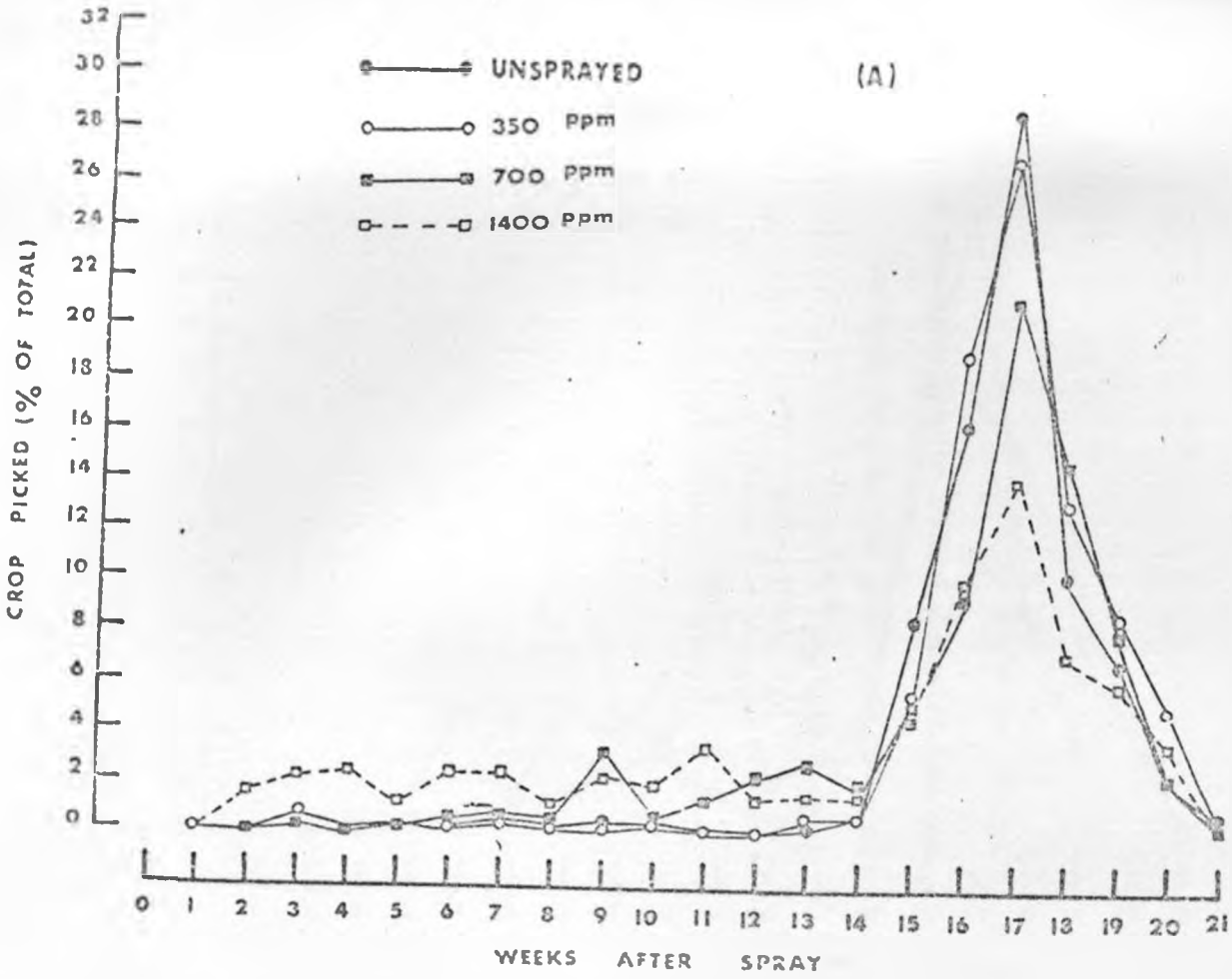
Table 27 also shows that the number of fruits ripened by CEPA at any one fruit stage increased as the CEPA concentrations applied increased. However, CEPA applied at 1400 ppm was always, at any fruit stage, able to cause earlier ripening than 700 or 350 ppm applications. As fruits grew older, the 700 and 350 ppm treatments were increasingly more able to promote ripening, possibly because endogenous ethylene by then reached high enough levels for even low concentrations of CEPA applied to trigger ripening.

The CEPA concentrations differed significantly (P=0.001) in their effect to promote ripening at various sites (Table 28). Hastening of ripening in terms of the number of fruits ripened early was significantly less (P=0.05) at the low site compared to the other two sites. However, there was no such difference between the other two sites. The reason for the difference of CEPA effect at the low altitude site compared to the other two sites is not known, but might be due to the difference between the time of the year when CEPA was applied at these sites. At the low site CEPA was applied between February and May 1974 and might have encountered less favourable conditions for CEPA action, than those present at the medium and high altitude sites where the late crop was treated between July and November 1975. This may be related to endogenous production of ethylene as affected by temperature.

Table 25: Effect of 2,3,5-triiodobenzoic acid (TIBA) on fruit abscission in Experiment VI. Percentages transformed to arcsine.

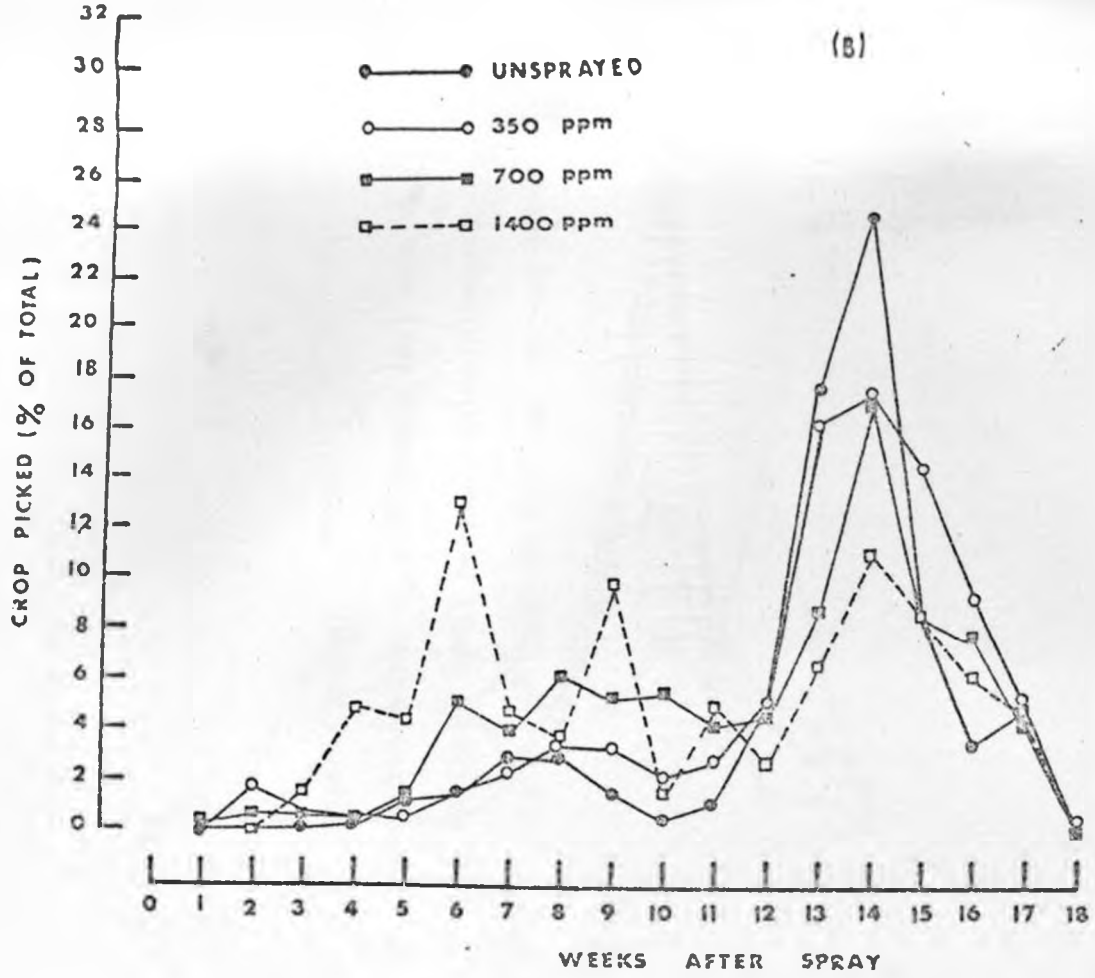
	TIBA treatments (ppm)				
	0	10	50	100	1000
TIBA alone, % abscission	8.24	35.23	26.90	21.43	37.31
TIBA + ethephon (1400 ppm) % abscission	25.27	40.21	50.57	37.62	41.66
	Values are means of 6 replications two recorded branches per replicate				
	SE = 4.21 (Transformed).				

Fig. 22 A Effect of CEPA at 350,700 or 1400 ppm applied 14 weeks before natural ripening on percentage of crop picked each week at Ruiru (1608 m).


UNSPRAYED
350 Ppm
700 Ppm
1400 Ppm
(A)
CROP PICKED (% OF TOTAL)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
WEEKS AFTER SPRAY

(b)

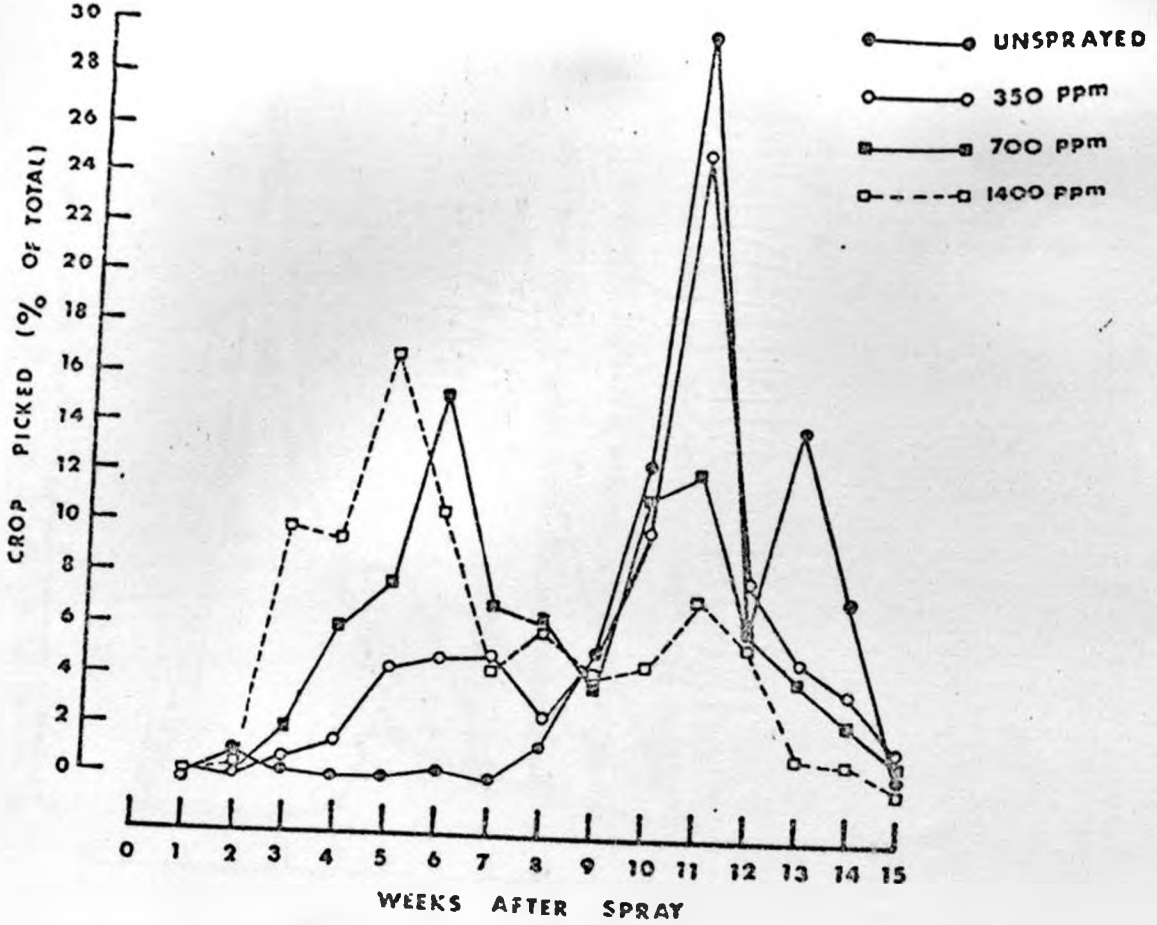
Fig. 22 B Effect of CEPA at 350, 700 or 1400 ppm applied 11 weeks before natural ripening on percentage of crop picked each week at Ruiru (1608 m).


(B)
UNSPRAYED
350 ppm
700 ppm
1400 ppm
CROP PICKED (% OF TOTAL)
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
WEEKS AFTER SPRAY

(c)

Fig. 22 C Effect of CEPA at 350, 700 or 1400 ppm applied 8 weeks before natural ripening on percentage of crop picked each week at Ruiru (1608 m).

(c)


UNSPRAYED
350 ppm
700 ppm
1400 ppm
CROP PICKED (% OF TOTAL)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
WEEKS AFTER SPRAY

(d)

Fig. 22 D Effect of CEPA at 350,700 or 1400 ppm applied 5 weeks before natural ripening on percentage of crop picked each week at Ruiru (1608 m).

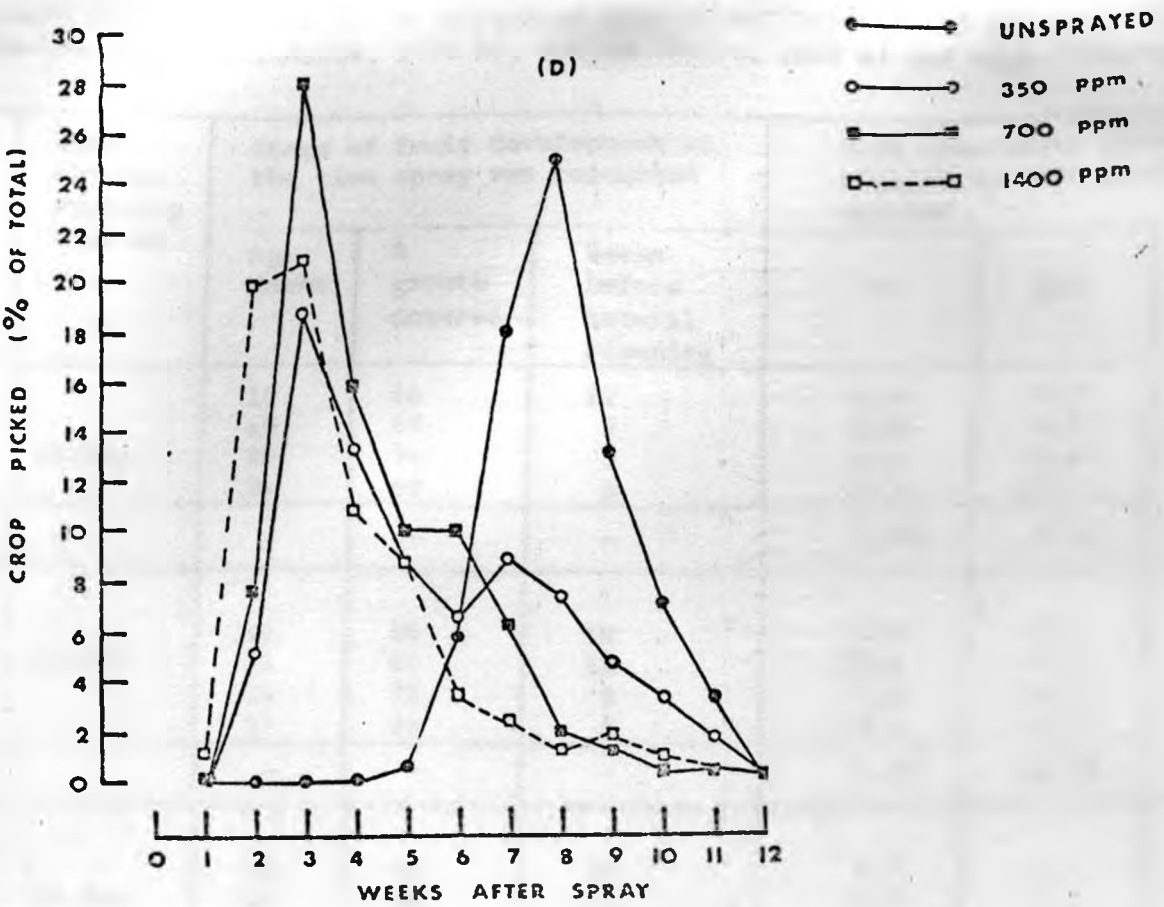

(D)
UNSPRAYED
350 ppm
700 ppm
1400 ppm
CROP PICKED (% OF TOTAL)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS AFTER SPRAY

Table 26: Influence of fruit stage on the effect of CEPA on acceleration of ripening and promotion of crop abscission at low (Kianzabe, 1479 m), medium (Ruiru, 1608 m) and high (Tinganga, 1829 m) altitude sites

Site altitude and date of flowering	Date natural ripening started	Stage of fruit development at the time spray was conducted			CEPA treatments (0-1400 ppm) and % of the fruits ripened by CEPA earlier before natural ripening started				
		Age Weeks	% growth covered	Weeks before natural ripening	0	350	700	1400	Mean
I Kianzabe 1479 m 7 Nov 1974	14 May	15 18 20 24	56 67 74 89	12 9 7 3	3.6 0.6 1.6 0.5	1.7 9.7 6.8 15.1	8.4 24.6 18.1 27.8	14.3 35.0 44.6 43.0	7.00 17.48 17.78 21.60
Mean		-	-	-	1.58	8.33	19.73	34.23	-
II Ruiru 1608 m 17 Mar 1975	27 Oct	18 21 24 27	56 66 75 85	14 11 8 5	2.8 12.2 3.1 0.5	2.9 19.5 19.2 45.3	13.7 33.8 44.5 61.2	22.9 47.9 57.9 60.9	10.53 28.35 31.18 41.98
Mean		-	-	-	4.65	21.70	38.30	47.40	-
III Tinganga 1829 m 20 Mar 1975	20 Nov	21 24 27 30	60 68 77 86	14 11 8 5	0.7 0.3 0.7 0.6	4.7 12.4 9.9 68.4	10.4 21.2 35.8 76.5	9.4 13.6 52.2 91.8	6.30 11.88 24.65 59.33
Mean		-	-	-	0.58	23.85	35.98	41.75	-

Table 26 (continued)

CEPA treatments (0-1400 ppm) and % of the fruits made to abscise by CEPA before natural ripening					Statistics
0	350	700	1400	Mean	
4.4	29.5	23.2	14.3	17.85	Abscission
0.8	20.0	11.2	8.9	10.23	Lsd 5% = 8.1
5.5	16.7	10.9	12.6	11.43	1% = 11.7
3.9	2.3	5.9	1.4	3.38	Ripening
3.65	17.13	12.80	9.30	-	Lsd 5% = 5.0 1% = 15.9
10.6	10.6	10.2	22.0	13.35	Abscission
7.0	7.3	6.5	7.8	7.15	Lsd 5% = 5.0
8.6	9.4	5.5	17.7	10.30	1% = 7.2
8.6	0.7	12.3	24.2	16.53	Ripening
8.70	9.50	8.63	17.93	-	Lsd 5% = 17.2 1% = 24.7
5.2	11.1	29.4	76.0	30.43	Abscission
4.8	12.2	57.1	66.4	35.13	Lsd 5% = 25.3
1.9	8.3	9.7	35.4	13.83	1% = 36.4
3.8	4.7	10.8	7.5	6.70	Ripening
3.93	9.08	26.75	46.33	-	Lsd 5% = 28.5 1% = 41.0

Abscission

Table 26 summarizes the effect of CEPA applied at different concentrations on the number of fruits abscised when CEPA was applied at different fruit stages at three altitude sites. If CEPA is applied to coffee fruits when they are rapidly expanding, the fruits abscise (Browning and Cannell, 1970; Adenikinju, 1975). It is clear from Table 27 that the amount of abscission differed significantly (P=0.05) from one stage to the other. The abscission was significantly correlated (r=0.99) with the stage of fruit development and it decreased as the fruits grew older. The percentage of fruit abscission increased as the concentrations increased (r=0.99). A high degree of correlation suggests that CEPA action may also be related to the release of endogenous ethylene in fruits. Using abscission figures as another criterion for the stage of CEPA application, it also appears that the 74-77% fruit stage would be the earliest stage at which it could be sprayed successfully.

Although analysis of variance indicated that altitude had no significant effect on the number of fruits abscised (Table 28), it appears that there might be a little more fruit abscission at higher altitude sites.

Quality

Shown in Table 29 are the mean fruit and bean weight and quality of bulked sub-samples obtained at the end of harvest

Table 27: Effect of CEPA applied at different fruit stages on ripening and abscission. Values are means of three different altitude sites and cumulative from the date of spray to the week natural ripening started.

Weeks before natural ripening	Fruit stage treatment	CEPA treatment ppm (a.i.) and % ripening					CEPA treatment ppm (a.i.) and % abscission					Statistics
		0	350	700	1400	Mean	0	350	700	1400	Mean	
15-21	56-60%	2.4	3.1	10.8	15.5	*7.95	6.7	17.1	20.9	37.4	**20.52	Abscission
18-24	66-69%	4.2	13.9	26.5	32.2	19.2	4.2	13.2	24.9	27.7	17.5	Lsd 5%=8.36
20-27	74-77%	1.8	12.0	32.8	51.6	24.55	5.3	11.5	8.8	21.9	11.82	1%=12.03
24-30	85-89%	0.5	42.9	55.2	65.2	40.9	5.4	5.9	9.7	11.0	8.00	Ripening
	Mean	2.2	18.0	31.3	41.1		*5.4	11.9	16.0	24.5		Lsd 5%=17.73
												1%=25.35

* The relationship found between fruit stages (y) and mean number of fruit ripened early (x) was $y = 54.5 + 1.1x$ $r=0.99$ ($P=0.01$).

** The relationship found between fruit stage (y) and mean number of fruit abscised (x) was $y + 46.2 - 0.4x$ $r=0.99$ ($P=0.01$).

Table 23: Effect of altitude site and CEPA concentration on fruit ripening and abscission. Values are means of 4 fruit stages (See Table 26) and cumulative from the date of the first spray to the week ripening begun.

Site and altitude	Weeks before natural ripening	fruit stage	CEPA treatment ppm (a.i.) and % ripening					CEPA treatment (a.i.) and % Abscission					Ripening Statistics
			0	350	700	1400	Mean	0**	350	700	1400	Mean	
Kianzabe 1479 m	12-3	56-89%	1.58	8.33	19.73	34.23	15.97	3.65	17.13	12.80	9.30	10.72	CEPA levels
Ruiru 1608 m	14-5	56-85%	4.65	21.7	38.3	47.40	29.01	8.70	9.50	8.63	17.93	11.19	Lsd 5%=8.8 1%=13.1 0.1%=21.5
Tinganga 1829 m	14-5	60-86%	0.58	23.85	35.98	41.75	25.54	3.93	9.08	26.75	47.33	21.52	Altitude Lsd 5%=7.8
Mean			2.27**	17.96	31.34	41.13		5.43**		11.90	16.06	24.52	

* The relationship found between CEPA concentration $\frac{(y)}{100}$ and mean number of fruit ripened early (x) was $y=6.4 + 2.7x$. $r = 0.95$ ($P=0.05$)

** The relationship found between CEPA concentration $\frac{(y)}{100}$ and mean number of fruit abscissed early before natural ripening started (x) was $y=5.2+1.3x$ $r = 0.99$ ($P=0.01$).

from different fruit stages treated with CEPA at the medium altitude site. The quality of all CEPA treated beans considered together was more or less the same as that of the unsprayed controls.

Under Kenya conditions, fruits of various ages are likely to be present on the same coffee tree at any one time. It is clear from this study that fruits sprayed with CEPA at any of the selected fruit stages, some of them were always made to ripen earlier than the control even though they were still physiologically immature. Beans from such fruits under UV light showed bluish white fluorescence (Gibson and Butty, 1975), which is a characteristic feature of stinkers (over-fermented beans). Although stinkers were not detected by the liquorers (Table 29), the presence of immature fruits on trees at the time of CEPA application is likely to lead to a poor quality crop. In fact, the overall quality standard of the treated fruits in one of the samples was slightly lower compared to the untreated controls (Table 29). It is also possible that CEPA might have imparted red colour to the skin andthe pulp of the fruits without accelerating other processes of ripening as observed in apples (Blanpied, et al., 1975) and in Robusta coffee (Snoeck, 1977).

5.23 Effect of tree size and leaves on fruit ripening response to CEPA

The peak of ripening due to CEPA application in Experiments I and II occurred after three weeks as has been

Table 29: Summary of quality results (means of 350,700 and 1400 ppm a.i.) CEPA applied at various stages of fruit development at Coffee Research Station, Ruiru

Details of fruit and beans quality records	Date and stage of fruit development at which CEPA was applied, 1975					
	21 July 56%	11 August 66%	1 September 75%	22 September 85%	Unsprayed Controls	SE
Fresh weight per fruit (g)	1.7	1.69	1.78	1.90	1.8	0.03
Parchment weight per bean (mg)	233.0	227.0	237.0	230.0	243.0	2.79
Outturn %	13.2	13.4	13.3	12.4	13.3	1.99
Dry weight per bean (mg)	170.0	180.0	187.0	177.0	183.0	2.87
Per cent Grade A bean (6.75 mm sieve)	71.7	69.7	57.0	58.5	65.1	2.93
Bean type used for gradings (U=Ungraded and G= Graded sample	G	G	U+G	U+G	U+G	
Quality gradings (Raw	2.9	2.7	2.9	3.0	2.5	0.09
(Roast	1.5	1.8	2.0	1.9	1.8	0.08
(Liquor	2.8	1.7	2.1	2.0	1.7	0.20
(Raw Defectives	1.9	1.3	1.6	1.1	1.5	0.14
(Liquor off flavours	0	0	0	0	2.0	0.40
(Roast Defectives	1.4	1.4	1.8	0.8	1.3	0.16
(Stinkers	0	0	0	0	0	0
Overall quality standard	4.0	3.0	4.5	4.0	4.0	0.24

SE= Standard error of the mean; Outturn= % clean coffee over total fresh weight;

Quality standard gradings range from 0-7, where 0= fine and 7 + very poor.

observed in earlier experiments (Browning and Cannell, 1970; Opile and Browning 1975). But where only branches were sprayed the peak of ripening was observed after 4-6 weeks.

Table 30 summarizes the effect of spray volumes and the tree size (age of tree heads) on the percentage of crop showing ripening period of three weeks. The volume of spray solution did not affect intensity of ripening. Ripening was significantly quicker on the 2 and 3-year old heads. The reasons for this difference was not investigated. But the results suggest that the effectiveness of CEPA may be related to the amount of crop on the sprayed trees as young trees normally carry heavier crops than old trees.

Defoliation only delayed ripening but did not affect the degree of acceleration of ripening following the application of CEPA (Table 31). In Experiment III the leaves were not removed while the CEPA sprays were being directed to either fruits only, to leaves only, or to both leaves and fruits. Compared to unsprayed controls, significantly ($P=0.01$) more acceleration of crop ripening was obtained where the sprays were directed to fruits only (Table 32) followed by the leaves and fruits treatment ($P=0.5$). It is not known why CEPA accelerated ripening faster when the spray was directed to "fruits only" than to "fruits + leaves". It is possible that CEPA might have accumulated in the treated fruits as has been observed in peach fruits (Lavee and Martin, 1974). It was also observed that in Experiment III where only leaves were sprayed, fruits on the node nearest to the treated

Table 30: Percent of total crop picked per treatment within 3 weeks following application of CEFA at 1400 ppm (a.i.).

Age of trees heads since stumping	Spray volume(ml/tree)						Row Statistics
	Unsprayed controls	250	500	750	1000	Mean	F 5%=4.48
2 years	27.50	56.14	72.78	69.38	39.44	38.23	
3 years	3.06	33.84	38.14	30.99	56.06	32.42	
4 years	8.73	24.17	14.00	41.45	19.56	21.66	
Mean	13.00	38.05	41.64	41.27	38.15		

SE of years mean = 6.22 *(P=0.05)
SE of spray volume mean= 8.03 NS
SE of difference =13.90
CV =39.06%
Lsd at 5% =32.11

Table 31: Percent of total crop picked per treatment within 3 weeks following application of CEPA

Defoliation Treatment (% Total leaves left on trees)	Treatment and amount of crop picked			Mean	CEPA Statistics
	0	700 ppm	1400 ppm		
0%	9.88	72.16	78.76	53.93	Lsd 5% = 23.47 1% = 35.54
25%	36.32	82.10	77.08	65.17	0.1%=57.1
75%	56.18	84.22	90.12	76.84	
100%	81.75	89.14	93.28	71.39	
Mean	36.53	81.91	85 06		

SE of defoliation means =5.53 NS (Not significant)
SE of CEPA treatment means =4.79*** (P=0.001)
SE of difference =9.58
CV% =14.33

Table 32: Percent of total crop picked from each treatment within 6 weeks following application of CEPA at 1400 ppm (a.i.)

Values are mean of five replications.

Treatment (Where the sprayers were directed	% crop picked
Leaves + Fruits	48.62
Fruits only	70.63
Leaves only	39.77
SE of mean = 8.04*** (P=0.001)	
SE of difference = 18.42	
CV = 44.88%	
Lsd at 5% = 40.15	
Lsd at 1% = 56.18	

leaves ripened first, followed by the fruits on the nodes further away from the treated leaves. This observation suggests that CEPA may be mobile in Arabica coffee tissues. It also implies that with judicious spraying, a good deal of saving on the chemical (CEPA) may be possible.

There is no information concerning translocation of ethylene in coffee. Similarly there is lack of information on ripening of coffee with respect to endogenous ethylene production. Therefore studies on mobility of CEPA in Arabica coffee need to be carried out to confirm the observation made in this study.

It should also be pointed out here that ethylene reduces auxin transport to abscission zone of tissues and subsequently other ethylene-induced events such as ripening occur (Pratt and Goeschl, 1969). It is therefore possible that CEPA applied to coffee leaves released ethylene which reduced auxin supply to fruit abscission zone and triggered the process of fruit ripening to nearby fruits.

CHAPTER VI

GENERAL DISCUSSION AND PRACTICAL IMPLICATIONS

As coffee is grown for seed, the study on fruit growth and development can be considered very important. It is unfortunate that apart from some fragmentary studies (Wormer, 1966; Cannell, 1974) no systematic work had yet been carried out on this subject. The present study cannot claim to be exhaustive but it has added some information on morphological features of various stages of fruit development, hormonal changes during its development, effect of exogenously applied hormones on fruit growth and thus on crop yield and finally on fruit ripening. Yield always has two components to be considered: quality and quantity. This study apart from looking at the quantitative aspect of yield it has also considered qualitative aspect.

Fruiting is dependent on how well the tree has flowered, which in turn is dependent on the nutritional supply and environment acting through hormonal changes in the tree. Coffee is known to exhibit gregarious flowering (Alvim, 1960 a) if the tree is in good vigour and environmental stimuli are favourable. Favourable environment and concomitant changes in the levels of hormones for various stages leading to flowering has been considered in several papers (Browning 1975 b; Barrows et al., 1979) and any imbalance can lead to flowering abnormalities of various kinds (Kumar ,1981) leading to crop losses. When flowering is normal and gregarious, fruit set is also very good as Arabica

coffee is mostly self -pollinated. No investigations were done on fruit-set in this work but normally fruit-set in coffee is not a problem except in some cases where, inspite of good flowering fruit-set has been disturbed. From recent work carried out by Kumar (1981) it appears that a very high level of endogenous gibberellin may be an important factor leading to the poor fruit-set. Also inadequate environmental stimuli giving rise to abnormal flowers, result invariably is poor fruit-set.

After anthesis young fruits do not show any perceptible growth upto about 9 weeks when they are called "pinhead". "Pinheads" have been found to respire normally (Cannell, 1971 d) show active cell division (Sybenga, 1960) and apparently are active sinks for assimilates (Cannell and Huxley, 1969). From the assessment of levels of various hormones it is clear that two important hormones involved in growth viz. cytokinins and gibberellins are in low supply at this stage whereas abscisic acid - an inhibitor of growth - is abundantly present. It is not to say that the hormones could be the only factor responsible for the lack of growth of pinheads. Assimilates preferetially move towards high hormone sink (Seth and Wareing,1967); it is possible that because "pinhead" are low in hormone content, perhaps lower than the growing shoots, they grow at the expense of pinheads . In most cases "flushing" and anthesis coincide. It appears that only after the completion of flushing that pinheads start expanding. Gibberellins and cytokinins levels start building up at this time and remain relatively high until the fruit expansion is completed. Abscisic acid level drops

at this time. That lack of growth hormones at the pinhead stage may be initially responsible for their quiescent nature, is substantiated from the experiment where externally applied hormones (gibberellin GA_3) accelerated the pace of development increasing finally the size of 11 week-old fruits. This aspect is further confirmed from the trial where GA_3 applied at the "pinhead" stage finally resulted in greater size beans. It appears that careful application of gibberellin at this stage can definitely help the farmer to harvest bigger size beans (A grade beans) and perhaps slightly higher crop yield/ha.

Quality has been an important consideration for Kenya coffee and in this investigation it was found that with the application of gibberellin at the correct stage, liquor quality of the beans may be enhanced.

This now leads to a more pertinent question as to whether or not gibberellins should be applied for the commercial coffee farming and whether in the long run it will be a viable economic proposition. Trials carried out to answer this question did not produce very consistent results on the crop yields. Coffee is perennial crop and its production in the current year is affected by several environmental, nutritional and management factors to which the plantation has been exposed to before, especially in the previous year. Perennials have usually a cycle of

fast growth and a period of quiescence when they show very little growth. Coffee normally flowers on the wood of the previous year. Therefore growth made in the previous year is an important determinant in the crop yield. Effect of gibberellic acid application on the crop yield in the following year may be due to increase in the previous year in the number of nodes which are the fruit bearing centres. In this connection it is interesting to note that at the lower altitude site a significant increase in the node number may have been responsible for an increase in crop yield whereas in case of medium and high altitude sites, as this did not happen, there was hardly any increase in the crop yield.

Timing of gibberellic acid application may be another important factor. This is clearly shown by the application of this hormone at medium and high altitude sites where March-May application increased yield but February to April application reduced yield. Although gibberellin is required for blossoming of coffee flower buds(Browning, 1973 a),too high gibberellin supply at this stage can disturb fruit-set (Kumar, 1980). Coffee normally flowers in these areas during February to April and it is possible that exogenously applied gibberellic acid may have disturbed fruit set resulting in lower crop yield. On the other hand application later in the season when fruits were in the "pinhead stage" may have increased fruit size and weight giving rise to slightly higher yield. This is also seen from the crop yield figures at the low altitude site where

February to April application resulted in 69% extra crop, but March to May application only 30%. In the first case gibberellic acid was probably applied at the pinhead and the fruit expansion stage, but in the second case at a later stage of development when fruits are less sensitive to the gibberellic acid application.

What has been consistently noted in this work from the application of gibberellic acid at medium and high altitude sites is that the "early crop" has been shifted to the "late crop". Coffea arabica L. being a short day plant (Piringer and Borthwick, 1955) gibberellic acid is an inhibitor of flower bud initiation (Salisbury, 1961). Therefore, gibberellic acid application, at the time when buds for the early flowering season are initiating, will inhibit flower bud initiation. It is only when the effect of exogenously applied gibberellin becomes minimal that flower buds initiation will begin in the late initiating season, which will then result in flowering in February-March giving rise to more "late crop".

Summarising the effect from the use of gibberellic acid in coffee it may be concluded that gibberellic acid has a good potential in increasing crop yield in coffee; however, not only the timing of application but the stage of crop development in the annual growth cycle must be taken into consideration. This, of course, will vary from year to year and from one altitude site to another altitude site. As mentioned before agronomic practices such as fertilizer application, irrigation and pruning will also have profound

effect on the results obtained from the use of gibberellic acid. Farmers who may decide to use gibberellic acid for boosting yield must take into consideration all the aspects mentioned above.

Considering what roles other hormones might play in the development of coffee fruit, it could be stated that work here is centred only to endogenous distribution of hormones like cytokinins and ABA at various stages of fruit development except in one case where a cytokinin (kinetin) was applied directly to the fruit. Cytokinins are important as a cell division factor (Letham , 1967) and a sharp rise in the level of endogenous cytokinins just before "pinhead" started expanding simply suggests its direct role in the fruit expansion process. It is unfortunate that externally applied cytokinins (kinetin) did not produce any sgnificant results. Perhaps the method of preparation of the solution could have been responsible for these results. Endogenous cytokinins appeared to be very similar to zeatin riboside and in fact similar to kinetin in its quenching and other characteristics and therefore kinetin should have normally worked. Otherwise, it is possible that endogenous level of cytokinin may have been enough to offset any effect of externally applied cytokinins. In comparing its effect with externally applied gibberellic acid at the pinhead stage it appears that the level of gibberellic acid continues to be low until about week - 15 but cytokinin level rises sharply only after week 7. This may have been the reason why it was possible to get effect of exogenously applied GA at the pinhead stage but not of

kinetin. Also, from the chromatographic characteristics at least one of the gibberellins in the fruit extract is gibberellic acid and therefore external application means supplying fruits with their own gibberellin. On the other hand kinetin has never been recognised as endogenous cytokinin. Nevertheless, kinetin worked to increase the bean dry weight by 50% when applied in combination with gibberellic acid. However, more critical studies involving all the stages of fruit development are demanded in this respect and it is only when the endogenous cytokinin is identified that externally applied cytokinin of similar nature could work in conjunction with gibberellin or alone.

It appears that during the expansion stage (week 9-18) both gibberellins and cytokinins are at moderately high levels although the peak of activity for the cytokinin comes earlier than gibberellin which is quite natural as cell division (a primary role of cytokinins) starts first which is then followed by cell elongation (a primary role of gibberellins). Abscisic acid level which was high at the "pinhead" stage drops during this period and continues to be low. Soil moisture stress which lowers fruit expansion must be acting through an increase in the ABA level in the fruits and irrigation, which helps to moderate the ABA level, has been found to increase bean quality by increasing the bean size (Cannell,1973). From week 18-24 all the three hormones maintain a low profile. Generally, the growth of the tree or branches, at this time is minimal and there is no competition for nutrients from the

other sources and therefore fruits are able to fill in very well. Just before ripening, there is a surge again/the ABA level which /in may boost ethylene level before ripening commences. ABA has been found to increase ethylene level in many cases (Milborrow, 1974). No attempts were made to measure ethylene in the fruits but work on CEPA clearly points out that ethylene is the ripening hormone in coffee. A small second peak in the cytokinin level just before ripening may be related to the second expansion stage of fruits, which takes place just before ripening.

Agricultural research must be geared to answer farmers problems. Very often due to coffee crop ripening all at one time congestion in the factory can cause loss in quality of the crop. On other occasions lack of labour for picking can result in the poor quality crop because over-ripening on the trees may reduce quality. In order to get over these problems extensive work was carried out to find out if CEPA, a chemical ripener, could be effectively used to bring forward ripening and stagger harvesting by applying it at a certain interval to different blocks of coffee. Because CEPA acts through ethylene produced after its application, apart from its role as a ripener it also causes abscission especially of developing buds, young fruits and relatively old leaves. Very often thinning is desirable especially where trees are going to overbear and cause subsequent dieback. Thus, CEPA applied at the right time can act as a chemical thinning agent for coffee. In discussing CEPA's primary

role as a ripener,results here have shown that, provided the chemical is applied carefully at the right time, CEPA can be used to stagger harvesting and concentrate ripening. Each point concerning the application of CEPA will now be considered separately.

Of all the concentrations of CEPA 1,400 ppm appeared to be the best for bringing forward ripening. Lower concentrations were less effective and higher concentrations caused unnecessary abscission of leaves, die-back and drying of branches essentially exhibiting some phytotoxic sysmptoms (Browning and Cannell, 1970). In this connection it may be mentioned that with relatively more mature fruits (e.g. 90% chronologically mature) lower concentrations of CEPA can effectively work. This confirms the observations of Snoeck (1973), who found out that in Robusta coffee, asthe fruits aged lower concentrations of CEPA could be used to hasten ripening.

(2-chloroethyl)phosphonic acid (CEPA) when applied to plants acts by releasing ethylene (Warner and Leopold, 1968). Apart from the role of ethylene as a ripener it also causes abscission (Lyons and Pratt,1964) and an observation to this effect was made when CEPA was applied to bring forward ripening. Young fruits, especially those which were expanding, were more prone to abscission caused by CEPA. Expanding flowers at the candle stage, were also susceptible to abscission. Leaves also abscised but in this case older leaves were affected. These are some of the problems

which could make the use of CEPA rather difficult.

Under Kenyan conditions trees always remain exposed to inductive day length conditions for the flower bud initiation and therefore flower buds get initiated and develop to flower any time of the year. However, moisture related factors play an important part in bringing flowering coinciding with the two main rains (Browning, 1975 a). Major flowering comes soon after the first showers of the main rains in about March and second major flowering with the onset of short rains in November. If trees had been exposed to enough drought and then irrigated or it rains, flowering can come any time. In essence, it often happens that on the same tree various stages of fruits may be observed. If developing berries are there along with mature berries and if CEPA is applied to bring forward ripening, expanding berries and young fruits are likely to be abscised. This presents a major problem in the use of CEPA. It clearly means that cropping has to be regulated if CEPA is recommended for the commercial use or this has to be applied only where cropping is uniform. Other problems related with the application of CEPA is the abscission of leaves as mentioned before. However, this can be minimised if NAA is also applied along with CEPA. In anycase, this is not a major problem as only relatively old leaves are affected, which are very low producers of carbohydrates (Kumar and Tieszen, 1976).

In utilizing abscission causing property of CEPA for abscission of ripe berries, it was foundthat this could be promoted if TIBA was used with CEPA, and as much as 50% of the berries could be abscised with this treatment. This finding is similar to that observed in cotton by Morgan and Durham (1972). Although this requires further experimentation, a possible application of this property for mechanical harvesting of coffee may be conceived.

Quality of CEPA sprayed beans is dependent on how mature the fruits were, before the ripener was applied. Berries which were chronologically less than 75% mature when sprayed with CEPA produced lower grade A beans and liquor quality was also poor. It is quite natural to find results like these as berries may not be filled in completely with endosperm tissue before this period which, although ripens under the influence of ethylene, results into lights giving low overall quality. This does not however happen when CEPA is sprayed when the fruits are fully mature and waiting to get ripe. In fact CEPA sprayed after fruits had attained 85% maturity in a trial resulted into slightly higher number of grade A beans although overall quality was graded similar to that for the unsprayed control.

Scientific study embraces continous uncovering of knowledge. This work has not managed to penetrate into more sophisticated and deeper realms of basic studies on the

physiology of fruit development. However, relationships with hormones in the development of coffee fruits have been shown to exist and the pattern has been established. Provided due care is taken in the application of hormones or synthetic growth regulators, they are likely to benefit growers in many ways. Work on cytokinin application requires more careful studies especially in relation to the timing of application. Again, variation in the time of application of GA, in relation to different altitude and growth pattern needs to be worked out. As gibberellic acid including its commercial products is expensive, economics must be known before this chemical is applied commercially.

Attempts should be made by further studies to minimize unwanted effects of CEPA in order to make this chemical a commercial propostion. More work on endogenous ethylene level should be taken up in order to find the peak of its production for a more rational approach concerning CEPA application.

APPENDIX I

CRITICAL FACTORS FOR ALLOWANCE FOR ONE WAY (BALANCE) DIVISION INTO GROUPS

1% RISK

No. of groups = No. of ranges = No. of treatments

Entries are to be multiplied by sum of ranges within groups to obtain allowances for group totals

	2	3	4	5	6	7	8	9	10
2	7.92	4.42	2.96	2.06	1.69	1.39	1.20	1.03	0.91
3	3.14	2.14	1.57	1.25	1.04	0.89	0.78	0.69	0.57
4	2.47	1.74	1.33	1.08	0.91	0.78	0.69	0.62	0.56
5	2.25	1.60	1.24	1.02	0.86	0.75	0.66	0.59	0.54
6	2.14	1.55	1.21	0.99	0.85	0.74	0.65	0.59	0.53
7	2.10	1.53	1.21	0.99	0.84	0.74	0.65	0.59	0.53
8	2.08	1.52	1.21	0.99	0.85	0.74	0.66	0.59	0.54
9	2.09	1.53	1.22	1.00	0.85	0.75	0.66	0.60	0.54
10	2.10	1.55	1.23	1.01	0.86	0.77	0.67	0.61	0.55
11	2.11	1.56	1.24	1.02	0.88	0.78	0.68	0.61	0.56
12	2.13	1.58	1.25	1.03	0.89	0.79	0.69	0.62	0.57
13	2.15	1.60	1.27	1.04	0.90	0.80	0.70	0.63	0.58
14	2.18	1.62	1.28	1.06	0.91	0.80	0.71	0.64	0.59
15	2.20	1.64	1.30	1.08	0.92	0.81	0.72	0.65	0.60
16	2.22	1.65	1.31	1.09	0.93	0.82	0.73	0.66	0.61
17	2.24	1.67	1.33	1.11	0.95	0.83	0.74	0.67	0.61
18	2.27	1.69	1.34	1.12	0.96	0.84	0.75	0.68	0.62
19	2.30	1.71	1.36	1.14	0.97	0.85	0.76	0.68	0.62
20	2.32	1.73	1.38	1.15	0.98	0.86	0.77	0.69	0.63

APPENDIX I Continued....

11	12	13	14	15	16	17	18	19	20
0.82	0.75	0.68	0.63	0.59	0.55	0.51	0.48	0.46	0.43
0.57	0.52	0.48	0.45	0.62	0.39	0.37	0.35	0.34	0.32
0.51	0.46	0.44	0.41	0.38	0.36	0.34	0.32	0.31	0.29
0.49	0.45	0.42	0.40	0.37	0.35	0.33	0.33	0.30	0.29
0.49	0.46	0.42	0.39	0.37	0.35	0.33	0.31	0.30	0.28
0.49	0.46	0.42	0.40	0.37	0.35	0.33	0.32	0.30	0.29
0.50	0.47	0.43	0.40	0.37	0.36	0.33	0.32	0.30	0.29
0.50	0.48	0.43	0.40	0.38	0.36	0.34	0.32	0.31	0.29
0.51	0.47	0.44	0.41	0.38	0.37	0.34	0.33	0.31	0.30
0.51	0.48	0.44	0.42	0.39	0.37	0.35	0.33	0.32	0.30
0.52	0.49	0.45	0.42	0.40	0.38	0.35	0.34	0.32	0.31
0.53	0.50	0.46	0.43	0.40	0.38	0.35	0.34	0.32	0.31
0.54	0.51	0.47	0.44	0.41	0.40	0.37	0.35	0.34	0.32
0.55	0.52	0.48	0.45	0.42	0.40	0.38	0.36	0.34	0.32
0.56	0.52	0.48	0.45	0.43	0.41	0.38	0.36	0.34	0.33
0.57	0.53	0.49	0.46	0.43	0.41	0.39	0.37	0.35	0.33
0.57	0.53	0.49	0.46	0.43	0.41	0.39	0.37	0.35	0.34
0.58	0.54	0.50	0.47	0.44	0.42	0.40	0.38	0.36	0.34

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