A STUDY OF GROWTH IN APRICOT FRUIT

by

D. I. JACKSON, M. Agr. Sc.(Hort.)
Department of Plant Physiology
Waite Agricultural Research Institute
University of Adelaide
South Australia

Thesis submitted for the Degree of
Doctor of Philosophy

April 1965

TABLE OF CONTENTS

I SUMMARY

A study has been made of growth in the fruit of apricot, cv. Moorpark, in Adelaide, South Australia. Morphological changes in the various tissues which constitute the fruit have been examined during the period from anthesis to maturity. Cell division continued in the mesocarp for approximately 15 days after anthesis; cell expansion continued from anthesis to maturity. Differences in the shape of fruit and of cells in the mesocarp have been related to stage of development and to various treatments imposed on the fruit.

Significant positive relationships have been shown to exist between the size of fruit at early pit-hardening, and that at the end of pit-hardening and at maturity. Large fruit at pit-hardening were also shown to ripen earlier. Fruit were shown to vary in size and in the number and volume of mesocarp cells, both within and between trees. Size differences in fruit within a tree were mainly due to differences in cell number, but between trees the contribution of cell number was less and cell size was relatively more important than within trees.

Factors have been tested for their effect upon fruit growth, and response has been measured in terms of fruit volume, cell number and volume in the mesocarp, and endogenous gibberellin.

Thinning flowers at full-bloom increased slightly the size of fruit at maturity, but no difference in size, cell number or cell volume could be detected at pit-hardening.

In 1964 fruit from blossoms which flowered early grew at a

slower rate than from blossoms which flowered late. It is suggested that the slower growth was due to the cooler conditions under which the former fruit developed. Application of heat to apricot branches at night for the first 10 days after anthesis increased the initial growth rate of fruit and of cells in the mesocarp, and produced more rapid cell division in this tissue. It did not affect final fruit size or the number and volume of cells in the mesocarp. No differences in the level of endogenous gibberellin in fruit tissues were produced by heat treatment.

Gibberellic acid injected into apricot branches, at or before full-bloom, increased early growth rate of fruit but subsequently depressed growth and final size. Cell numbers in fruit from treated branches were significantly lower than controls. The level of endogenous gibberellin in fruit tissues was not affected by gibberellic acid application.

Endogenous gibberellin was estimated using the barley endosperm test. There was a positive relationship between growth rate of seed, endocarp, and mesocarp and the level of gibberellin found in crude extracts of these tissues. An hypothesis is suggested to explain the role of gibberellin in apricots. Using a partitioning procedure and paper chromatography some properties of this gibberellin were obtained and are discussed. Only one zone of activity was recovered on paper chromatograms and this ran at an Rf of 0.28 in isopropanol: ammonia:water (10:1:1). The compound is non-basic and more polar than gibberellic acid, but it does not resemble any of the known gibberellins.

II STATEMENT

This is to certify that the thesis here presented is the authors own work, that it contains no material previously published (without due reference in the text), and that no part of the same has been submitted or accepted for any other degree.

III ACKNOWLEDGEMENTS

The author is indebted to the counsel and guidance of his supervisors, Dr. B. G. Coombe and the late Professor L. H. May.

He expresses thanks to Mr. B. Falk for all the photographic work, and to Mrs. F. H. Randles for assistance in preparing some of the diagrams. The advice and help from members of the Statistical Department, Waite Institute is gratefully acknowledged.

Financial assistance was given by the Australian Government under the Commonwealth Scholarship and Fellowship Fund. For this he is most grateful.

IV INTRODUCTION

The attempt has been made in this study of the apricot to gain insight into some of the mechanisms controlling fruit growth. Three aspects of growth were of particular interest: volume increase of the fruit as indicated by diameter measurements; growth as manifested by cellular changes within the mesocarp; gibberellin changes within the developing fruit. It was hoped that if overall growth could be influenced by external factors, in a predictable manner, the subsequent examination of these fruits on the basis of cell number and size, and gibberellin content, might give more insight into mechanisms of growth than the same determinations on untreated fruit. The project was an attempt to study, in one fruit, certain aspects of growth, and factors affecting growth, which had more often been investigated separately, or with different fruit. Such results are more meaningful, and any hypotheses drawn bear closer scrutiny. This is particularly so in work dealing with growth substances wherein conflicting data and conclusions abound. Such observations might indicate that no one mechanism is present in all fruit, but that each has evolved its own particular system. It would thus appear more productive to examine one such system.

Among the treatments applied to apricots, the more

predictable in terms of fruit response appeared to be the effect of temperature and the effect of gibberellic acid. The project has, therefore, as its main thesis: the study of apricot* fruit growth, in terms of fruit volume, cell number and volume, and endogenous gibberellin, and its response to temperature differences and applied gibberellic acid.

---

* A few experiments were conducted with Elberta peaches, but the main body of this research was concerned with Moorpark apricots.

V REVIEW OF LITERATURE

A. A General Description of Fruit Growth

The morphological changes, both preceding and subsequent to fertilisation, which lead to the formation of a fruit are described in most botanical text books. These changes will not be detailed here, but certain aspects of these changes are discussed because they are relevant to the project.

1. Rates of growth

If the overall growth of fruit in terms of weight, volume, length, or diameter, is plotted as a function of time after anthesis, the curve produced is either sigmoid or double-sigmoid in character. Among the fruit mentioned in this review the apple, pear, almond, tomato, strawberry, orange, and avocado have simple sigmoid growth curves. In contrast the curves for apricot, peach, plum, cherry, fig, and grape are double sigmoid. As pointed out by Crane (1964), the type of curve bears no relationship to its morphological structure; thus simple fruits, as almond and peach, or compound fruits, as fig and apple, are found in both groups.

2. Methods of growth

A fruit can grow by cell division, cell enlargement, or by

the proliferation of air spaces or other inclusions within the existing framework. In any fruit there is often one tissue which is predominantly responsible for size increase. In stone fruits, excluding almond, it is that portion of the ovary wall called the mesocarp, in the apple and pear it is the cortex, which, according to the appendicular theory (MacDaniels 1940), is tissue derived from the fused bases of the sepals, petals, and stamens, and in the strawberry it is the fleshy receptacle. It is with these tissues that this work is primarily concerned.

The parts which will eventually form the fruit grow, prior to blossoming, mainly by cell division. As anthesis is approached, growth slows down and may cease (see Luckwill 1957). After anthesis some fruit grow by cell division alone, but the majority grow by both cell division and expansion. Crane (1964) summarises the reported length of cell division in various fruits. In the apple, peach, and plum it continues for three to four weeks, in the pear for six to eight weeks, and in the avocado and strawberry it continues until maturity. Houghtaling (1935) concluded that in the tomato (Lycopersicum esculentum) cell division ceases at anthesis; McCombs (1955) and Simons (1960) however, found cell division to continue in the pericarp of the same species for about 12 days after anthesis and in other tissues it continued even longer. Bradley (personal communication), from observations on mitosis in the Tilton variety of apricot, found no evidence of cell division in the mesocarp of that fruit after four weeks from anthesis.

B. Factors affecting fruit growth.

This review will be concerned primarily with reports on modification of fruit growth, by methods similar to those successful in these apricot experiments.

1. Tree factors

Denne (1963) studied the tree factors affecting the growth of apples in New Zealand: she found that the "size of apple fruits at harvest is influenced by position of the fruit on the spur, seed number, spur size, number of fruit set on the spur, position of the spur on the branch, and the date of flowering within the blossom season". Several workers have investigated the cause of within- and between- tree differences in fruit size as related to cell number and cell size. Denne (1961) concluded that, in dwarf Cox's Orange at East Malling, variation in fruit size within a tree and between trees, was related to both cell number and size. In contrast, for Granny Smith in Australia, Bain and Robertson (1951) showed variation in size of mature apples on a single tree to be due to differences in cell number, the cell size remaining relatively constant. Differences in size for several apple varieties in Tasmania, between similar trees bearing either light or heavy crops, was found by Martin and Lewis (1952) to be influenced by cell size rather than cell number. Seasonal differences in fruit size have been related to both cell size and cell number (Smith 1950; Pearson and Robertson 1953). There are more and larger cells in apple fruit

in seasons when mean fruit size is large, than when fruit size is small. In peach the studies of Bradley (1959) showed a tendency for the larger fruit to have larger cells. This however was not invariable, and cell size differences were not generally sufficient to account for differences in fruit size. She concluded that probably cell number was more important than cell size in accounting for fruit differences.

Although Lilleland (1930) was unable to demonstrate any relationship between the size of apricots at pit-hardening, and that at maturity between several seasons, other reports have shown that, for peaches, such a relationship does exist (e.g. Davis and Davis 1948; Prochsting 1962). This knowledge is used to predict the size of fruit at maturity, and, if necessary, to attempt to modify it by thinning. By this time cell division in the peach has stopped (Addoms et al. 1930; Ragland 1934), and it would seem probable that the number of cells within the mesocarp at this stage can limit the ultimate size to which a fruit grows. It might be postulated that any factor which affects fruit growth by its effect upon cell number, is operative before, or within the first few weeks after antheais. A factor affecting growth by modifying cell size can conceivably be operative at any stage during the growing season.

2. Thinning

It has long been understood that thinning a tree will not only increase fruit size, but often will affect the crop in the

following year. It is further known that the earlier the tree is thinned, the more advantageous it is in both respects. Davis (1962) thinned peach trees at consecutive two week periods and demonstrated this convincingly. One report (C. S. I. R. O. Annual Report 1957 - 8) showed that hand thinning apple fruitlets in the cell division stage increased both cell number and fruit size. Also in the apple, Denne (1960) produced an increase in both cell size and cell number by severely thinning trees at the pink-bud stage of blossom. Later she showed that thinning after cell division had normally ceased, would stimulate further cell division in the cortex of apple (Denne 1961).

3. Temperature

In 1929 Tufts, investigating the growth of Royal apricots in relation to temperature, concluded that minimum night temperature was the most important factor in hastening or delaying maturity; the higher the temperature the earlier the maturity. By artificial heating Lilleland (1935) was able to shorten Stage I in the apricot. Likewise Tukey on sour cherry (1952), McIntosh apples (1956, 1960) and Concord grapes (1958) altered the rate of growth, and in many cases the final size, of these fruits by artificially regulating the night temperature; application of heat increased the rate of growth until an optimum temperature was reached, and above which growth rate declined. Although the most effective time for the application of heat was in the early growth stages, no details were given of its effect upon cell

division or cell growth.

4. Gibberellic acid (GA_3) application

A recent review by Crane (1964) on growth substances in fruit setting and development has, among other things, summarised the research to date on the effect of gibberellic acid applied to fruit. He showed that one of the most remarkable properties of gibberellic acid is its ability to stimulate parthenocarpy in many fruit. It has been proved successful on many fruits which do not respond to auxin, eg. citrus and some Prunus species, and often more successful than auxin on fruits which do respond to the latter. Although other fruit, produced parthenocarpically with gibberellic acid, are smaller than normal, parthenocarpic peaches have been produced which, except for lack of seeds, are almost identical with open-pollinated fruit. Crane concludes: "These results provide indirect evidence that endogenous gibberellin-like growth-regulators are present in peach fruits as a result of pollination and fertilisation, and that they are involved not only in the development and sclerification of the endocarp but also in regulating cell division and enlargement in the mesocarp.... The practically normal growth and development of parthenocarpic peaches produced by GA_3 application further indicates that gibberellin or gibberellin-like substances are the only growth factors produced in the seeds of pollinated fruits that are lacking in unfertilised ovaries of this species". This, it is felt, is a reasonable hypothesis. It should be noted however that

evidence from other fruit does not always support similar conclusions and it would appear possible that different fruit have different hormonal requirements for growth.

In contrast to the above results, GA_3 applied to open-pollinated fruits has generally reduced growth. Carlone (1962), by application of GA_3 to J. H. Hale peaches when the fruit was 4.3 cm diameter, produced an initial stimulation of growth, which lasted for one month, followed by a depression of growth. Ripe fruit were small and russetted. Citrus treated with GA_3 are generally smaller and later-maturing than untreated fruit (Crane 1964). Strawberries on the other hand respond to pre-bloom applications of GA_3 by the acceleration of both flowering and ripening; early yields are increased, while at optimal concentrations total yield is equal to control: high concentrations of GA_3 produced small elongated fruit (Singh et al. 1960; Smith et al. 1961; Turner 1963). Seedless varieties of grapes respond to GA_3 by increased fruit size whereas seeded grapes do not; both are accompanied by reduced fruit set (Crane 1964). Lavee (1960) showed that spraying grapes (var. Queen of Vineyard) with GA_3 , 23 days after full-bloom, increased the weight of berries, containing no seeds, by over 100%, berries with one seed were increased 50%, and those with 2-3 seeds were no different in size.

If GA_3 can, in some cases, replace the stimulus of pollination and fertilisation, it would not appear to complement the stimulus following normal pollination and fertilisation. The response, in fact, is one of growth depression or modification. Fruit may

be smaller and mature earlier, or later, they may be misshapen (Griggs and Iwakiri 1961), elongated (Weaver and McCune 1959), or born on long pedicels (Coggins et al 1960; Walker and Donoho 1959).

C. Endogenous Gibberellins within Developing Fruits.

Hypotheses to explain growth, based on the response of the fruit to applied growth regulators, should be substantiated by parallel work on endogenous growth regulators. Crane (1964) has summarised the recent findings on endogenous gibberellins (and auxins and kinins) within fruit. Seeds generally contain the highest amount of extractable gibberellin and most workers have concentrated on these tissues. Crane states:- "In general, a positive correlation was found between growth of the seed and increase in amount of gibberellin-like substances..... However, as in the case of auxins, no relationship between levels of these substances and fruit growth was apparent".

As previously mentioned, for some species of Prunus it was found that the stimulus to growth following fertilisation could be replaced by applied GA_3. It seemed possible that the seed supplied gibberellin or was somehow responsible for its release to the fruit. Weaver et al. (1962) found seedless grapes would respond to gibberellic acid application by increased growth, whereas GA_3 often depressed growth in seeded varieties. This might suggest that the latter, by virtue

of their seed content, were able to supply adequate gibberellin. When, however, Coombe (1960) tested crude extracts of both seeded and seedless grapes he was able to detect gibberellin activity in the latter only. The obvious difficulty in interpreting such results indicates the possibility, that what is measured as endogenous gibberellin, may not be operative within the fruit, or that a high gibberellin content may not be indicative of a high utilisation rate. Such difficulties in research into endogenous hormones will be considered further in the Discussion.

Much work in identification of gibberellins within plants has been done by workers in the ICI group, England. Jones (1964) detected gibberellins A_1, A_3, A_5, A_6, and A_8, and the lactones of two unknown gibberellins, in the seed of Phaseolus multiflorus. Seeds were dissected into embryonic axis, cotelydons, and seed coats, all the gibberellins were found in each tissue, but 80% of the total activity was concentrated in the seed coats. He discovered that, with the exception of GA_5, which reached its peak activity early, gibberellins were most abundant when the seed was about 25% of its total size.

VI MATERIALS AND METHODS

A. The Trees, the Climate, and the Soil

Unless otherwise stated, experiments were performed on Moorpark apricots and Elberta peaches at the Waite Agricultural Research Institute, Glen Osmond, South Australia. Apricot trees were planted in 1954 and peaches in 1955. Forty-eight apricot and 12 peach trees were available for experimentation. Peaches were 12 ft high and in full production (no yield records are available). An identical description would apply to 50% of the apricots in 1961; the remainder were infected to a greater or lesser extent with the limb-killing fungus disease, gummosis (Eutypa armeniacae). By 1963 the number of trees unaffected was only 12.

Peaches had been regularly pruned to form vase-shaped trees, about half the fruiting laterals generally being cut back. Apricots were unpruned: this is now a common practice in South Australia due to the greater likelihood of gummosis infection in cut or damaged trees.

The main commercial plantings of apricots and peaches in South Australia are in the Murray River districts north-east of Adelaide, where annual precipitation is 22.5 - 25.0 cm, and irrigation is essential.

The climate of Adelaide is less extreme than that of the

Murray districts, but the summers are hot and dry; January, the hottest month, has a mean maximum of 27.8°C and a mean minimum of 16.1°C. Winters are mild and damp, and frosts are not common; July, the coldest month, has a mean maximum of 14.0°C and a mean minimum of 7.5°C. Total precipitation is 62.3 cm to which January contributes 2.4 cm and July 8.2 cm. The above figures for Adelaide have been extracted from information recorded at the meteorological station at the Waite Institute.

Soils are classified as Urrbrae Loam within the Red Brown Earth soil group.

Established trees in Adelaide will survive and bear fruit without irrigation, but optimal production can only be gained with regular summer irrigation. In the Waite Institute orchard, regular irrigation had not been practised: during the experimental period, water was applied several times each summer but at less-than-optimum frequency. No observable effects of drought were however noticed.

B. Sampling and Fruit Measurement

Three measurements at right-angles to each other were used to characterise the fruit in terms of size. These are shown in Fig. 1A; "mean diameter", when used in the text, refers to the mean of the two dimensions a and b, length refers to the dimension shown as c. Measurements were made with a pair of vernier calipers. Fruit volume was found by water displacement.

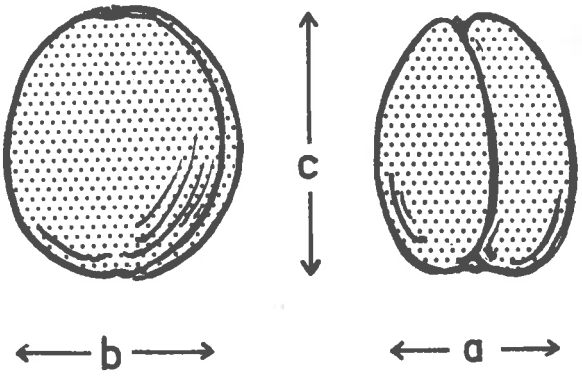


Fig. 1A.- The three major axes of apricot fruit.

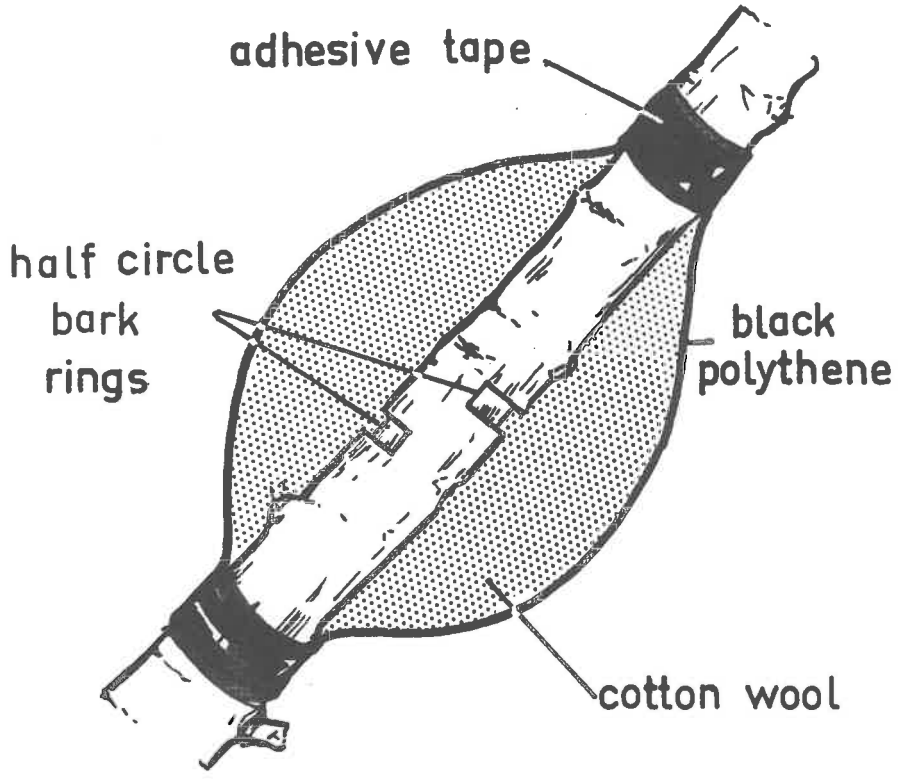


Fig. 1B.- Method for injection of chemicals into apricot branches.

Ripeness of fruit was estimated on a scale from 0 (fruit showing no signs of ripening) through to 5 (ripe). This was entirely subjective:- an unripe fruit is green and hard, while the ground colour of a ripe fruit approximates to "Yellow ochre 07/2" in the Royal Horticultural Society's Colour Charts, and the fruit is soft. Both firmness and colour were taken into account when making these estimations.

Fruit taken from the tree for subsequent cell number and size determinations were stored in standard formalin - aceto-alcohol fixative (FAA). Samples for gibberellin bioassay were kept in plastic bags, quick-frozen with dry-ice and stored at -12°C. Within two months samples were lyophilised, the bags sealed, and samples stored at 2°C.

C. Girdling

Girdling or bark-ringing refers to the method of removing a circle of bark, 5 mm wide, down to the cambial layer of a branch.

D. Application of Chemicals to Trees

Chemicals were sprayed on to, or injected into, branches. Spraying was performed with a small hand atomiser on selected branches until "run-off". The method of injection is shown in Fig. 1B. Two parallel semi-circular rings were cut around the

bark, with a sharp knife, and the tissue between these was removed down to the cambial layer; another half ring of bark was removed on the opposite side of the limb, two centimetres above or below the first. A wad of cotton wool, sufficient to absorb 20 ml of the injection liquid, was placed around the two cuts and the portion of branch so treated was wrapped in black polythene, the ends being sealed with adhesive plastic tape. The plastic and cotton wool were not removed until after fruit were harvested.

E. Measurement of Light

Friend (1961) describes the measurement of integrated light values in the field using Sepia Oxalid paper, No. 33 NT, 8½ x 14 in. This paper becomes bleached on exposure to light, and light will penetrate a stack of papers in proportion to the amount received, the bleached papers acting as a series of density filters. After dry development in ammonia the yellow unbleached area becomes deep blue.

A local firm, Harding & Halder, make a similar paper which they specify as "2 M, 10 Ammonia Paper". Fifteen 3 cm squares of this were stacked yellow side upward, edges were sealed from light with black tape, and a 1 cm diameter circle at the top was exposed to sunlight. These were positioned on branches for 24 hours. Up to six papers were penetrated by light and it was found that the eye could detect bleaching of the yellow paper

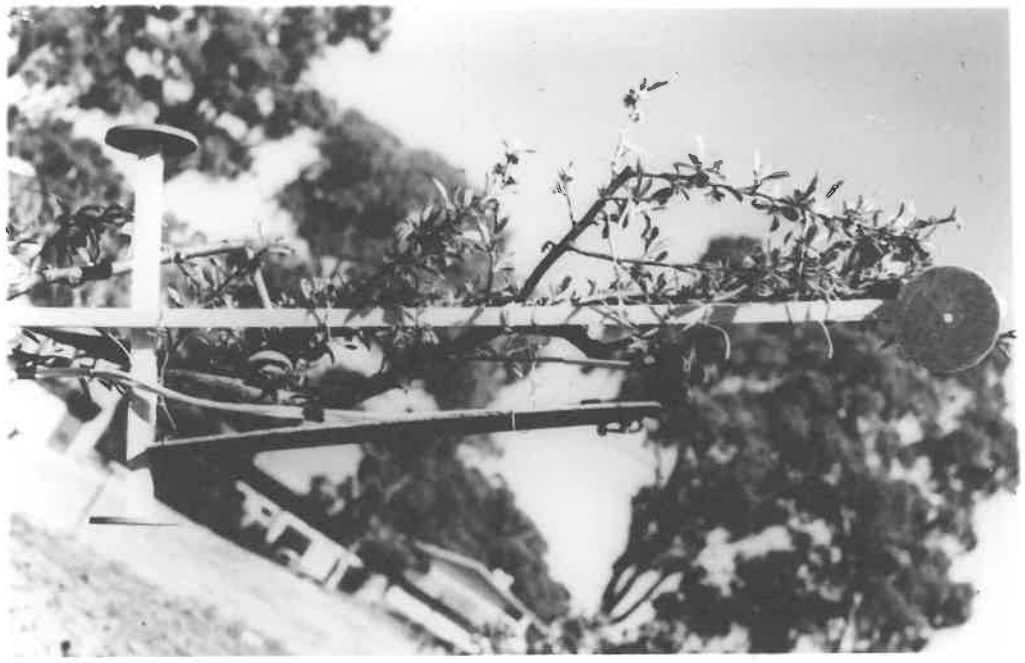
and treatment with ammonia was not necessary. Number of papers penetrated was used as a direct measure of the amount of light received over those 24 hours.

F. Artificial Heating of Branches

The two methods of branch heating are shown in Plates 1 and 2. Method 1 (Plate 1) consisted of a cylinder of wire netting, 1.5 x 0.6 m, covered with plastic film. This was placed over the branch as shown, and temperature was regulated by means of a strip-heater and thermostat. Because of inadequate air circulation damage was inflicted on leaves, fruit, and branches close to the strip-heater, and this method was replaced by Method 2. Method 2 (Plate 2) involved a cylindrical metal cage, 1.35 m long, and 0.65 m in diameter. Ends were covered with plastic and hardboard. When required, a plastic sheet, contained on a roller blind beneath, was drawn around the cylinder as shown. A hair-dryer circulated warm air and temperature in four cages was controlled by a thermostat in one. Variation in temperature over the four cages was rarely greater than $\pm$ 2°C; when, however, on windy nights gaps appeared between plastic and the framework, discrepancies of $\pm$ 3.5°C were occasionally noted.

G. Methods of Cell Counting

Several methods of estimating cell numbers in developing



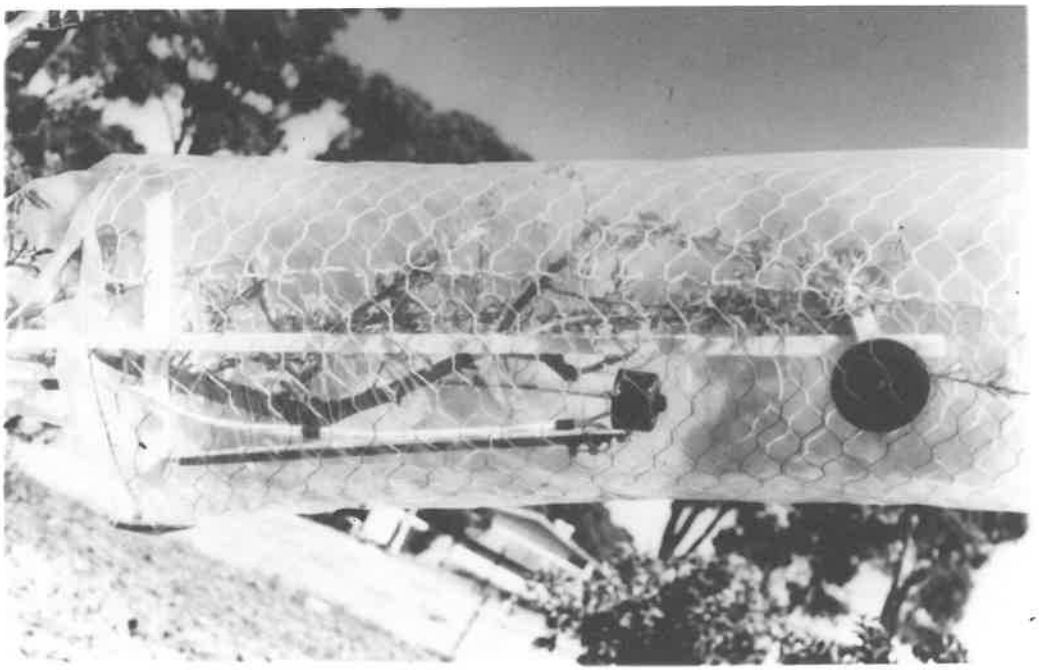
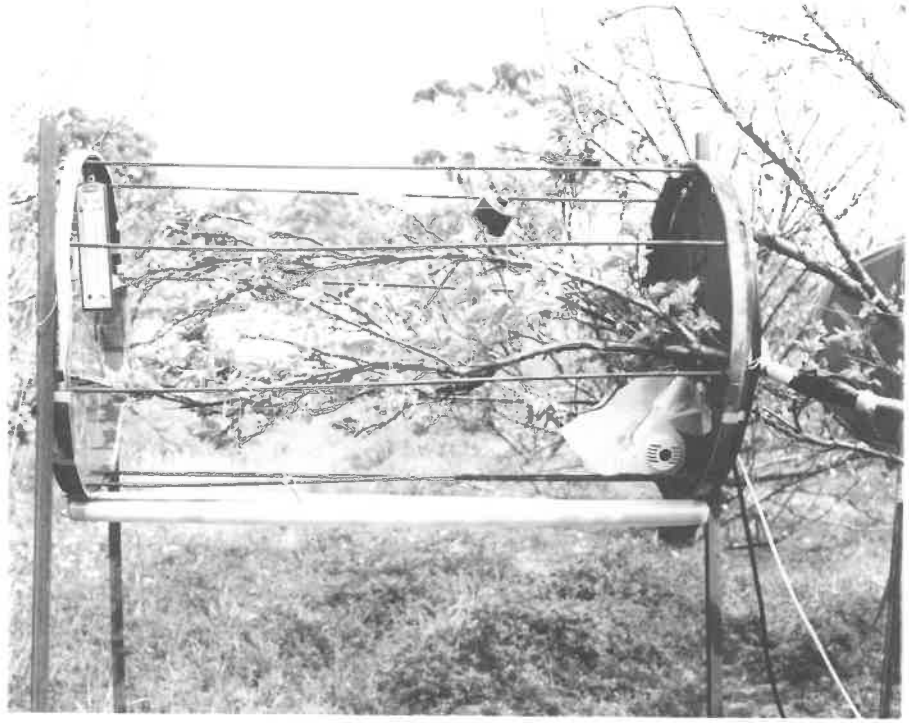


Plate 1.- Branch heating apparatus (Method 1).



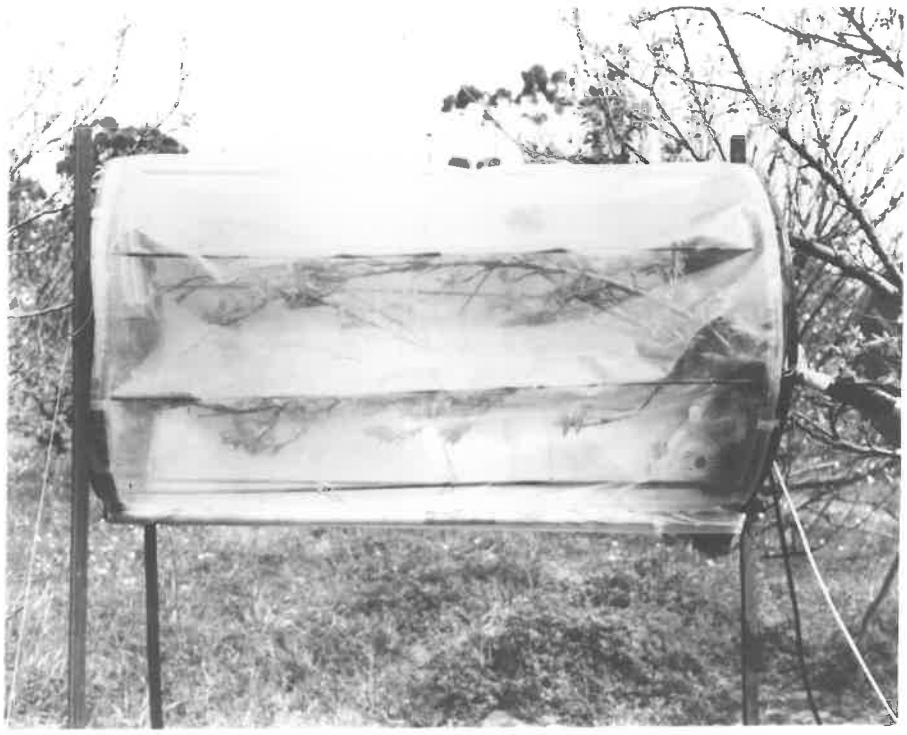


Plate 2.- Branch heating apparatus (Method 2).

fruits have been described in the literature. Smith (1940) estimated the cell numbers in the fruit of several varieties of apple by dividing the tissue volume by the cell volume estimated from cross-sections of cells 0.5 cm below the skin at the equator. The assumption was made that cells approximated to spheres and the cells at this point contributed most to fruit size. Bain and Robertson (1951) used a similar method: size of cells in the mid cortex of the Granny Smith apple were shown to be almost equivalent to the mean for the whole fruit. Form of cells was found to approximate to spheres or oblate spheroids and volume was calculated using the formula $4/3\ \pi\ a^2 b$, where a is half the length of the major axis and b half the length of the minor axis. Cell number was found by dividing fruit volume by cell volume. Bradley and Crane (1955) compared apricot fruit on the basis of cell numbers by counting the cell layers across the mesocarp, presuming that a greater cell number in fruit would be reflected in a greater number of layers across the mesocarp. A further method of estimating cell numbers in a fruit is by removing a representative portion of that fruit, macerating to produce a single-cell suspension and estimating, by various methods, the number of cells in the suspension, thereby finding, by proportion to fruit volume, the number of cells per fruit (eg Bradley 1959).

The examination of sections has the obvious advantage that the position of cells in relation to the whole fruit is not lost. In stone fruits for instance, the endocarp and the mesocarp

can be differentiated microscopically at an earlier developmental stage than the two tissues can be readily dissected. For this reason, a microscopic technique was adopted when cell numbers in the mesocarp of apricots were required before pit-hardening.

1. Microscopic technique for cell counting

(a) Preparation of slides

Sections were cut on a freezing microtome from samples stored in FAA. They were mounted in 50% glycerine and the coverslip sealed with nail varnish. No staining was found necessary. It was possible to cut small fruit to a thickness of 5 to 10 μ, but the larger samples disintegrated after cutting unless a thicker section was cut. In practice a range of thicknesses from 10 to 50 μ was used, depending on the size of the fruit, the larger the fruit the thicker the section.

(b) Estimation of cell numbers and cell size from sections

Sections were cut across the fruit through the point of maximum cheek diameter, and transects were examined along the plane at right-angles to the suture (dimension a, Fig. 1A). The transect itself contained cells of varying dimensions, and whereas, close to full-bloom, cells near the endocarp and exocarp were similar in shape, at maturity, the former had a much greater radial diameter than the latter. It was decided to estimate cell numbers in the mesocarp by determining the number of cells across

the mesocarp at different dates and for different samples, assuming, as did Bradley and Crane (1955), that increase in cell numbers in the fruit would be reflected in the number of cells across this transect.

On the transect across the mesocarp from the endocarp to the epidermis ten cells were selected at equidistant points. Radial diameter was measured for each cell and, to calculate the number of cells in that tenth portion of the mesocarp, the radial cell diameter was divided into one-tenth of the mesocarp diameter. The sum of all ten values obtained was the estimate termed "number of cells across the mesocarp". It should be noted that an estimate could be gained by dividing mesocarp diameter by mean cell diameter; this is accurate where cells towards the endocarp are of similar diameter to cells near the epidermis, but where gradation of cell diameter exists, this estimate is less reliable.

Cell size has been expressed in terms of radial diameter. This, however, is but one of the dimensions contributing to cell volume and fruit volume; suture diameter and fruit length may in fact be dependent on a different pattern of cell growth and division. A detailed study of other dimensions was not made, but a few general observations of mitosis and cell size in those planes indicated that the pattern was similar.

2. The counting of cells in macerates

Cornwall and Davison (1960) describe equipment based on a

photo-electric detection method, for the rapid counting of apple cells in suspension. This, in essence, entails passing a suspension of cells through a constricted glass tube mounted on the stage of a microscope. Light is passed through the constricted area focusing an image on to a photo-transistor mounted, together with a two-stage amplifier, over the eye-piece. The image of cells passing through the constriction produces electrical pulses which are amplified and used to trigger an electronic counter. To cope with the large number of cell determinations envisaged, a machine was constructed on the specifications of Cornwall and Davison. The following descriptions refer to the maceration procedures used to prepare a single-cell suspension, to the standardisation and modification of the equipment, and to its method of use. Counts were checked periodically with a haemocytometer used in the normal way.

(a) Maceration of apricot fruit tissue

Three maceration techniques were tested :-

Method I. Jeffrey's method (Johansen 1940), using a mixture of 10% aqueous nitric acid and 10% aqueous chromic acid, caused rupture of 50% of cells. In spite of good separation its use was therefore abandoned.

Method II. Ethylenediaminetetraacetic acid (EDTA) 0.05% at pH 10.5 (Cotham 1960) produced excellent maceration of apricot tissue. Over 95% of cells were as single intact units; some of the remainder were ruptured and a few were in small clusters, generally of not more than three cells.

Method III. Sodium hexametaphosphate, 20g/L at pH 4.1 (Letham 1962), produced maceration equivalent, but not superior, to Method II; therefore EDTA was used.

Cell counting by this procedure was confined to apricot tissue sampled at pit-hardening and stored for several months in FAA. Circular plugs of tissue, 8 mm in diameter, were removed with a cork-borer from opposite cheeks of the apricot. Tissues immediately adjacent to the endocarp and epidermis were removed one millimetre from each surface with a razor blade. Plugs were quickly dried with tissue paper and weighed to the nearest milligram; weight of the two plugs together was 500 $\pm$ 100 mg. Each plug was then cut into two semicircular sections and the four pieces placed in a 50 ml conical flask containing 10 ml, 0.05 M EDTA, the pH having previously been adjusted to 10.5 with sodium hydroxide. Flasks were shaken with a motion reciprocating 15 cm at 1 r.pm. for 24 hours in a water bath at 45°C. Final maceration was achieved by shaking vigorously for three minutes in a lidded plastic container with ridged sides.

(b) Preparation of sample for counting

The vascular tissue, which resists maceration, was contained in the suspension described above. This tissue, constituting about 4% by weight of the plug, could be readily removed by passing through a 200μ wire sieve, and washing the vascular tissue remaining with distilled water to dislodge cells adhering to it.

The maximum diameter of the largest cells was rarely more than 150μ and it was felt that virtually all cells were recovered. For convenience washing was used to make the volume up to 200 ml; 2 ml of Trypan Blue (0.5% in 45% glacial acetic acid) was included in this volume. After 10 minutes a 10 ml aliquot was diluted to 200 ml to give the solution used for cell counting; one tenth of this volume was used for each determination.

(c) Use of the cell-counter

The mode of operation and the construction of this instrument has been described by Cornwall and Davison (1960). Comments here relate only to modifications required for dealing with a different fruit at a different stage of development.

Diameter of apricot cells examined was 50 - 100μ, considerably smaller than the 70 - 500μ recorded for apple cells by Cornwall and Davison. Because of this a smaller constriction (375μ diameter capillary, rather than 500μ) and a greater magnification (x150 instead of x40) were used. To cope with increased magnification the light source was lowered below the casing and its light focused onto the capillary by a lens placed between the bulb and the microscope stage.

The circuit of the eye-piece unit has been modified by Cornwall and Davison since publication of their work to include a sizing control; the revised circuit which has been incorporated in the construction of the machine used here is shown in Fig. 2.

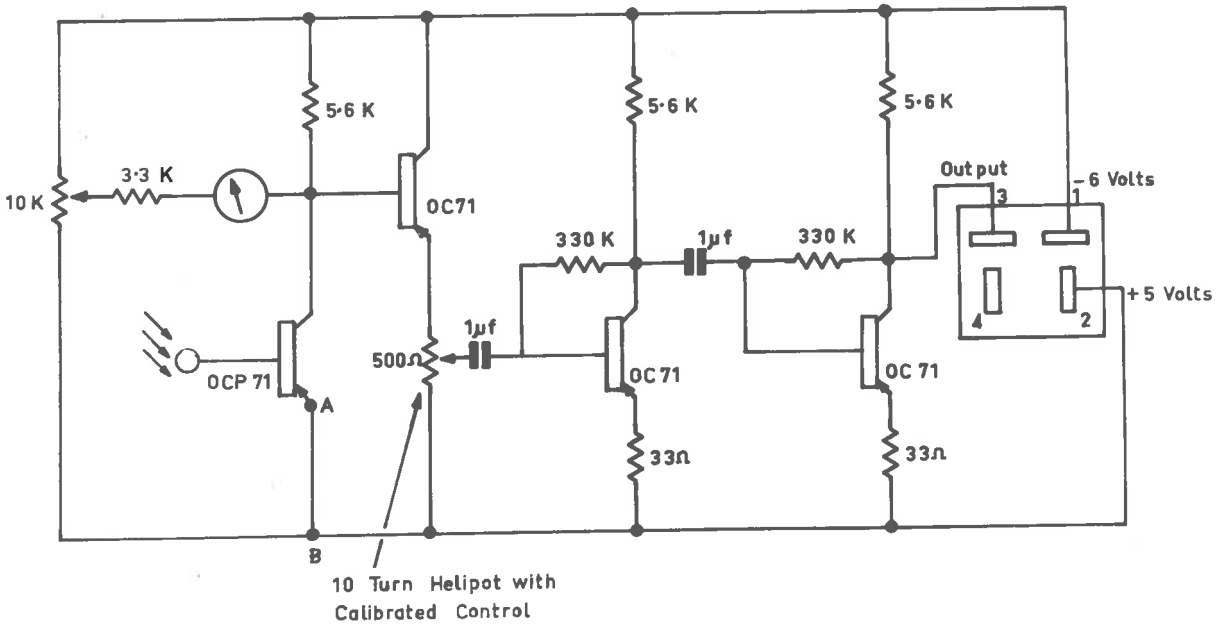


Fig. 2.- Eyepiece unit of cell counter, modified to include a sizing control (Cornwall and Davison, private communication).

The photo-transistor was found to be more heat sensitive than expected. Counts were generally higher on hot than cold days and heat accumulated in the eye-piece unit from the light source gradually increased the total count obtained. A 2.2 K ohm collector bias resistor placed between points A and B overcame this problem.

The cell suspensions contain a proportion of fragmented material which is mostly smaller than the smallest cells, accordingly some method of selection for size is necessary. This can be done by narrowing the sensitivity range of the instrument. Use of the light control or the sizing control incorporated in the revised circuit enables this to be done readily. Pure water run through the instrument will not record any count if the amplifier gain is below 70%; above this value a count is recorded which becomes excessive at 100% gain. Slight vibrations or knocks will cause the instrument to record pulses, but, under normal conditions, these extra counts are negligible when the amplifier gain is below 70%. By stabilising the light to produce a 40μA reading on the ammeter it was possible to obtain satisfactory counting rates below 70% gain. The graph in Fig. 3 shows the count obtained at different amplifier gains, together with an estimation of cell number, in the same sample, using a haemocytometer. Counts obtained at 45% amplifier gain at the light intensity specified above gave in this, and in other samples, closest agreement with haemocytometer counts. Points on this and later graphs are the means of four counts from 20 ml

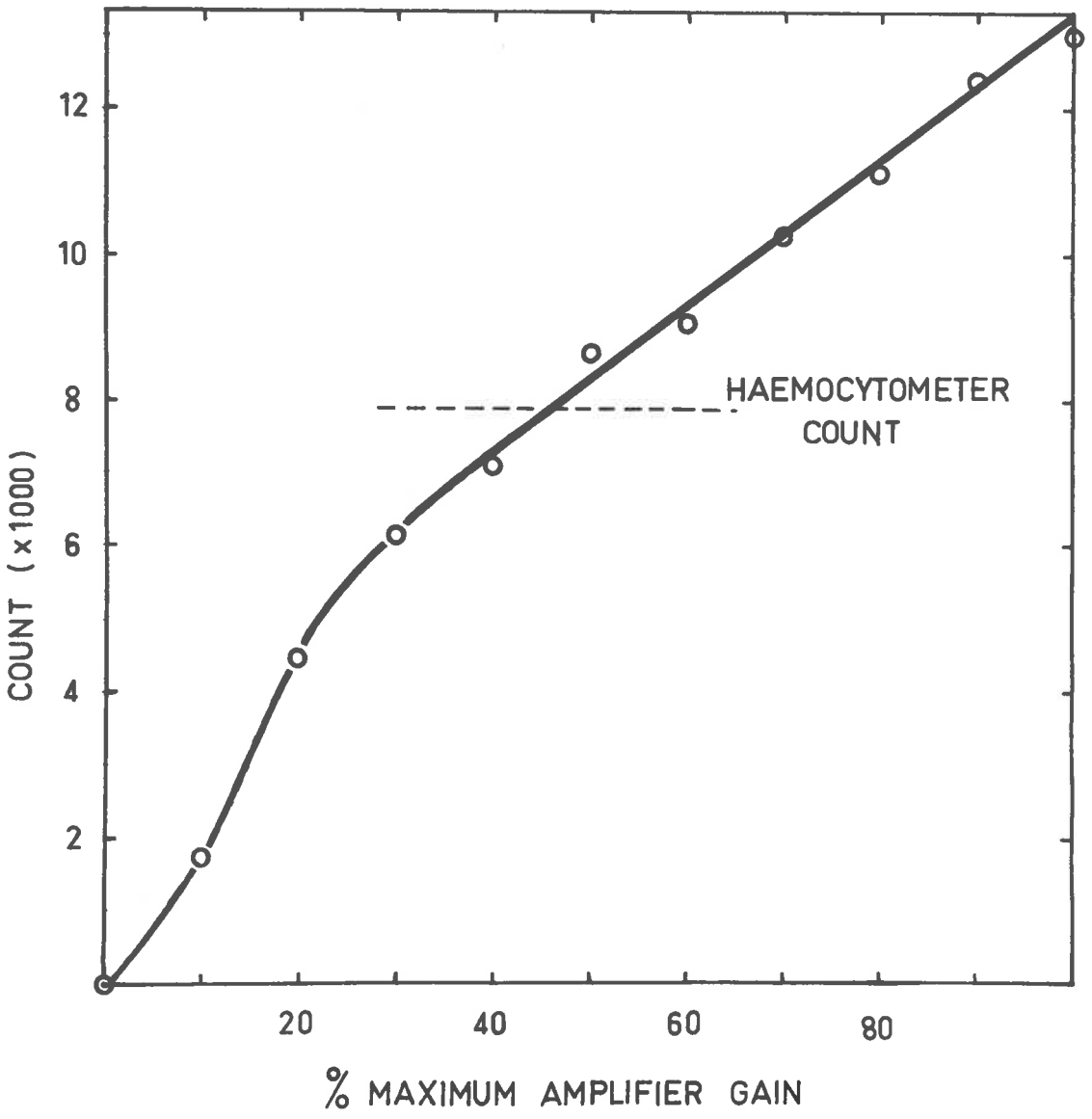


Fig. 3.- Count of cells at different amplifier gain settings of the cell counter.

aliquots of the same sample (standard deviation 3.42%).

Cornwall and Davison (in their Fig. 4) demonstrate loss of counting efficiency at higher cell concentrations; their explanation is that cells in close proximity tend to adhere to one another and record one pulse instead of two or more. An almost identical curve was obtained using various concentrations of apricot cells in 20 ml of liquid (Fig. 4). In practice samples were chosen containing about 5000 cells per 20 ml.

Operation

Liquid containing the cell suspension was contained in a 200 ml measuring cylinder and mixed by blowing air through a 20 ml pipette immediately before taking a 20 ml sample with that pipette. The sample was passed through the counter until the cut-out terminals were exposed and the flow was stopped: 3.8 ml of the suspension was retained beneath the cut-out terminals and 1.0 ml was contained in the tube before the constriction. This amount of liquid was always present before counting took place and each initial count was followed by two washes using 10 ml water each time. It is calculated that, using this procedure, 98.5% of cells were counted from each sample, the remaining 1.5% would be recorded in the next sample. The whole operation took less than five minutes.

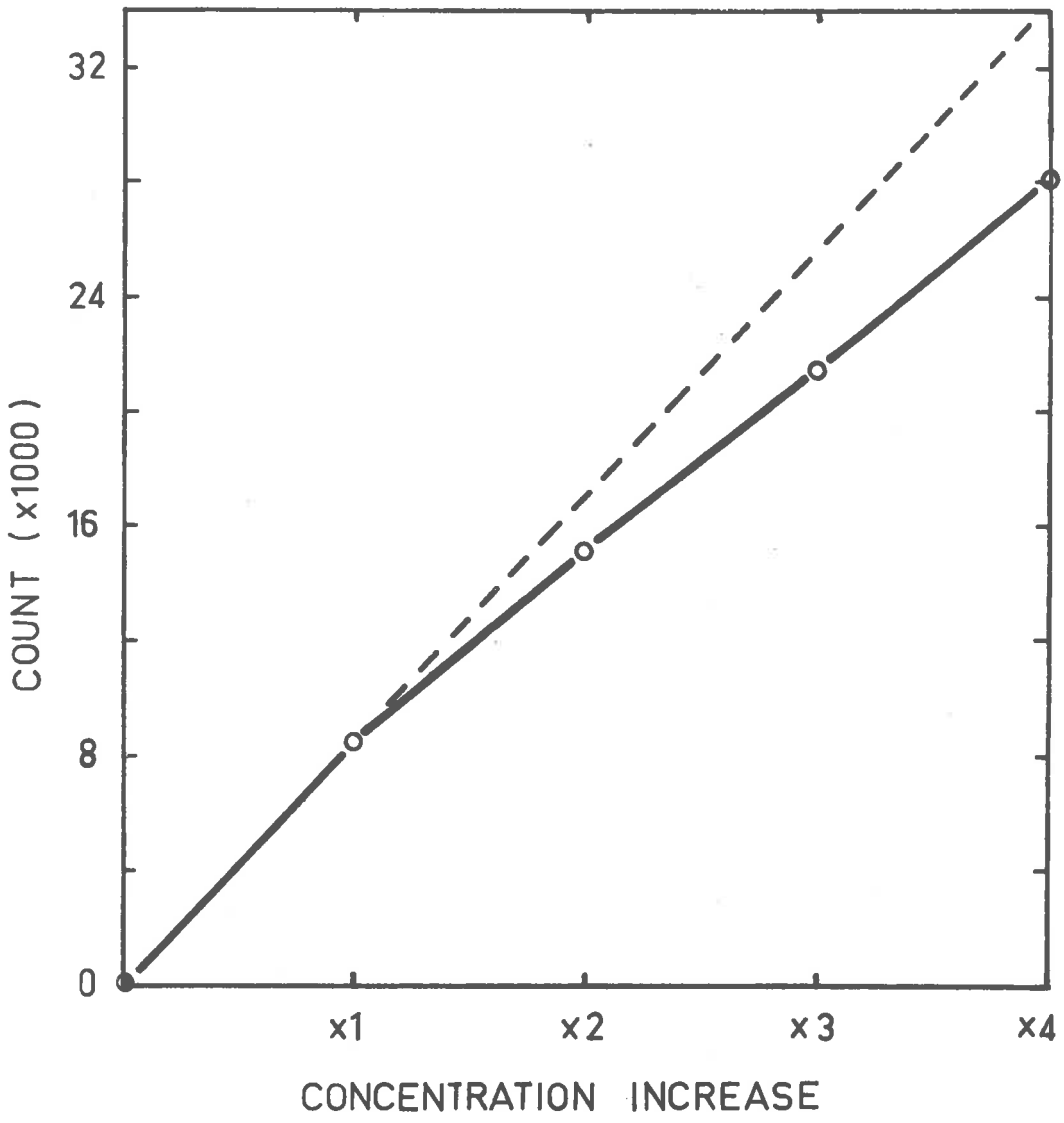


Fig. 4.- Effect of suspension concentration on counting efficiency in the cell counter.

H. Preparation of Tissue Extracts for Gibberellin Bioassay

Extraction and bioassay of plant tissues took place in the winter months following the season in which the tissue was collected and freeze-dried.

The most common extractants used in gibberellin work are ethyl acetate, acetone, methanol, and ethanol. For the present study ethyl acetate was initially used, but it was discovered later that methanol would remove from tissue, already extracted with ethyl acetate, further and often greater gibberellin activity. Preliminary work also showed that the activity from both extracts ran at different Rf's on a chromatogram, and these two peaks were not apparent in a normal methanol extract (see Fig. 14, seed at five days after full-bloom). Consequently most material was extracted first with ethyl acetate and, after drying, the residue was extracted with methanol. The two extracts were separately tested for gibberellin activity.

In all cases described, where ethyl acetate has been used for extraction, it has received no treatment to remove traces of water. Previously it had been the custom in this Department to dry ethyl acetate with excess anhydrous sodium sulphate (B.D.H. Analar), but recently Cohen (private communication) found that this treatment interfered with the barley endosperm test.

1. Extraction

Freeze-dried apricot tissue (usually 10 mg) was ground to a powder with a mortar and pestle, and shaken continuously with

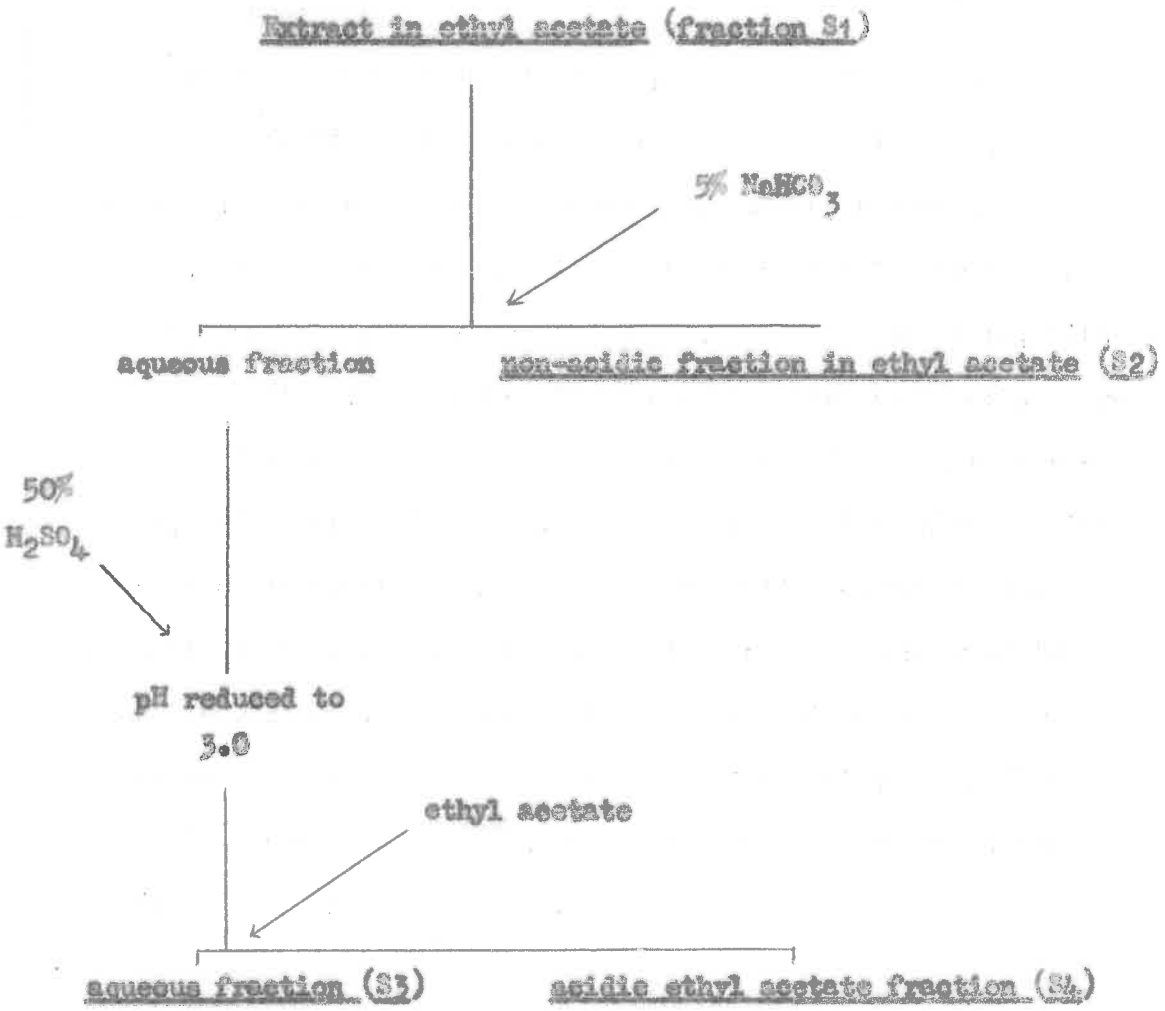
10 ml ethyl acetate in a 75 x 25 mm vial at room temperature for two hours. The mixture was filtered and the residue, with filter paper, re-extracted with another 10 ml for 22 hours at 23°C. This was filtered and the residue washed with a further 10 ml and again filtered. Filtrates were combined and evaporated under reduced pressure at 30°C to dryness. For chromatography the volume was reduced to 3 ml, transferred to 7 x 1 cm test-tubes, and evaporated to dryness. Vials were washed twice with 1 ml ethyl acetate, the washings being transferred and evaporated in the test-tubes.

After drying the residue and filter papers were extracted similarly with absolute methanol

2. Partitioning

A common method of purification (and sometimes separation) used by many authors (Wheeler 1960; Radley 1958, 1959; Frankland and Wareing 1962; West and Phinney 1959; Hayashi and Rappaport 1962) utilises the fact that, at low pH, ethyl acetate will generally remove activity from an aqueous phase. If, however, the water phase is made alkaline, activity will usually return to this phase. Using this information the scheme shown on the following page was devised.

Partitioning Procedure



The extract in 30 ml ethyl acetate was shaken three times with 20 ml sodium bicarbonate to give an aqueous fraction of 60 ml. After acidification this was shaken three times with 40 ml ethyl acetate. An equivalent crude extract (S1) was made and fractions S1, S2, and S4 were evaporated as previously described; the aqueous fraction (S3) was reduced to dryness by

freeze-drying.

3. Chromatography

Ascending paper chromatography, using Whatman's No. 1 paper and isopropanol:ammonia(density 0.88):water (10:1:1), was selected. Extracts were run on single paper chromatogram strips, 2.5 cm wide; they were dissolved in three drops of ethyl acetate or methanol and streaked across the paper, 5 cm from the base, with a fine capillary; a further two drops were used as a wash. Pure GA_3 was dissolved in ethyl acetate evaporated in 7 x 1 cm test-tubes, and treated similarly. Two strips were hung side by side in a 2 l cylinder at 23°C. After equilibration overnight the chromatograms were developed to about 15 cm and then dried in a cold air stream. Each chromatogram was divided into 10 or 15 parts and each section was placed in 1 ml autoclaved distilled water in vials for bioassay; the paper remained in the vials during the bioassay procedure.

I. Gibberellin Bioassay

1. Barley endosperm test

Nicholls and Paleg (1963) describe a bioassay for gibberellin activity based on the release of sugar from barley endosperm. It is capable of detecting amounts equivalent to 10 µµg GA_3 in a ml of solution and contrasts favourably with the dwarf corn and

dwarf pea tests, where a response is rare below 10^{-3} µµg GA_3. The test is specific to gibberellins and does not respond to either kinetin or IAA. The response to various gibberellins has been demonstrated by Paleg et al. (1964): GA_1 and GA_3 initiated release of sugar at the lowest concentrations, GA_4 was only slightly less active, allogibberic acid, GA_5, and GA_6 had lower activity, while GA_8 and GA_9 were considered inactive, or nearly so. The method as described by Nicholls and Paleg (1963) has been subsequently modified by B. G. Coombe and D. Cohen in this Department, rendering it quicker and easier, without loss in sensitivity. This modified method was adopted and, as it is yet unpublished, is described below.

(a) Seed preparation

Barley seed (variety Prior, 1960 harvest) was soaked in 50% H_2SO_4 at 23°C for three hours; this removes the husks and sterilises the seed. Acid was decanted off and the seed shaken vigorously and decanted ten times with distilled water and five times with autoclaved distilled water, to dislodge husks and remove acid. Seed was then soaked for 21 hours in autoclaved distilled water at 2°C. Excess seed, 100%, was used to enable selection of undamaged, even-sized seed; in an average test 600 seeds were soaked in 250 ml 50% acid, followed by 250 ml water.

(b) Seed cutting and incubation

All steps from this time until incubation were performed under as sterile conditions as possible. Glassware, forceps,

blades etc. were dry-heated to 140°C overnight before use, aqueous solutions were made with autoclaved distilled water, and containers were covered whenever possible.

After soaking, water was decanted and seed was cut 4 mm from the distal end (away from the embryo) with a razor blade. To do this a brass block with 20 half-circle grooves across it, each 4.5 mm wide and 1.5 mm apart, was used. Seeds were placed so that the distal end abutted a stopping plate and the blade was then run down a fine groove 4 mm from this plate, seed being held firmly with a brass bar. The endosperm pieces (without embryo) were washed four times with autoclaved distilled water and kept on sterile filter paper in a petri dish until use.

The subsequent operations have been speeded considerably by using automatic syringe pipettes (B - D Cornwall, Continuous Pipetting Outfit), wooden racks holding ten 50 x 25 mm vials for incubation, and brass racks holding twenty 150 x 12 mm test-tubes for immersion in the boiling water bath. The racks have facilitated handling and labelling of vials and tubes.

One ml of water, containing known or unknown amounts of gibberellin, was added to 50 x 25 mm vials, and two endosperm pieces were added to each vial. It was made certain that these pieces were lying horizontally so that cut surfaces were exposed to liquid. Vials were capped, incubated in the dark at 30°C for 24 hours, and then frozen until sugar determination (usually the next day).

(c) Measurement of Sugar

About 1 cc Amberlite IR - 120 (H) ion exchange resin was added to each vial to remove amino acids and other positively-charged entities from solution (these interfere with the Somogyi test). To this was added 9 ml distilled water and the vials were recapped and shaken horizontally, end to end, for five minutes.

A filter cup, formed by folding a 9 cm circle of Whatman's No. 1 filter paper over a 2 cm tube, was slowly lowered into the liquid: 0.5 ml was pipetted from inside the filter cup into a 150 x 12 mm test-tube containing 0.5 ml distilled water and 1 ml Somogyi copper reagent (see Paleg 1959). Tubes were covered with marbles placed in a boiling water bath for 15 minutes, and then cooled in cold running water for five minutes.

Arseno-molybdate reagent (1 ml) (see Paleg 1959) was added to each tube and the contents shaken vigorously until colour development was complete. Volume was made up to 10 ml and, after mixing, absorption was read in a Unicam SP 1400 prism absorptionometer set at 560 mμ and adjusted to zero on a reagent blank.

Estimation of sugar equivalents of known and unknown gibberellin solutions was made by comparing their absorbency with two tubes containing 40 μg glucose and another two containing 80 μg. Four such tubes were included every time a batch of tubes was boiled, since significant differences in absorption values do occur between batches. For this reason also, when more

than one batch had to be boiled, each extract or GA_3 standard was, whenever possible, replicated equally into all batches.

In all cases described, standard solutions were in triplicates containing 0, 10, 10^2, 10^3, 10^4, 10^5 µµg GA_3 per vial. Coombe (private communication) could detect no difference in response from standard solutions with or without chromatogram paper equivalent in size to those used for elution from chromatography. When chromatography was used, equivalent pieces were not included in vials containing standard gibberellic acid solutions. For each test a standard curve, log sugar concentration against GA_3 standards, was constructed. One such curve is shown in Fig. 16. The test was rarely capable of detecting amounts as low as 10 µµg, reported by Nicholls and Paleg (1963). Although the curve is often linear between the points 10^2 and 10^4 µµg GA_3, the overall shape is usually sigmoid. To obtain, statistically, the line of best fit for such data is difficult, hence, a line has been drawn by eye and GA_3 equivalents of unknown solutions have been estimated from this. It is obvious from the general appearance of the curve that interpolations of responses between the levels 10^2 and 10^4 µµg GA_3 will be more accurate than responses above or below. Dilution curves have been constructed from data obtained when representative extracts of apricot tissue have been diluted by $^1/_{10}$ and $^1/_{100}$. Parallelism has been shown in all cases between these curves and the standard curves. Concentrations of unknown solutions obtained by interpolation,

are therefore likely, within normal experimental error, to be accurate in terms of GA_3 equivalents.

2. Dwarf corn test

The following procedure was used in the dwarf corn gibberellin bioassay.

d - 5 dwarf corn seed (supplied by Dr. B. O. Phinney, University of California, Los Angeles) was soaked for one day in running tap water. Seed flats were filled to 4 cm from the top with John Innes No. 1 Compost. They were firmed and seed was sown closely in drills, 4 cm apart, and covered with 2 cm of river sand. Plants were grown under 12 hour days at 29°C in a growth cabinet giving light of 1750$\pm$ 200 foot-candles. Plants segregated 'tall' : 'dwarf' in the ratio 4 : 1. Seven days after planting 'talls' were cut off at soil level and 'dwarfs' treated. An aqueous solution (0.1 ml) containing the extract or standard to be tested, was applied by automatic syringe pipette to the base of the first leaf blade. If this would not hold the required amount of liquid the remainder was applied to the base of the second leaf blade. Where leaves had not expanded the liquid was introduced down the centre of the unexpanded leaves. In all cases the cuticle was slightly scratched to facilitate penetration into the plant. Watering was necessary daily; on the day of treatment it was so arranged that treatment should lie midway between two water applications. After a further seven days the lengths of first and second leaf sheaths were measured, that is,

the length of sheath between the prop-roots and the base of first or second leaf blade. That of the second leaf sheath showed the most consistent response to gibberellic acid standard solutions, and hence the figures presented refer to this parameter.

VII. EXPERIMENTAL

A. Gross Morphological Changes during the Development of the Apricot Fruit

This description of Moorpark apricot growth in Adelaide is from data collected in 1963. Examination of fruit in 1961 and 1962 showed a similar developmental sequence, although there was, between seasons, slight variation in the duration of each stage.

Anthesis, the maturation of the sexual parts of the flower, occurred at the time when the flower was fully open, ie. full-bloom. The terms anthesis and full-bloom are used in the text to designate a stage in ovary development; in this context therefore they may be regarded as synonymous.

Stone fruit growth is generally described as taking place in three stages, Stages I, II, and III (Conners, 1920). Following full-bloom, size of apricot fruit increases rapidly for a period of about 30 days: this is termed Stage I. There follows a period of approximately 40 days when overall growth of fruit is slow, Stage II. During this period the endocarp (the pit, or the stone) hardens; thus its alternative name - pit-hardening. Growth of the fruit is completed in Stage III when size again increases rapidly for a further 20 days, and the fruit matures. This double-sigmoid growth curve has already been described in

the Review of Literature; several figures show it clearly, e.g. Figs. 7 and 9. Ripeness, involving softening of the flesh and a change in skin colour from green to yellow-ochre, occurred at about 90 days after anthesis.

Major changes within the seed of apricots occur during Stages I and II; these are illustrated diagrammatically in Fig. 5. By 30 days the seed has reached its full size and the integuments have finished growing. The endosperm remains in the free-nuclear condition until 20-25 days after anthesis, at which stage cell walls form and the tissue begins to expand rapidly. The embryo begins its period of rapid growth at 30 days, the stage when the seed has reached its full volume. Between 35 and 45 days the space occupied by the endosperm diminishes and it is replaced progressively by embryo. The endosperm never occupies the same volume as the nucellus, and, in fact, disappears from the seed before the nucellus. Embryo growth is completed by 60 days, i.e. towards the end of Stage II, when it completely fills the space within the integuments.

B. Factors affecting the Growth of Apricot and Peach Fruit

1. Tree factors

To correlate certain tree factors with the growth of apricot fruit, 15 branches, approximately 30 cm long, were selected on each of four trees after anthesis in 1961. Later branches on which fruit drop had been excessive, were excluded

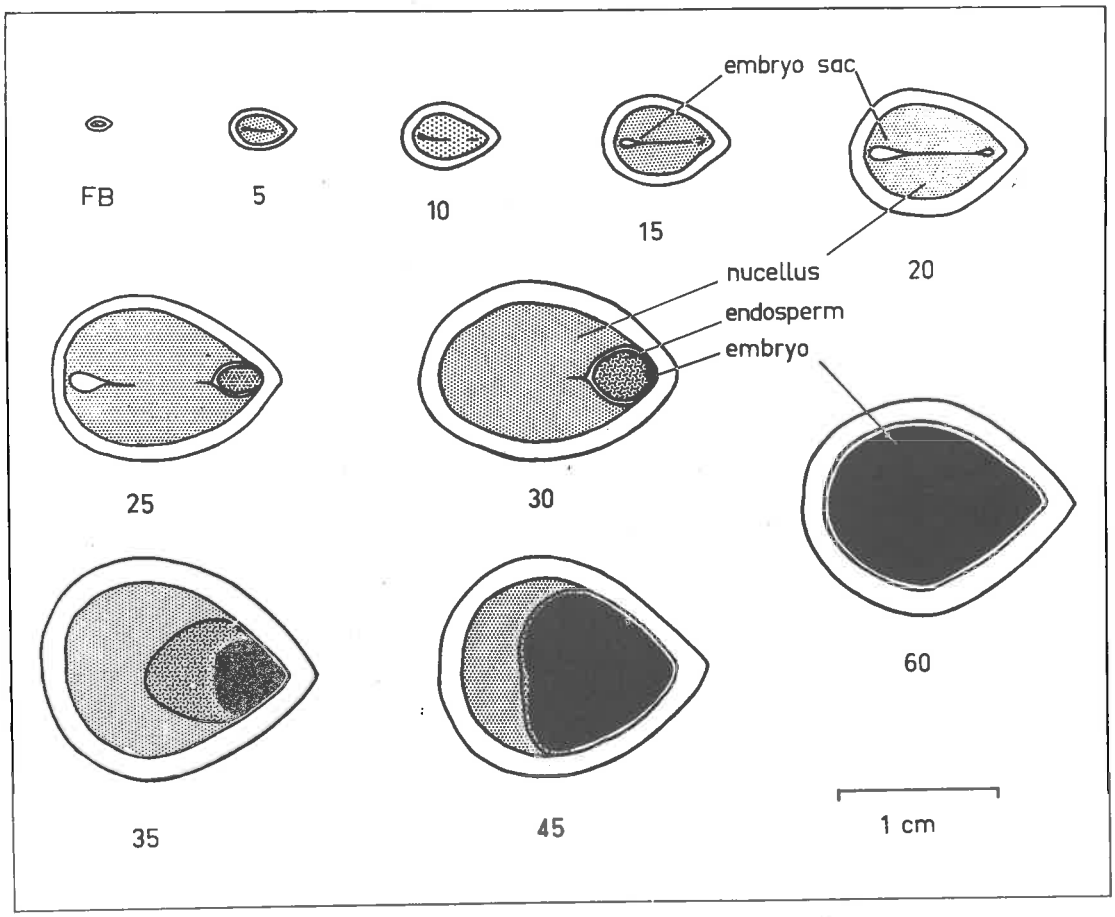


Fig. 5.- Seed development in apricots; diagrams prepared from longitudinal sections in 1963.
(Nunbers refer to days after full-bloom, FB.)

and on 13 branches in tree I, 12 each in trees II and III, and 9 in tree IV the following data was recorded.

1. Branch diameter at base.
2. Leaf number at harvest.
3. Fruit number at harvest.
4. Ratio $\frac{\text{leaf number}}{\text{fruit number}}$.
5. Ratio $\frac{\text{leaf number}}{\text{fruit number x branch diameter}}$.
6. Cumulative light intensity at each branch.
7. Diameter of fruit at the beginning of pit-hardening (Oct. 30).
8. Diameter of fruit at the end of pit-hardening (Nov. 28).
9. Diameter increase of fruit over the period of pit-hardening.
10. Diameter of fruit at maturity (Dec. 19).
11. Diameter increase from Oct. 30 to Dec. 19.
12. Diameter increase from Oct. 30 to Dec. 19 expressed as a percentage of the first diameter.
13. Classification of fruit ripeness at Dec. 19.

The mean value of each factor was calculated for all branches, and, with these as replicates and trees as variables, an analysis of variance was done for each factor. Trees varied significantly in the following :-

P below 0.001

9. Diameter increase over period of pit-hardening.
11. Diameter increase from Oct. 30 to Dec. 19.

12. Diameter increase from Oct. 30 to Dec. 19 expressed as a percentage.

P below 0.01

7. Diameter of fruit at Oct. 30.

10. Diameter of fruit at Dec. 19.

13. Ripeness at Dec. 19.

P below 0.05

6. Light quantity.

Simple regressions were calculated (by computer) for the following factors, both in individual trees and over all trees:-

Factor 1 was correlated with factors 2, 3, 4, 7, 8, 10, 11, and 12.
2 " " " " 3, 7, 8, 10, 11, 12, and 13.
3 " " " " 7, 8, 10, 11, 12, and 13.
4 " " " " 7, 8, 10, 11, 12, and 13.
5 " " " " 7, 8, 10, 11, 12, and 13.
6 " " " " 7, 8, 10, 11, 12, and 13.
7 " " " " 8, 9, 10, 11, 12, and 13.
8 " " " " 10, 11, 12, and 13.
9 " " " " 10, 12, and 13.

Correlations and their significance are shown in Table 1.

It might be noted here, that when such a high number of correlations are made, 2 - 3 pairs of variables would, by

TABLE 1

TREE FACTORS AND FRUIT GROWTH

Growth correlations between thirteen variables on four apricot trees in 1961

Variable 1	Variable 2	Tree Number I		II		III		IV		All trees	
		P	Sign	P	Sign	P	Sign	P	Sign	P	Sign
Branch diameter	Leaf/fruit ratio	x*	+	xx	+	x	+	x	+	xxx	+
" "	Leaf number	xxx	+	xxx	+	xx	+	xx	+	xxx	+
" "	Fruit number	NS	+	NS	+	NS	-	x	+	xx	+
Leaf number	Fruit number	NS	+	NS	+	NS	-	xx	+	xx	+
Fruit number	Diameter at Nov. 28	NS	-	NS	-	NS	-	NS	-	x	-
" "	Diameter at Dec. 19	NS	-	NS	-	NS	-	NS	-	x	-
" "	Increase Oct 30-Dec 19	NS	-	NS	+	NS	-	NS	-	x	-
Leaf number / Fruit number x branch diameter	Diameter at Dec. 19	NS	+	NS	+	NS	-	x	+	x	+
" " " "	Increase Oct 30-Dec 19	NS	+	NS	+	NS	-	xx	+	x	+
Light received	Increase Oct 30-Dec 19	NS	-	NS	-	NS	-	NS	-	xx	-
" "	% " " " " "	NS	-	NS	-	NS	-	NS	-	xx	-
Diameter at Oct 30	Diameter at Nov. 28	xxx	+	xxx	+	xxx	+	xxx	+	xxx	+
" "	Diameter at Dec. 19	xxx	+	xxx	+	xxx	+	xxx	+	xxx	+
" "	Increase Oct 30-Dec 19	xx	+	NS	+	NS	+	NS	+	x	+
" "	Ripeness at Dec. 19	x	+	x	+	NS	+	x	+	xxx	+
Diameter at Nov. 28	Diameter at Dec. 19	xxx	+	xxx	+	xxx	+	xxx	+	xxx	+
" "	Increase Oct 30-Dec 19	xxx	+	NS	+	x	+	NS	+	xxx	+
" "	Ripeness at Dec. 19	x	+	x	+	NS	+	x	+	xxx	+
Increase Oct 30-Nov 28	Diameter at Dec. 19	xx	+	x	+	NS	+	NS	+	x	+
" " " " "	% Incr. Oct 30-Dec 19	xx	+	x	+	NS	+	NS	+	xxx	+

*Probability levels:- x = P below 0.05; xx = P below 0.01; xxx = P below 0.001 .

chance, be expected to show either significance at the 5% level, or have their true significance reduced or obliterated. For this reason only overall correlations which are highly significant and show significance in all or most trees will be considered further.

A definite difference was shown between trees, in the rate of fruit growth from the beginning of pit-hardening to maturity, in the size of fruit at the beginning of pit-hardening and at maturity, and in speed of ripening. From factor correlations it was shown that fruit, within trees, which are large at early pit-hardening, tend also to be large at the end of pit-hardening and at maturity; they also ripen earlier. Large fruit at early pit-hardening probably add more to their size than smaller fruit; large fruit at the end of pit-hardening almost certainly do. As expected, number of leaves, number of fruit, and leaf / fruit ratio are positively related to branch size.

2. Effect of thinning and date of flowering on fruit diameter, cell number, cell size, and maturity of apricots during 1961.

(a) Experimental design

Ninety-five percent of the flower buds on apricot trees in 1961 opened over a two week period. Maximum blossom was noted on Sept. 17, which was the middle of the flowering period, and flowers opening on Sept. 12, 13, and 14 were, for this experiment, termed early, those opening on Sept. 20, 21,

and 22, late. The following treatments were adopted :-

Treatment 1. Early and late blossoms were labelled with small 'tie-on' cardboard tags on four trees.

Treatment 2. Four trees were thinned heavily over the blossoming period to about one fifth of the total number of blossoms; early and late flowers were then tagged.

Treatment 3. Three trees were thinned to leave only blossoms opening over the period designated as early.

Treatment 4. Three trees were likewise thinned to leave only late blossoms.

It was estimated that about four-fifths of blossoms were removed in treatments 3 and 4, i.e. treatments 2, 3, and 4 had an equivalent number of flowers per tree.

(b) Results

(i) Fruit diameter and maturity

For each treatment the diameters of forty randomly selected fruit per tree were measured at full-bloom, full-bloom plus four days, and thence at seven day intervals until pit-hardening. Different growth patterns of "early" and "late" fruit were shown in treatments 1 and 2, and between "early" fruit of treatment 3 and"late" fruit of treatment 4. Up to the stage of pit-hardening no significant differences between the various thinning treatments were found. Diameters of early and late

flowering fruit on unthinned trees during Stage 1 are shown in Fig. 6 (together with results from a similar comparison in 1962). "Late" fruit grew at a more rapid rate in 1961 and reached pit-hardening in a shorter number of days than "early" fruit.

In November a severe storm caused great loss of fruits from trees on the windward side of the orchard; two trees from treatment 2 and one from treatment 1 were badly affected, and it was considered that further records from these trees might be biased by the severe thinning induced. In Table 2 treatment 2 has been omitted and comparisons between 1, 3, and 4 are shown on the basis of three tree replications. The table shows only diameter measurements at pit-hardening and maturity. A comparison of fruit ripeness at a date approximately five days before general maturity is also shown.

Analysis of this data was complicated by the fact that unthinned "early" and "late" fruit were on the same trees, whereas thinned "early" and "late" fruits were on different trees. For the purpose of analysis it was assumed that each treatment was applied to different trees. Results of analysis of variance for this data showed the only significant difference to be the greater diameter of thinned, compared with unthinned fruits at maturity (P below 0.05). There was no significant difference between fruit from early and late flowers in diameter or in ripeness classification.

TABLE 2

EFFECT OF THINNING ON FRUIT FROM EARLY AND LATE APRICOT FLOWERS

Diameter at pit-hardening and maturity, and ripeness at Dec. 14, 1961.

Treatment	Diameter at beginning of pit-hardening (mm)				Diameter at maturity (mm)				Ripeness Classification (0-5)			
	Tree			Mean	Tree			Mean	Tree			Mean
	1	2	3		1	2	3		1	2	3	
Early flowers	30.0	28.9	29.3	29.4	40.9	39.2	42.1	40.7	2.05	2.85	3.05	2.65
Late flowers	28.5	29.4	28.1	28.7	39.9	38.7	41.2	39.9	1.90	2.35	2.35	2.20
Thinned to Early flowers	28.4	30.1	30.7	29.7	41.0	45.0	45.6	43.9	4.55	2.62	3.32	3.50
Thinned to Late flowers	29.9	28.7	30.6	29.7	42.6	43.4	48.7	44.9	3.50	3.55	2.10	3.05
L.S.D., P below 0.05 =				NS				4.3				NS

(ii) Cell number and cell volume

Samples collected at pit-hardening, prior to the storm, enabled a comparison to be made between date of flowering and effect of thinning in treatments 1 and 2.

There was found to be a significant correlation (P below 0.001) between mesocarp volume and the total volume of fruit measured by water displacement. The line of linear regression was:-

$Y = 0.878X - 0.984$, where Y = mesocarp volume and X = fruit volume in ml. In all cases mesocarp volume was computed from the more easily measured parameter, fruit volume. Cell numbers were obtained using the cell counter, and cell volume was calculated as mesocarp volume/ cell number. Results in terms of mesocarp volume, cell volume, and cell number are shown in Table 3: each figure is the mean calculated from four fruits. Analysis of this data showed the following:-

1. There was no significant effect of thinning upon any variable at pit-hardening. The variance ratio for the effect of thinning on mesocarp volume, cell volume, and cell number was 4.26, 3.02, and 1.86 respectively, the significance level, where P = 0.05, being 5.99 in each case. The effect of thinning may have affected mesocarp volume after pit-hardening, since at maturity thinning significantly increased fruit diameter (Table 2). This would be through its effect on cell size, for cell division in the mesocarp stops before pit-hardening (see Fig. 10).
2. There was a significant (P below 0.05) difference in mesocarp volume and a highly significant (P below 0.01) difference in cell number attributable to date of flowering. Early flowers

TABLE 3

EFFECT OF BLOSSOM THINNING* AND DATE OF FLOWERING ON MESOCARP VOLUME, CELL NUMBER, AND CELL VOLUME OF APRICOTS AT PIT-HARDENING, 1961

Parameter	Stage of Flowering	Unthinned					Thinned					F test Early v late
	Tree No.-	1	2	3	4	Mean	5	6	7	8	Mean	
Mesocarp Volume (ml)	Early	10.35	10.28	9.95	11.15	10.43	10.91	10.76	13.65	11.16	11.62	P below 0.05
	Late	10.55	9.90	8.56	9.25	9.57	10.66	9.90	12.60	11.65	11.20	
	F test, thinning effect - NS .											
Cell Volume (ml x 10^{-8})	Early	43.56	41.12	42.66	45.75	43.27	46.00	43.54	58.81	44.60	48.24	NS
	Late	51.75	47.02	42.89	45.95	46.90	48.68	51.13	51.28	44.73	48.96	
	F test, thinning effect - NS .											
Cell Number (millions)	Early	24.25	25.26	23.83	25.44	24.69	24.55	24.76	23.70	24.98	24.50	P below 0.01
	Late	20.00	21.35	19.92	21.05	20.58	22.20	19.91	24.56	26.21	23.12	
	F test, thinning effect - NS .											

* Trees thinned during blossoming to 20% of flowers.

produced fruit with larger mesocarp volume and more cells at pit-hardening, despite the previous indication that fruit diameter at pit-hardening and maturity were similar in "early" and "late" fruit (Table 2). Without further evidence it is impossible to know whether this is due to compensatory growth of "late" fruit after pit-hardening, or to the failure of the data in Table 2 to reveal these differences.

3. A highly significant (P below 0.001) effect of trees, within thinning treatments, on mesocarp volume was found; there was, however, no effect upon cell number or cell size.

In an attempt to discover which factor, cell number or cell volume, contributes more to differences in mesocarp volume, certain regression analyses were performed between cell number or size and mesocarp volume. Since results shown in paragraph 1 above indicated no significant effect of thinning, the thinning treatments were not separated; results in paragraph 2, however, demonstrated that fruit from early and late flowers had significant differences in mesocarp volume and cell number, and, for this reason, "early" and "late" were considered either together, as one population, or as separate populations. Coefficients of correlation are presented below for between-tree differences and for within-tree differences; the latter being the coefficients for individual fruit after the effect of trees had been removed.

Correlation coefficients, cell number and cell volume on mesocarp volume for early- and late-flowering fruit, within and between trees.

		r_n *	r_v **	Significance
Between Trees	Early	-0.327		N.S.
			0.972	P below 0.001
	Late	0.791		P below 0.05
			0.475	N.S.
	Early and late combined	0.554		P below 0.05
			0.622	P below 0.01
Within Trees	Early	0.495		P below 0.01
			0.167	N.S.
	Late	0.775		P below 0.001
			0.384	P below 0.05
	Early and late combined	0.612		P below 0.001
			0.248	N.S.

* r_n - Correlation coefficient, cell number on mesocarp volume.

** r_v - Correlation coefficient, cell volume on mesocarp volume.

It seems that, between trees, early-flowering fruit vary in size mainly because of differences in cell volume, yet in late-flowering fruit the situation is reversed and cell number is a more important factor than cell size. The results, within trees, suggest that cell number contributes more to differences in mesocarp volume for both early- and late-flowering fruit. When, in the analysis, the two types of fruit were not separated results suggest that cell size and cell number influence between-tree differences, but that

the latter has more effect in determining within-tree differences.

3. Date of flowering and its effect upon fruit growth in 1962.

In 1961 fruit from early and late apricot flowers grew at different rates over the first growth period. To investigate this effect in a different season, 1962, five trees were selected and flowers were tagged as early or late. Early flowers opened on Sept. 15, 16, or 17, while late ones opened Sept. 22, 23, or 24. Measurements were made on five fruits of each type, removed from the tree at ten day intervals beginning at full-bloom.

Unlike the previous season, the pattern of fruit growth from early and late flowers in 1962 was almost identical when expressed on the basis of number of days from full-bloom. This is shown in Fig. 6 and compared with results from 1961 (unthinned fruit). Below, the number of days each class of fruit took to reach maturity are tabulated.

Year	Time of flowering	Days to maturity
1961	Early	93
	Late	84
1962	Early	98
	Late	97

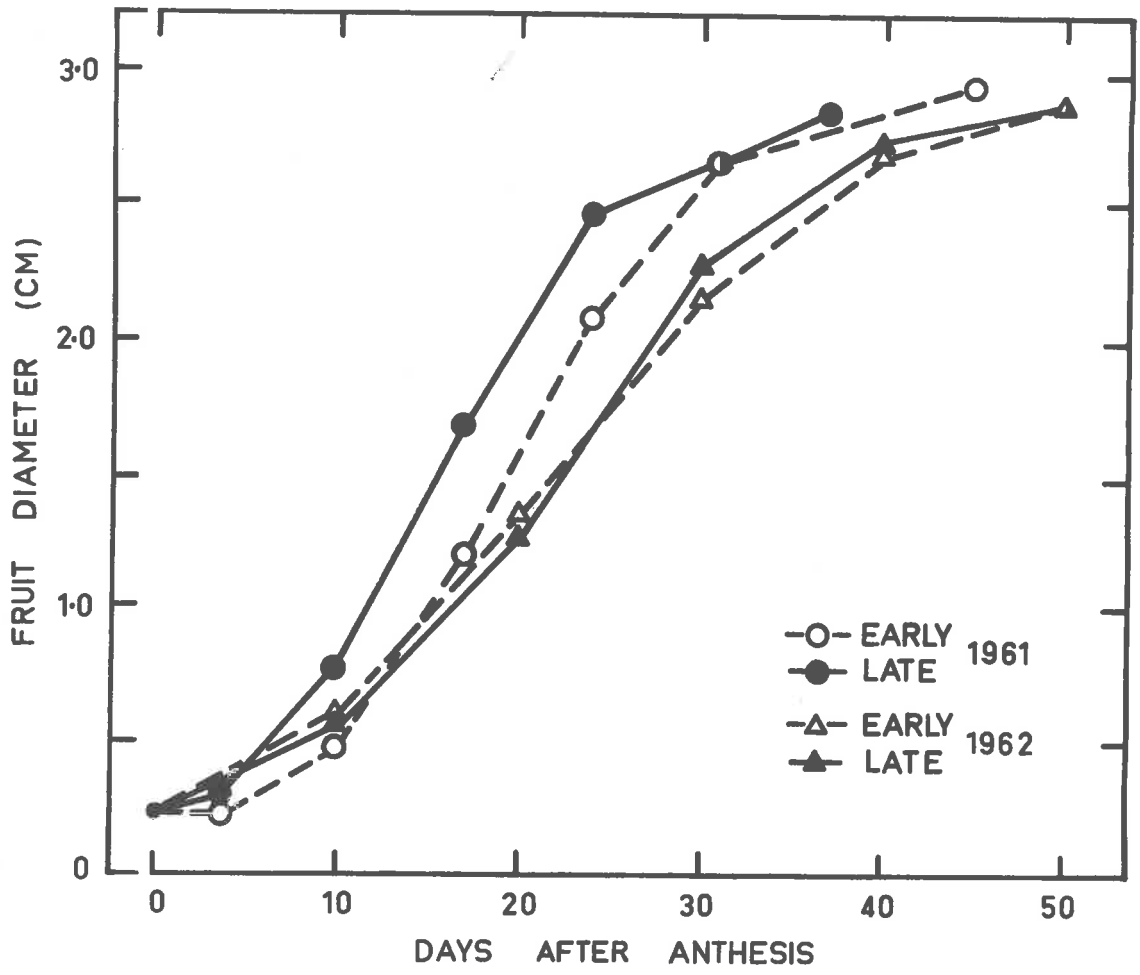


Fig. 6.- Growth of early- and late-flowering apricots during Stage I in 1961 and 1962.

Fruits grew more rapidly and took less time to reach maturity in 1961 than 1962. Effect of time of flowering in 1961 was manifest both in rate of growth in stage I and in time taken to reach maturity. No effect of flowering date was noted in 1962.

Cell size and cell number were determined for "early" and "late" fruit at pit-hardening in 1962. No significant difference was found and the data is not presented.

The cause of differences in growth rate for the two types of fruit in 1961, and the general differences in growth between 1961 and 1962, may have been due to temperature in which the various fruit grew. Up to a diameter of 2.3 cm, fruits, though still rapidly expanding, are approaching the period of pit-hardening. For each curve (Fig. 6) mean daily temperature (taken as the mean of daily maximum and daily minimum) was calculated over the number of days taken to reach this size. It was discovered that there was a negative correlation (significant at the 5% level) between mean temperature and number of days taken. This data will be presented later in conjunction with results from temperature experiments.

4. The effect of girdling on size and maturity in peaches

Eight branches, about one metre long and distributed over five trees, were girdled nine days after full-bloom. Eight similar branches on the same trees were ungirdled and served as control. At maturity ten fruit per limb were sampled for

diameter and ripeness. No variation between mean fruit size or ripeness could be attributed to treatment.

5. Bud development and the early stages of fruit growth in apricot

On June 1 and Aug. 23, 1962 it was shown that diameter of apricot flower buds was positively correlated with diameter of the ovary they contained. In both instances the correlation was highly significant (P below 0.001). Where X = bud diameter and Y = ovary diameter (mm), the linear regression equations were $Y = 0.018X + 0.037$ and $Y = 0.175X - 0.104$ respectively. It was decided to follow the growth of ovaries from two types of buds, labelled as large or small, on girdled branches

Eight branches on five trees were girdled, 30 days prior to full-bloom, at a position where the volume of the branch, distal to the girdle, was 37.5 ml, as determined by water displacement. (Girdling severs the phloem connections, and is generally thought to restrict movement, across the girdle, of materials normally translocated in the phloem.) Each branch was differentially thinned so that half had 32 and the remainder 16 flower buds. On each branch half the buds were labelled as large (diameter 2.7 - 2.9 mm) and the remainder as small (2.4 - 2.6 mm). Four chemical treatments were applied to the heavily thinned, and the same four to the lightly thinned branches on each tree.

It was planned to follow growth of fruits developing

from these buds. Unfortunately growth was severely inhibited by girdling, and most buds dropped at, or near, flowering. Full realisation of this experiment was therefore impossible. Details of the effect of chemicals on growth below the girdle will be considered later; apart from this, only the following information was gained :-

(a) Large buds flowered 2-5 days before small buds.

(b) More small buds dropped before flowering than large buds.

(c) Eleven large buds, but only one small bud, produced mature fruit.

The examination of buds and flowers at different dates before and after the general date of full-bloom (Sept. 16, 1962) provided an opportunity to measure the percentage of flowers with undeveloped ovaries and the mean fresh weight of developed ovaries at three flowering stages, pink-bud, full-bloom, and petal-fall (Tables 4A and 4B). The same five trees were used, but flowers were selected from ungirdled branches. The figures shown in the two tables are means of 100 - 150 randomly selected flowers. It was noted that, whereas developed ovaries had a mean diameter of 2 - 3 mm, undeveloped ovaries were normally below 1 mm in diameter and often showed signs of blackening and death; they were therefore readily distinguished.

Analysis of variance for Table 4A shows significant differences between number of flowers with undeveloped ovaries at pink, full bloom, and petal-fall. It seems likely that the

TABLE 4A

PERCENTAGE OF FLOWERS WITH UNDEVELOPED OVARIES AT DIFFERENT DATES AND IN DIFFERENT STAGES OF FLOWER DEVELOPMENT, 1962

Stage of Flower Formation	Date of Sample					Means	L.S.D. (Stage of Flower Formation)
	7/9/62	11/9/62	15/9/62	19/9/62	24/9/62		
Pink-bud	43	49	53	18	74	47.4	P below 0.05 16.8
Full-bloom	28	53	50	21	36	37.6	
Petal-Fall	13	14	6	5	15	10.6	P below 0.01 24.4
Mean	28.0	38.7	36.3	14.7	41.7		
Differences due to Date of Sampling are not significant							

TABLE 4B

MEAN FRESH WEIGHT OF OVARIES(mg) AT DIFFERENT DATES AND IN DIFFERENT STAGES OF FLOWER DEVELOPMENT, 1962

Stage of Flower Formation	Date of Sample					Means	L.S.D. (Stage of Flower Formation)
	7/9/62	11/9/62	15/9/62	19/9/62	24/9/62		
Pink-bud	9.23	6.74	9.54	8.22	11.90	9.13	F below 0.01 4.66
Full-bloom	17.46	17.89	12.94	17.11	18.21	16.72	
Petal-Fall	19.04	21.25	22.06	25.53	27.01	22.98	
Means	15.24	15.29	14.85	16.95	19.04		
Differences due to Date of Sampling are not significant							

low number at the later stage of development is due to abscission of a number of flowers or flower buds with undeveloped ovaries. As expected there is a highly significant difference between ovary weight at the different stages of development. Between dates of sampling there is no significant difference between any variable.

6. The effect of chemicals on the growth of apricots and peaches

(a) Chemicals sprayed on apricots and peaches

Eight days after full-bloom of apricot and 12 days after full-bloom of peaches, an array of chemicals, which it was suspected might influence fruit growth, were sprayed on small branch units in four trees. Branches were approximately 26 cm long and each was sprayed to "run - off" with a small hand atomiser. Each of the four trees had one branch sprayed with one of the following chemicals or mixtures of chemicals.

Material	Concentration (in g / l, unless otherwise stated)
Water	-
Adenine sulphate	0.05
Asparagine	2.0
Ascorbic acid	0.05
Uracil	0.10
5-fluorouracil	0.10
Sucrose	2000
Urea	500
Coconut milk	33%
6-furfuryl amino-purine (kinetin)	0.02
Maleic hydrazide	1.00
Gibberellic acid (GA_3)	0.10
2, 4, 5-trichloroacetic acid	0.05
2, 3, 5-tri-iodobenzoic acid	0.20
p-chlorophenoxyacetic acid	0.05
Beta-naphthoxyacetic acid	0.05
α-naphthaleneacetic acid	0.05
3-indolyl-acetic acid (IAA)	0.05
GA_3 and IAA	at above concentrations
Kinetin and IAA	" " "
Kinetin, GA_3 and IAA	" " "

Measurements of fruit diameter were made at the completion of each growth phase. On the last date an estimation of ripeness was also made.

No size or ripeness differences were noted in apricots or peaches in this experiment, hence the values are not presented.

(b) The injection of chemicals into apricots

(i) Preliminary trial

In Experiment B5 it was stated that, in addition to bud thinning, certain chemicals were applied to certain branches on five trees. These materials were applied over a complete girdle, in contrast to the two semi-circular bark rings described under Materials and Methods. In other respects the method was identical. To two branches on each of five trees the following were applied 30 days before full-bloom.

1. Water.
2. Kinetin, 20 ppm; GA_3, 50 ppm; 2, 4-dichlorophenoxyacetic acid (2, 4-D), 50ppm.
3. Sucrose, 2% and urea, 0.5%.
4. Sucrose, 2% plus White's medium (White 1943) minus organic constituents.

As was indicated previously most fruits above the girdle dropped; other effects, however, which were attributed to the substances injected, were noted both above and below the

girdle. These are summarised below.

1. Kinetin, GA_3, and 2, 4-D mixture tended to induce bud drop, especially in those buds less advanced. Those remaining had the following abnormalities: Leaf buds opened earlier and produced spindly shoots with long internodes and slightly chlorotic leaves. Flower buds opened at the normal time, but all flower parts were larger than normal; in addition flowers and the subsequent fruit were borne on an elongated pedicel. The contract between a normal flower and one so treated is shown in Plate 3. This latter fruit is actually from a branch treated with GA_3 alone (Experiment 6b(ii)), but the effect is the same.

2. Sucrose and urea mixture had the effect of delaying flowering and producing flowers which were smaller than normal. These flowers had a high rate of abscission, but fruit when they developed were apparently normal.

3. The complete nutrient solution produced growth and development equivalent to water control. Assuming no interaction between the sucrose and urea of treatment 3, it would appear that the abnormal flowering of that treatment was due to urea.

Growth measurements taken at ten day intervals throughout the season revealed no significant modifications in growth or maturity.

(ii) Subsequent trial

When it was realised that injection of kinetin, GA_3, and

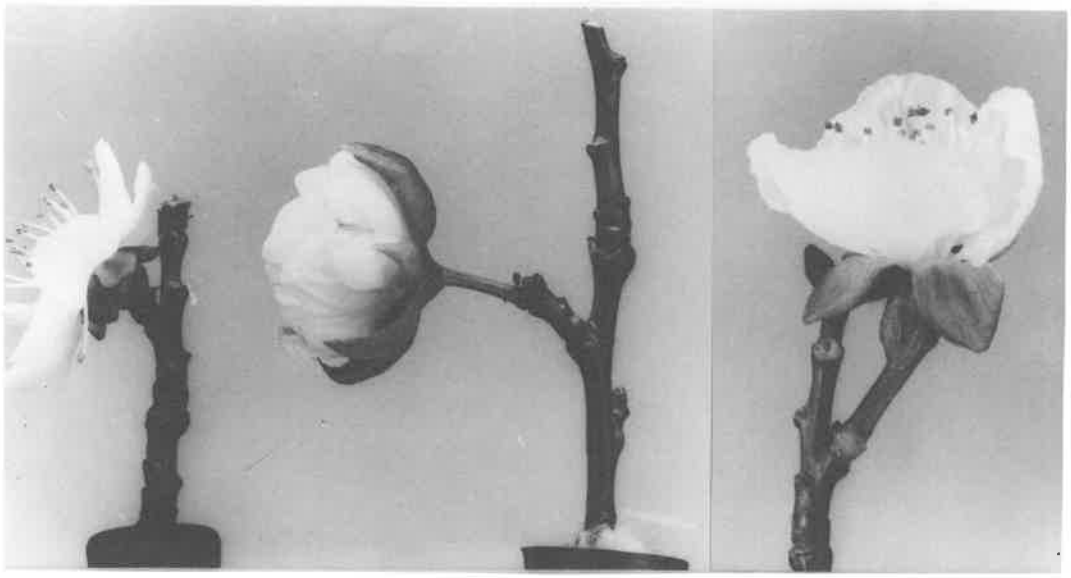


Plate 3A.- Left to right, a. normal apricot flower, b. flower opening three weeks later than normal, c. flower from gibberellic acid-treated branch.

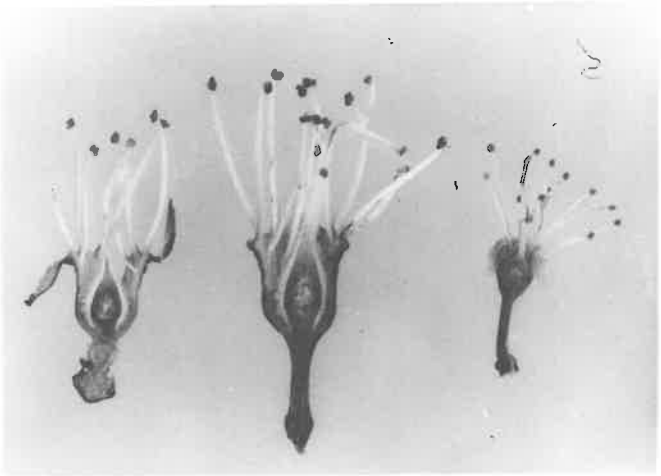


Plate 3B.- Longitudinal sections; left to right, a. normal apricot flower, b. flower from gibberellic acid-treated branch, c. normal flower of cherry plum (Prunus cerasifera).

2,4-D was producing perhaps the most significant growth modifications yet recorded and when, too, the likely failure of the experiment investigating flower-bud thinning was realised, another experiment was designed. Four trees in the orchard were flowering later than others and it was possible to apply chemicals to these approximately six days before full-bloom. The following materials were injected at the base of branches, one metre long, one treatment per tree.

1. Water.
2. Kinetin, 20 ppm.
3. GA_3, 50 ppm.
4. 2, 4-D, 50 ppm.
5. Kinetin, GA_3, and 2, 4-D at above concentrations.
6. Coconut milk, undiluted.

Treatment 5 produced effects similar to those noted in the preliminary trial for the identical treatment. It soon became obvious that most of the modifications in growth were due to the GA_3 component. Gibberellic acid applied alone produced all the modifications described above, although a peculiar chlorosis along the veins, which was present in the combined treatment and with 2, 4-D when applied alone, was absent. Later, exudations of a resin-like substances, at cut surfaces and bud scars, were noticed in the 2, 4-D and combined treatments.

Diameter of fruit was measured at ten-day intervals, beginning 12 days after full-bloom. By maturity only 2 - 5

fruit remained on each branch and the means, at ten day intervals, for branches and trees were calculated from the sizes of these fruit only.

The graph (Fig. 7) shows the growth curve of fruit from control and GA_3 - treated branches. Kinetin, coconut milk, and 2, 4-D treatments were very similar to control, and have been omitted. Two branches of treatment 5 lost all their fruit and results from this treatment are also omitted. At 12 days after full-bloom fruit from GA_3 - treated branches had a significantly greater diameter than other treatments. This size advantage was gradually lost and eventually GA_3 - treated fruit were smaller, although not significantly so. Fruit from branches treated with GA_3 appeared to ripen about five days earlier than other fruit.

At pit-hardening two fruit per branch were removed and determinations of cell size, cell numbers, and mesocarp volume were made. No significant differences were apparent in any of these parameters.

It became apparent later in the season that GA_3 was affecting fruit bud production. In the axils of leaves, particularly towards the tip of strongly growing shoots, there were often four or five flower buds instead of the normal quota of two (Plate 4). Unfortunately the production of flowers and fruit from these buds was not investigated the following season.

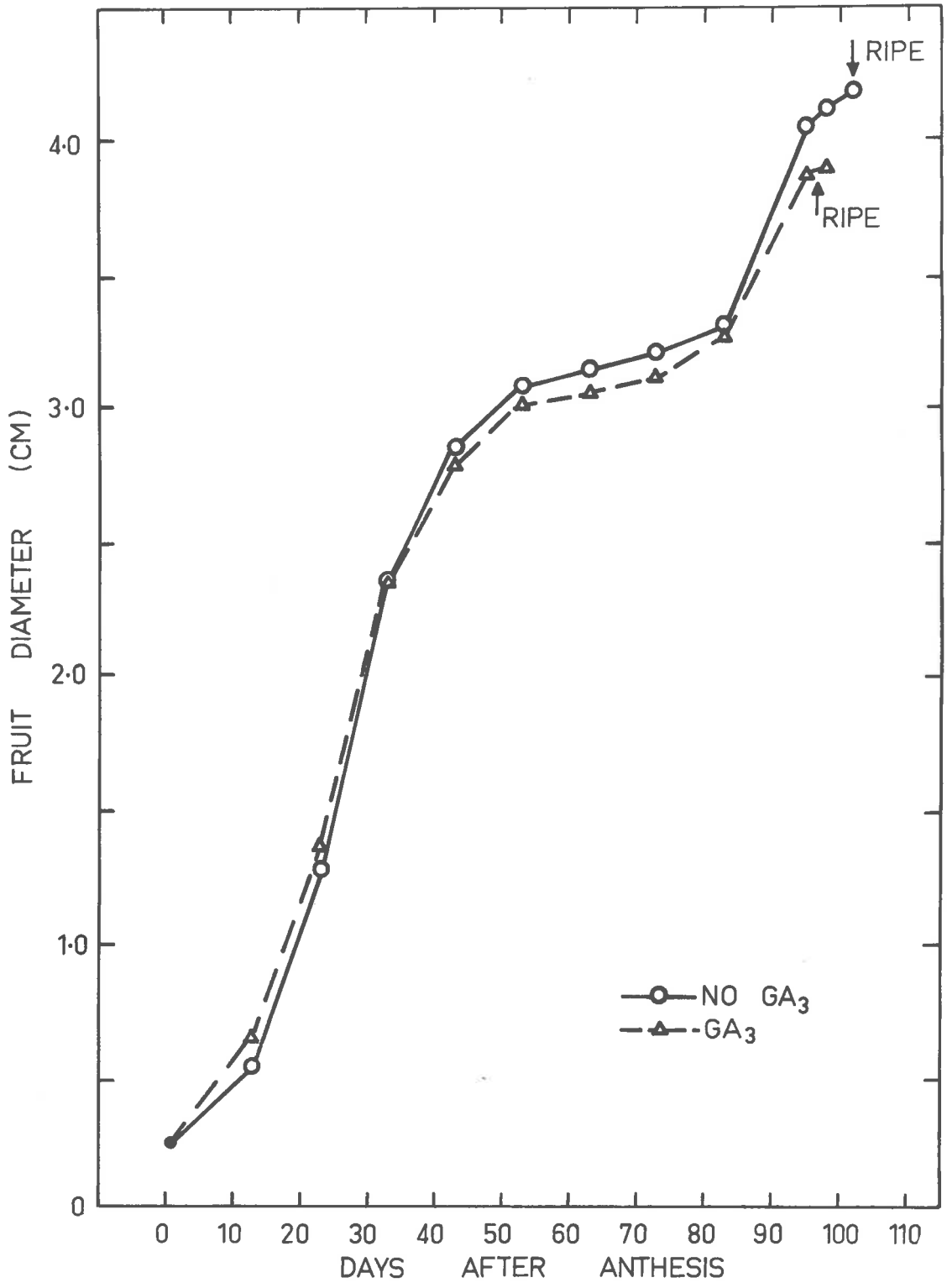


Fig. 7.- Growth of fruit from branches injected with water or gibberellic acid (GA_3), 50 p.p.m.

Plate 4.- The effect of gibberellic acid on bud production. Left, gibberellic acid-treated shoot, right, untreated shoot.

(c) Effect of concentration and time of injection of gibberellic acid on bud and fruit drop, and upon fruit growth

A trial was designed and set out at Waikerie to test the effect of various chemicals on bud and fruit drop, and on growth of apricots.

To one branch, approximately one metre in length, on each of five trees, the following chemicals were injected:-

1. Water.
2. GA_3, 15 ppm.
3. GA_3, 50 ppm.
4. GA_3, 150 ppm.
5. 2-chloroethyltrimethylammonium chloride (CCC), 50 ppm.
6. CCC, 2000 ppm.
7. Alpha-naphthaleneacetic acid (NAA), 10 ppm.

These treatments were applied to three different sets of five trees on the following dates: April 4, June 14, and August 17, 1963. Full-bloom was estimated as September 12.

Gibberellic acid at all concentrations, when applied in June or August, hastened burst of leaf buds by about five days and produced the characteristic effects on flower and shoot growth previously described. The effect was slightly more noticeable in the June treatment and at higher concentrations.

One hundred flower buds were counted on every branch prior to the first application in early April. Fruit buds retained at full-bloom and fruit 35 days after full-bloom were

recorded. Percent bud drop between April and full-bloom, and percent fruit drop between full-bloom and full-bloom plus 35 days, were calculated. No effects of CCC or NAA were apparent and results from GA_3 treatments only are shown in Table 5. Each figure is a mean from five branches over five trees, and significance levels were calculated using branch means as replicates.

The effect of increasing the concentration of GA_3 is to increase both bud drop and fruit drop. In both cases the overall effect is significant at the 1% level. Between adjacent means the greatest differences are between 15 and 50 p.p.m. for bud drop, and 0 and 15 p.p.m. for fruit drop; these are in fact, the only adjacent values which are significantly different (both at the 5% level, but approaching 1%). Lower concentrations may therefore be required to induce fruit drop than to induce bud drop.

Date of application significantly affected bud drop but not fruit drop, earlier applications being more effective. The overall effect on bud drop was significant at the 1% level. Differences between April and June were highly significant (P below 0.01), but between June and August there was no significance. The interaction between concentration and date of application was not significant, therefore there is no reason for supposing that date of application alters the effect of GA_3, it may simply indicate a differential response to the time of girdling or injection of liquid.

Length and mean diameter of fruit were measured 5, 15, 25, and 35 days after full-bloom for the water and GA_3 50 p.p.m. injection treatments only; results are shown in Table 6. There were no obvious effects of NAA or CCC on growth and GA_3 at 15 or 150 p.p.m. did not appear, from general observations, to differ from GA_3, 50 p.p.m.

An analysis of variance showed significant differences in length and mean diameter due to date of measurement, and a significant interaction for both these parameters in measurement date x application date x GA_3 concentration, all at the 0.1% level. L. S. D. values for the interaction showed GA_3 applied in April did not significantly affect fruit size. June application significantly increased both dimensions at 5, 15, and 25 days but the effect was lost at 35 days. August application increased length and diameter at five days but did not affect it subsequently.

Differences attributable to date of water application only rarely approach the 5% significance level, any differences, therefore, due to date of GA_3 application are likely to be because of the chemical, rather than the process of injection. The greater increase in early growth induced by GA_3 injection in June, compared to April or August, is confirmed by comparing dates of application within the GA_3 series. All differences are lost by 35 days after anthesis.

TABLE 5

EFFECT OF GA_3 INJECTED AT THREE DIFFERENT DATES AND AT THREE DIFFERENT CONCENTRATIONS ON BUD DROP BETWEEN APRIL AND FULL-BLOOM AND ON FRUIT DROP IN 35 DAYS AFTER FULL-BLOOM

First figure indicates % drop; figures in parenthesis are logit transformations of % drop / 100 - 0.01 (natural logs)

Effect	Date of GA_3 Application		GA_3 Concentration (p. p. m.)				Date of Application	
			0	15	50	150	Means	(L. S. D. transformation)
% Bud Drop	April		84.4 (1.79)	88.0 (2.39)	93.2 (3.04)	92.8 (3.36)	89.6 (2.65)	5% - 0.50 1% - 0.76 0.1% - 1.22
	June		78.6 (1.30)	72.4 (0.96)	91.4 (2.30)	90.6 (2.69)	83.5 (1.81)	
	August		65.6 (0.65)	75.6 (1.17)	80.0 (1.44)	85.6 (1.78)	76.7 (1.26)	
	GA_3 conc.	Means	76.2 (1.24)	78.7 (1.51)	88.2 (2.26)	89.7 (2.61)		
		L. S. D.	5% - 0.58; 1% - 0.87; 0.1% - 1.40					
% Fruit Drop	April		69.4 (1.29)	78.8 (1.69)	96.0 (3.94)	100 (4.60)	86.1 (2.93)	N. S.
	June		69.4 (0.82)	81.8 (2.04)	92.6 (3.06)	100 (4.60)	86.0 (2.63)	
	August		59.2 (0.35)	97.2 (3.78)	95.2 (3.65)	100 (4.60)	87.9 (3.08)	
	GA_3 conc.	Means	66.0 (0.82)	85.9 (2.56)	94.6 (3.55)	100 (4.60)		
		L. S. D.	5% - 1.30; 1% - 1.97; 0.1% - 3.17					

TABLE 6

EFFECT OF GA_3 INJECTED AT THREE DIFFERENT DATES AND AT THREE DIFFERENT CONCENTRATIONS ON LENGTH AND MEAN FRUIT DIAMETER AT 5, 15, 25, AND 35 DAYS AFTER FULL-BLOOM (F.B.)

First figure, length or diameter in mm; figures in parenthesis, log transformations.

Dimension	Date of Application	GA_3 Conc.	Date of Measurement - Days after F.B.				L.S.D. (log transformations) GA_3 x Application date x Days after F.B.
			5	15	25	35	
Length (mm)	April	0	7.2 (0.86)	14.7 (1.19)	26.6 (1.43)	29.5 (1.47)	5% - 0.02 1% - 0.03 0.1% - 0.04
		50	6.9 (0.84)	15.0 (1.18)	26.1 (1.41)	30.7 (1.49)	
	June	0	7.1 (0.85)	15.2 (1.18)	26.2 (1.42)	30.1 (1.48)	
		50	9.3 (0.97)	18.8 (1.27)	27.7 (1.44)	30.9 (1.49)	
	August	0	7.2 (0.86)	14.1 (1.15)	25.6 (1.41)	31.0 (1.49)	
		50	8.3 (0.92)	14.1 (1.15)	25.4 (1.41)	30.3 (1.48)	
Mean Diameter (mm)	April	0	4.3 (0.63)	10.1 (1.00)	23.0 (1.36)	28.8 (1.46)	5% - 0.02 1% - 0.03 0.1% - 0.04
		50	4.2 (0.61)	10.3 (1.01)	22.1 (1.34)	29.2 (1.46)	
	June	0	4.1 (0.61)	10.4 (1.02)	22.2 (1.35)	28.6 (1.46)	
		50	5.4 (0.73)	13.8 (1.14)	23.6 (1.37)	28.5 (1.46)	
	August	0	4.4 (0.64)	9.3 (0.97)	21.9 (1.34)	28.8 (1.46)	
		50	4.7 (0.67)	9.0 (0.95)	21.2 (1.33)	27.2 (1.44)	

7. The effect of night temperature on the growth of Elberta peaches

Eight branches, 1.0 - 1.2 m long, were selected on one peach tree and four were heated by the method described (Method 1). Heat was given at night between 6 pm and 8 am, of the fifth day after full-bloom, and continued for 25 consecutive nights. Temperature was adjusted to a mean of 27°C, while the mean ambient night minimum over this period was 12.8° C. On removal of the heating apparatus, fruit were labelled on the eight branches and diameter was recorded then, and subsequently, as shown in Fig. 8.

The method of heating caused damage to fruit, leaves, and branches close to the heater and some leaves and fruit dropped for this reason. Fruit numbers were also severely and unequally reduced by a severe storm two weeks after termination of heating. It was possible to analyse statistically only those measurements taken on Oct. 20 and Nov 3, 1961. Immediately after the cages were removed heated fruit was significantly larger than unheated fruit (P below 0.01). On Nov. 3 this situation was reversed, non-heated fruit being larger (P below 0.05).

Although high temperatures have significantly increased the early growth rate, they have caused no increase in final size, and may, in fact, have reduced it. Heating, however, does appear to have hastened the maturity of the fruit. Because of the low, and unequal, fruit numbers per branch later

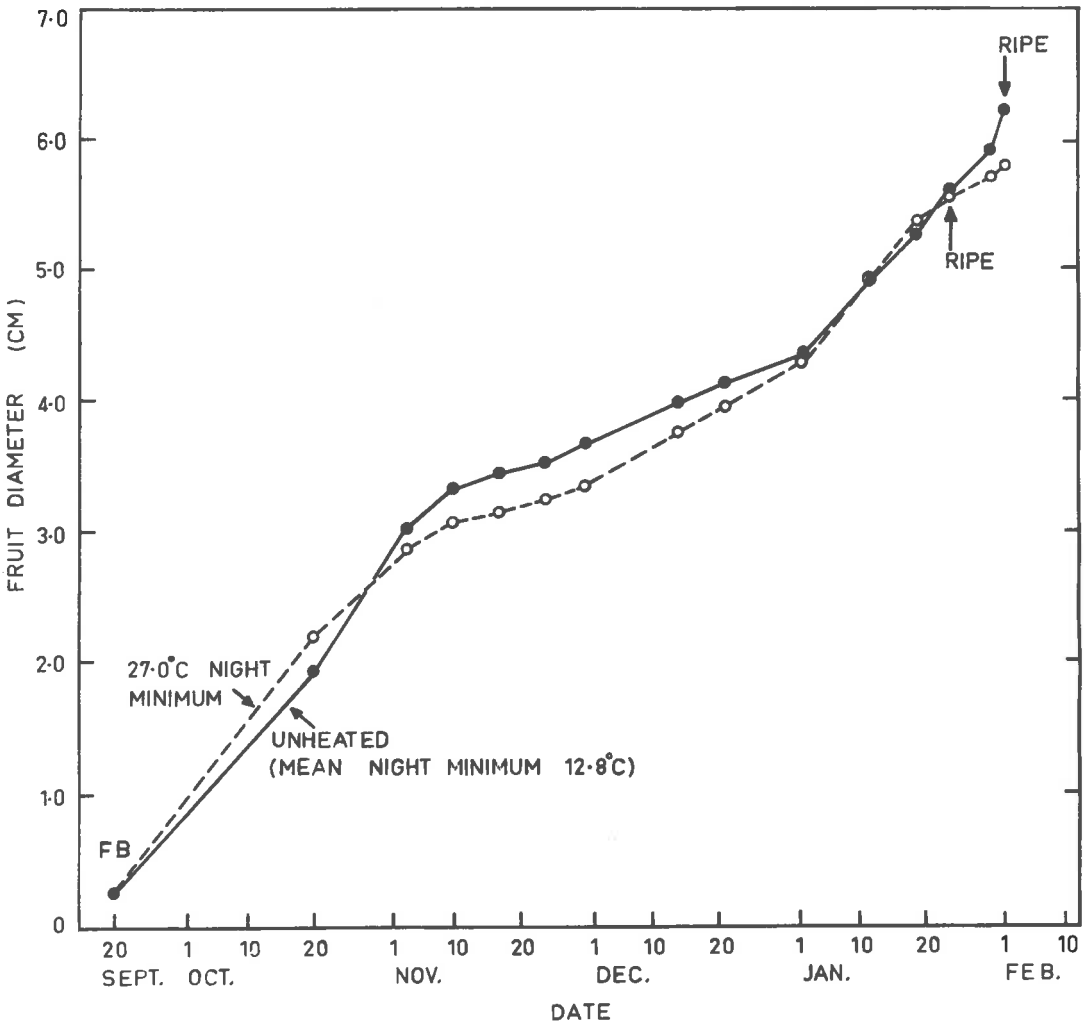


Fig. 8.- Effect of temperature on the growth of Elberta peaches; heat applied at night between Sept. 25 and Oct. 19, 1961 (inclusive).

in the season, doubt could be expressed on the validity of these conclusions, however, the early increase in growth rate and the hastening of maturity are interesting in that they support later results with apricots. The reduction in final fruit size indicated here was not found in later experiments on the effect of heat on apricots (see Fig. 9).

8. The effect of gibberellic acid and different night temperatures on growth and endogenous gibberellins in apricots

(a) Introduction

Although experiments in 1961 and 1962 did not demonstrate any predictable method of altering final fruit size or cell number, certain methods of altering growth in Stage I were suggested. It was therefore decided to study more closely the effect of certain treatments on the fruit during this period.

Two factors were investigated for their effect upon apricot, viz temperature and GA_3 injection. Response was measured in terms of fruit growth, rate of cell multiplication and expansion, and content of endogenous gibberellin. The latter was measured because of its intrinsic interest and because preliminary trials indicated that gibberellin activity was present in extracts of apricot fruit tissue, was greatest during Stage I, and appeared to parallel fruit growth rate at this time

(b) Preliminary experiment

A preliminary experiment was designed to test the equipment of Method 2, the method of GA_3 application, and different temperature regimes.

On Aug. 5 (5 - 6 weeks before flowering) four limbs on each of four trees were enclosed in cages from 6 pm to 8 am of each night, and temperature was adjusted to 16°C. Within four days some buds were showing signs of expansion and by Aug. 26 many flowers were at full-bloom and the following treatments were applied :-

Treatment 1. Night temperature maintained at 18°C.

Treatment 2. Night temperature, 18°C; GA_3, 50 ppm, injected Aug. 26.

Treatment 3. Night temperature, 18°C; GA_3, 500 ppm, sprayed Aug. 26.

Treatment 4. Night temperature, 25.5°C.

At this date no flowers were open except on the heated limbs, which had flowered two weeks earlier than normal. Two types of flower were labelled; early flowers, opening between the dates 26 - 29 Aug. inclusive, and late flowers, between Aug. 30 - Sept. 2; all other flowers were removed. It was hoped that such a selection would determine whether it was better to apply GA_3 0 - 3 days or 4 - 7 days before anthesis.

Heating was discontinued on the evening of Sept. 4 and fruit diameters were measured at seven day intervals,

beginning seven days after early and late full-bloom respectively.

By Sept. 4 the effect of the higher temperature treatment in increasing fruit size was apparent. Up to Sept. 9 no significant effect of GA_3, injected or sprayed, could be found.

Growth curves from this experiment are not shown because there were too few fruits remaining on many branches to give very reliable results. Nevertheless the results did tend to support the next experiment and are as follows. The high-temperature treatment (Treatment 4) increased early growth rate and hastened maturity, compared with the low-temperature treatment (Treatment 1). GA_3 applications caused a depression of growth rate, a reduction in final size and hastened maturity relative to the low temperature, no - GA_3 treatment; this was more pronounced with the injection, than with the spray treatment. No conclusions could be drawn on differences between fruit from early and late flowers.

(c) Main experiment

The previous experiment had confirmed the suspicion that heat would increase the early growth rate of fruit. It did not indicate, until later in the season, whether spraying or injection was the superior application method for GA_3. At Waikerie, injected GA_3 at 15 p.p.m. was giving growth modifications similar to GA_3 at 50 p.p.m, and a trend towards less bud drop with the former was suspected. In consequence

GA_3 application was made by injection at 15 p.p.m. Three temperature regimes, with and without GA_3, were combined in a factorial as follows :-

Night temperature	Injection liquid	
	Water	GA_3 - 15 ppm
Unheated - branches not caged (Mean night minimum 11.4°C)	A	B
Branches caged Mean night temperature 18°C	C	D
Branches caged Mean night temperature 25.5°C	E	F

Six similar branches were selected on each of four trees. They were injected on Sept. 9 at the base of the branch, just outside the cage. Full-bloom was taken as Sept. 13 and flowers opening more than two days on either side of this date were removed. Heating was applied at night between 6 pm and 8 am, for a period of ten days, beginning at full-bloom.

At five days after full-bloom as many fruit as possible

were tagged and their diameter recorded then, and at five day intervals over the growth period. It was hoped to take four fruits for cell determinations at 5, 10, 15, 20 days after full-bloom, and at maturity, and to take eight fruit at five days and three fruit at 15 days for gibberellin estimations. However, in some branches there were too few fruit remaining to take a full sample and, in such cases, numbers were proportionally reduced. Table 7 gives the number of fruit on each branch after the ten day sample had been removed.

TABLE 7

NUMBER OF FRUIT RETAINED BY BRANCHES ON FOUR TREES, UNDER DIFFERENT TEMPERATURE AND GA_3 TREATMENTS, 10 DAYS AFTER ANTHESIS.

Treatment		Tree 1	Tree 2	Tree 3	Tree 4
Unheated	No GA_3	21	23	34	40
	GA_3	28	15	31	14
18°C	No GA_3	25	26	24	27
	GA_3	8	7	9	31
25.5°C	No GA_3	16	13	14	39
	GA_3	1	2	8	16

It will be seen that there is a tendency for both GA_3 and high-temperature treatments to increase fruit drop in the early part of the season.

(i) Mean fruit diameter

Results in terms of mean fruit diameter per treatment are shown in Fig. 9. An analysis of variance was done on the data obtained at each sampling date using trees as replicates. The probability figures at the top of Fig. 9 refer to overall effects of temperature and GA_3 and do not necessarily apply to individual points shown on the curves.

High temperatures have clearly increased the growth rate early in the season; they have possibly increased growth at the beginning of Stage III, and have hastened maturity by 2 - 5 days. Temperature does not appear to have affected the final size to which the fruit grew. After a stimulation to growth at five days, GA_3 has significantly depressed growth over the remainder of the season. Final size of fruit receiving GA_3 is significantly less than untreated fruit (P below 0.01). Interactions GA_3 x temperature are not generally significant.

(ii) Relative fruit dimensions

Examination of the original measurements for the three major fruit axes (see Fig. 1A) showed that the relative shape of the graph constructed from each was similar to that

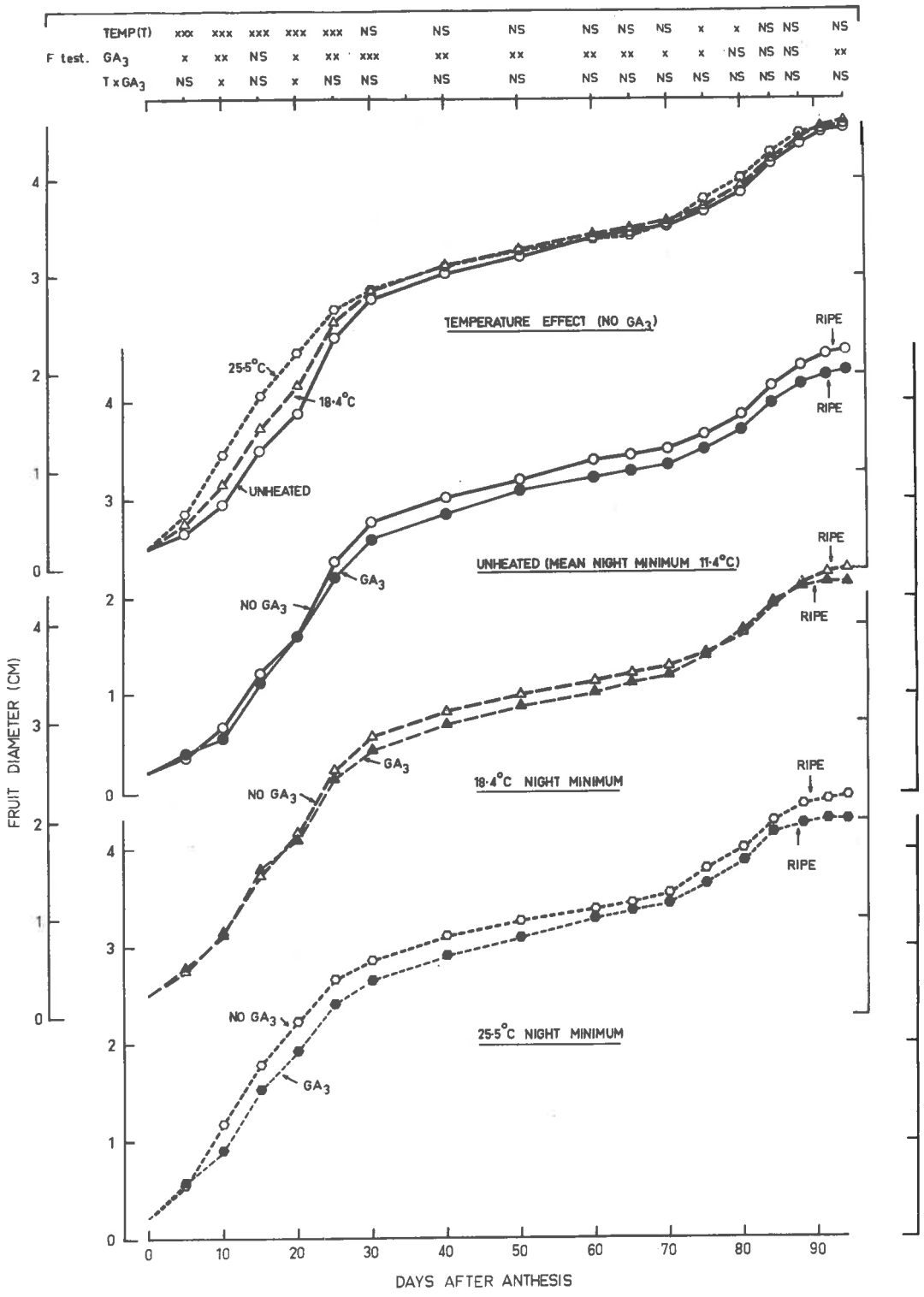


Fig. 9.- Effect of three night temperatures, with and without applied gibberellic acid (GA_3), on the growth of apricot fruit.

drawn from mean diameter in Fig. 9. It is therefore safe to say that the treatment effects are produced in three dimensions. Table 8 shows the ratio length / mean diameter and Table 9, the ratio suture diameter / non - suture diameter (b/a) at different stages after anthesis.

It is clear that fruit develops from a shape which is oval, in longitudinal - section and in cross - section, to an almost spherical structure. High temperatures appear to hasten this change but do not alter the final shape. Gibberellic acid has, if anything, delayed the change, but again has not altered the final shape.

(iii) Effect on ripening

The dates at which fruit ripen are shown in Fig. 9 for each treatment. At 88 days after anthesis ripeness of all fruits was estimated as previously described. Results were analysed as the factorial- temperature x GA_3 x trees, and are as follows :-

Treatment	Ripeness means	Significance
No GA_3	2.47	P below 0.05
GA_3	3.30	
11.4°C night minimum	2.04	P below 0.01
18.4°C " "	2.80	
25.5°C " "	3.80	

There was no GA_3 x temperature interaction.

TABLE 8

RATIO LENGTH / MEAN DIAMETER OF APRICOT FRUIT AT DIFFERENT DATES AND UNDER DIFFERENT TEMPERATURES AND GIBBERELLIC ACID TREATMENTS

Days after FB	No GA_3			GA_3		
	11.4°C	18.4°C	25.5°C	11.4°C	18.4°C	25.5°C
5	1.72	1.70	1.67	1.87	1.84	1.80
10	1.66	1.58	1.22	1.87	1.62	1.54
15	1.40	1.33	1.19	1.49	1.31	1.37
20	1.34	1.26	1.14	1.33	1.25	1.27
25	1.14	1.14	1.08	1.19	1.15	1.14
30	1.08	1.07	1.05	1.12	1.09	1.09
40	1.03	1.03	1.02	1.05	1.05	1.05
50	1.02	1.03	1.00	1.04	1.03	1.03
60	1.02	1.01	1.01	1.03	1.02	1.02
65	1.00	1.02	0.99	1.03	1.01	1.02
70	1.00	1.01	0.99	1.02	1.01	1.02
75	1.00	0.99	0.98	1.02	1.00	0.99
80	1.00	0.98	0.97	1.00	0.98	0.98
84	0.96	0.96	0.96	0.99	0.97	0.95
88	0.95	0.96	0.94	0.98	0.97	0.94
94	0.94	0.95	0.94	0.96	0.97	0.94

TABLE 9

RATIO SUTURE DIAMETER / NON - SUTURE DIAMETER OF APRICOT FRUIT AT DIFFERENT DATES AND UNDER DIFFERENT TEMPERATURE AND GIBBERELLIC ACID TREATMENTS

Days after FB	No GA_3			GA_3		
	11.4°C	18.4°C	25.5°C	11.4°C	18.4°C	25.5°C
20	1.13	1.11	1.08	1.12	1.15	1.13
25	1.13	1.11	1.07	1.14	1.13	1.13
30	1.13	1.10	1.06	1.11	1.11	1.10
40	1.11	1.09	1.05	1.09	1.10	1.07
50	1.10	1.09	1.05	1.09	1.10	1.08
60	1.08	1.07	1.05	1.07	1.09	1.05
65	1.09	1.07	1.05	1.06	1.08	1.05
70	1.08	1.07	1.04	1.07	1.07	1.04
75	1.07	1.04	1.02	1.05	1.04	1.01
80	1.04	1.02	0.99	1.02	1.02	1.00
84	1.01	1.00	0.99	1.01	1.02	1.00
88	1.00	1.00	0.98	1.00	1.03	0.99
94	1.01	1.00	0.98	1.00	1.04	0.99

It is interesting to note that, despite different effects on fruit growth, both GA_3 and temperature treatments hastened ripening. It can be seen from Fig. 9 that this was from 2 - 5 days.

(iv) Cell numbers in the mesocarp

Numbers of cells across the mesocarp were measured at 5, 10, 15, and 20 days after full-bloom, and at maturity. A minimum of eight fruit per treatment (i.e. two per branch) were used to give the points shown in Fig. 10. * The data was analysed as the factorial:- temperature x GA_3 x time, using the four trees as replicates. Overall effects of temperature, GA_3, and time were all highly significant (P below 0.001) but the only significant interaction was temperature x time (P below 0.01). Means (with corresponding L.S.D's) are given in Table 10 and are helpful in interpreting Fig. 10.

The analysis shows that before 15 days, temperature has significantly increased cell number; thereafter, however, this effect is lost. Between 10 and 15 days the unheated

---

* At both 15 and 20 days after full-bloom, only one of the four tree replicates, for the treatment 25.5°C with GA_3, had fruit for cell determinations. These two points are omitted on the graph and figures in brackets in Table 10 have three missing values and should be treated withcaution.

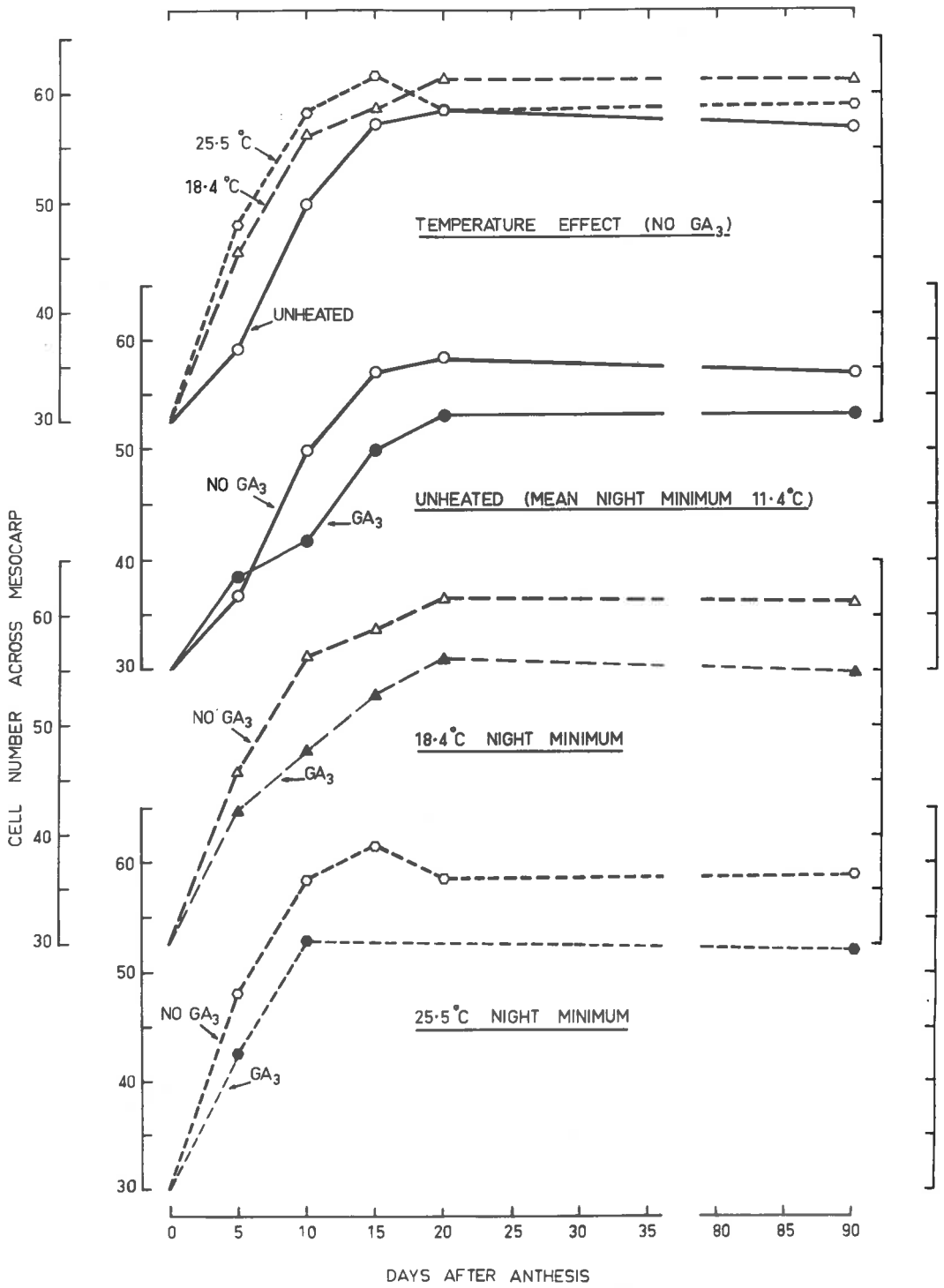


Fig. 10.- Effect of three night temperatures, with and without applied gibberellic acid (GA_3), on cell numbers across the mesocarp of apricot fruit.

TABLE 10

EFFECT OF TEMPERATURE, GA_3, AND TIME ON CELL NUMBER ACROSS THE MESOCARP IN APRICOTS

Effect	Means						L. S. D.
GA_3	No GA_3			GA_3			(P below 0.001)
	55.11			50.10			
Temperature	Unheated		18.4°C		25.5°C		P= .05 - 1.63 P= .01 - 2.16 P= .001 - 2.80
	32.98		35.77		36.46		
Time	Days after FB	5	10	15	20	Maturity	P= .05 - 2.10 P= .01 - 2.79 P= .001 - 3.61
		42.25	50.94	56.59	57.07	56.18	
Temperature X Time	Unheated	37.61	45.31	53.44	55.85	55.16	P=.05 - 3.62
	18.4°C	43.90	52.00	55.67	58.72	57.97	P= .01 - 4.81
	25.5°C	45.24	55.50	(60.66)*	(56.64)*	55.40	P= .001 - 6.23

* Figures in parenthesis have three missing values.

fruit show a highly significant (P below 0.001) increase in cell numbers, those under "medium" heat are just significantly different while under high temperature there is probably no significant difference. Thus higher temperatures induce more rapid cell division but do not significantly alter final cell number in the mesocarp. The overall effect of applied GA_3 is to reduce the amount of cell division. In a preliminary trial it was found that GA_3 at 50 p.p.m., applied two weeks before full-bloom, increased cell numbers at full-bloom. It is suggested that GA_3 increases early growth rate (Figs. 7, 9) and cell number (Fig. 10) but later causes a decrease; these depressive effects are hastened by high temperature.

(v) Cell size

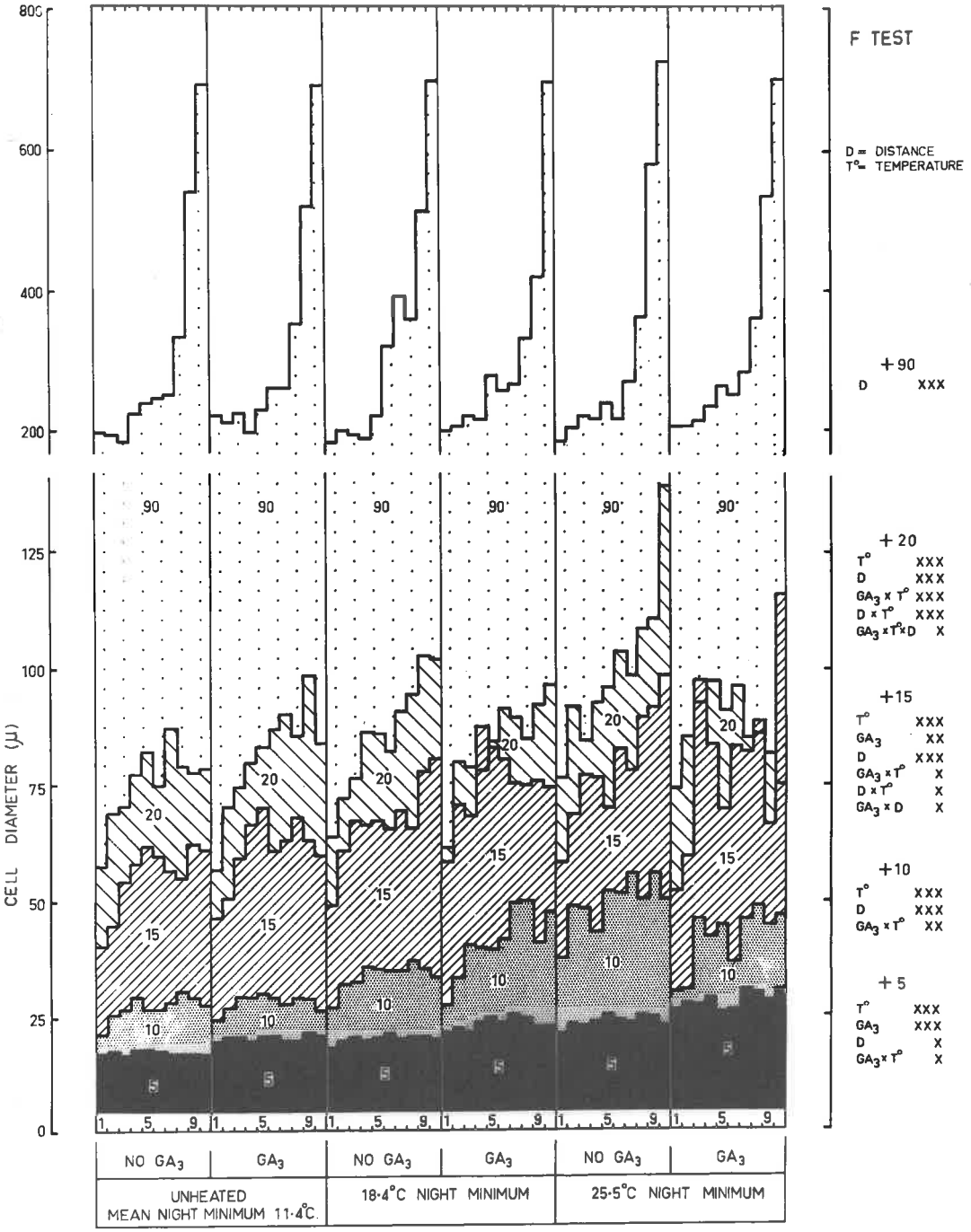
It will be remembered that the calculation of cell numbers was made by selecting single cells at 10 equidistant points across the mesocarp, measuring their radial diameter and, together with the knowledge of mesocarp width, computing the number of cells across that transect of mesocarp. The values of radial diameter so gained are presented in Fig. 11. Diameters of cells near the skin, notated as cell number 1, to those close to the endocarp, cell number 10, are presented as histograms for all treatments at each date. Figures from each date after full-bloom were analysed as the factorial : temperature x GA_3 x distance from skin (1 - 10), and

Fig. 11.- Effect of three night temperatures, with and without gibberellic acid (GA_3), on the radial diameter of cells across the mesocarp of apricot.

Histograms, left to right, give the size of cells at 10 equidistant points from epidermis to endocarp. Age of fruit in terms of days after anthesis are shown directly beneath each histogram. Significance in the F test of the analysis of variance for the factorial is shown at the right of this diagram; non-significant results are omitted.

x = P below 0.05, xx = P below 0.01,

xxx = P below 0.001.


800
600
400
200
F TEST
D = DISTANCE
T° = TEMPERATURE
+90
D XXX
125
100
75
50
25
0
CELL DIAMETER (μ)
90
20
15
10
5
+20
T° XXX
D XXX
GA3 x T° XXX
D x T° XXX
GA3 x T° x D X
+15
T° XXX
GA3 XX
D XXX
GA3 x T° X
D x T° X
GA3 x D X
+10
T° XXX
D XXX
GA3 x T° XX
+5
T° XXX
GA3 XXX
D X
GA3 x T° X
1 5 9
NO GA3
GA3
UNHEATED
MEAN NIGHT MINIMUM 11·4°C.
18·4°C NIGHT MINIMUM
25·5°C NIGHT MINIMUM

significant effects are shown at the right side of the diagram.

The results show the differential growth in radial cell diameter of cells close to the skin and endocarp respectively, from five days after anthesis to maturity. Cells close to the endocarp expand more in this plane than do cells close to the skin. There is no treatment effect on cells at maturity, but up to 20 days, temperature does increase diameter and also interacts significantly with the effect of distance of cells from the skin. Thus high temperature induces an earlier difference in radial diameter between cells in the different positions across the mesocarp. The effect of GA_3 is even briefer; at five days it has increased radial diameter, but thereafter it is barely significant, or is not apparent. Up to 20 days there is a significant interaction between GA_3 and temperature; GA_3 seems to reduce the relative increase due to high temperatures. That this may be due to its effect in disrupting the smooth transition from nearly equal cells near the skin and endocarp at five days, to the unequal radial cell diameter later in the season, is indicated by the appearance of the histograms and the interaction: temperature x GA_3 x distance at 20 days.

Fig. 11 shows that, at maturity, the radial diameter of cells close to the endocarp is 3.5 times that of cells near the epidermis; yet other observations, made at the same time, revealed that the tangential diameter of cells near the endocarp was 0.58 times those near the skin at the same stage. Assuming the volume of the cells adjacent to the epidermis approximates

to oblate spheroids and those near the endocarp to prolate spheroids, it can be calculated that the cells near the endocarp are only 1.16 times the volume of those near the epidermis. Thus the differences shown in Fig. 11 are largely differences in cell shape rather than volume. The change in cell shape at the two positions with growth can perhaps be appreciated from the observation, that, at five days after full-bloom the ratio radial to tangential diameter was unity at both position, at pit-hardening it was 0.69 and 2.14, and at maturity 0.70 and 4.26, for cells adjacent to the skin and endocarp respectively.

(vi) Effect of temperature on growth in Stage 1 of the apricot fruit

In Experiment 3 it was found that, if the mean daily temperature was taken over the period from full-bloom until the fruit diameter was 2.3 cm, it was negatively correlated with the number of days taken to reach that diameter. The comparison was made between "early" and "late" fruit of 1961 and 1962. In Table 11 the results from this experiment (Experiment 8) plus data from "early" and "late" fruit, 1963 were included. Analysis proved a highly significant negative correlation between number of days and mean temperature (P below 0.001). Where Y = number of days and X = mean temperature (^{o}C)

TABLE 11

THE NUMBER OF DAYS TAKEN BY APRICOT FRUIT TO REACH A MEAN DIAMETER OF 2.3 cm, COMPARED WITH MEAN DAILY TEMPERATURE OVER THAT PERIOD.

Data from various experiments at the Waite Institute over a period of three years

Experiment	Date of F.B.	Days	Temperate (°C)
Early *	16/9/62	32	13.8
A,** Late- 18.4°C	31/8/63	31	13.9
Late *	23/9/62	30	13.4
A, Early- 18.4°C	28/8/63	26	15.2
A, Late- 25.5°C	31/8/63	26	15.6
Early	12/9/61	26	16.6
Early	11/9/63	25	15.4
A, Early-25.5°C	28/8/63	25	16.2
B ** - Unheated	13/9/63	25	16.2
B - 18.4°C	13/9/63	23	16.9
Late	16/9/63	22	16.5
Late	20/9/61	21	17.8
B - 25.5°C	13/9/63	21	18.1

* "Early" refers to fruit from flowers which opened early
"Late" " " " " " " " "

** "A" refers to preliminary heating trial 1963
"B" " " main " " "

After these letters in the heating trial is given the night temperature at which the fruit was maintained.

the linear regression equation is $Y = 66.29 - 2.57X$. This line is probably a portion of a rectangular hyperbola whose slope approaches the vertical at a temperature where no growth occurs. If this particular temperature is taken as zero and temperatures above are appropriately adjusted, the product XY should be constant. Reasonable constancy is in fact obtained for the data, if a base temperature of $5^{\circ}C$ is chosen. (This, incidentally, is similar to that utilised by Brown (1952) for predicting harvest time of apricots from temperature data in the first 42 days after full-bloom.) The product, days x mean daily temperature minus 5, is approximately 273 "Degree-days".

(vii) Endogenous gibberellins

Seed or pericarp (10 mg) from samples of all six treatments at 5 and 15 days after anthesis was extracted with ethyl acetate and the residue re-extracted with methanol. Extracts were chromatographed and the chromatograms divided into ten equal pieces and tested for gibberellin activity in the barley endosperm test. Twelve chromatograms, i.e. 2 extractants x 2 GA_3 treatments x 3 temperature treatments, plus another , spotted with 10^3 µµg GA_3, were tested each week. Over four weeks this was repeated with seed or pericarp at 5 or 15 days after anthesis. The whole series was repeated to provide duplicates during the following four weeks.

Activity from GA_3 - spotted chromatograms was always contained between Rf 0.2 - 0.6 with a peak estimated as 0.4. Activity from all extracts was contained between Rf 0.1 - 0.5 with peak activity at about 0.28. No difference in the position of the peak due to extractants or treatments was apparent but there was variation between tissue and time. Activity was always present in the methanol extracts and for each chromatogram the Rf of maximum activity was estimated. The twelve figures - duplicates (2) x GA_3 (2) x temperature (3) were treated as replicates and the data analysed with tissues and time being treated as independent variables. Rf values and L.S. D's were as follows:

Treatment	Mean Rf value	L. S. D. (Tissue x Time)
Seed	0.261	
F.B + 5		P below 0.05 - 0.020
Pericarp	0.326	
		P below 0.01 - 0.027
Seed	0.286	
F.B + 15		P below 0.001 - 0.036
Pericarp	0.268	

The greatest difference is shown in pericarp at five days which has a greater Rf (P below 0.001).

The total activity in GA_3 equivalents was calculated for

each chromatogram and activity from equivalent ethyl acetate and methanol chromatograms was summated. The total activity per 10 mg tissue so obtained was analysed as the factorial : tissue x time x GA_3 x temperature (2 x 2 x 2 x 3), with two replicates. Only two significant points were realised; a very highly significant difference in activity of tissue and a highly significant tissue x time interaction. These are shown in Table 12; for comparison, activity in ethyl acetate and methanol extracts is also shown, but L.S.D. values refer only to total activity. None of the other variables, nor their interactions, were significant; hence the gibberellin and temperature treatments had no measured effect on the content of endogenous gibberellin per unit of dry weight of seeds or pericarp.

Methanol, it will be seen, extracts more activity from the residue than ethyl acetate originally extracted from the tissue. Seed has significantly more activity than pericarp at both dates, and from 5 to 15 days activity increases in the seed but decreases in the pericarp; both the increase and the decrease are close to the 5% level of significance.

C. Endogenous Gibberellins in Apricot Fruit

1. Gibberellin activity in crude ethyl acetate and methanol extracts of apricot tissue from anthesis to maturity.

TABLE 12

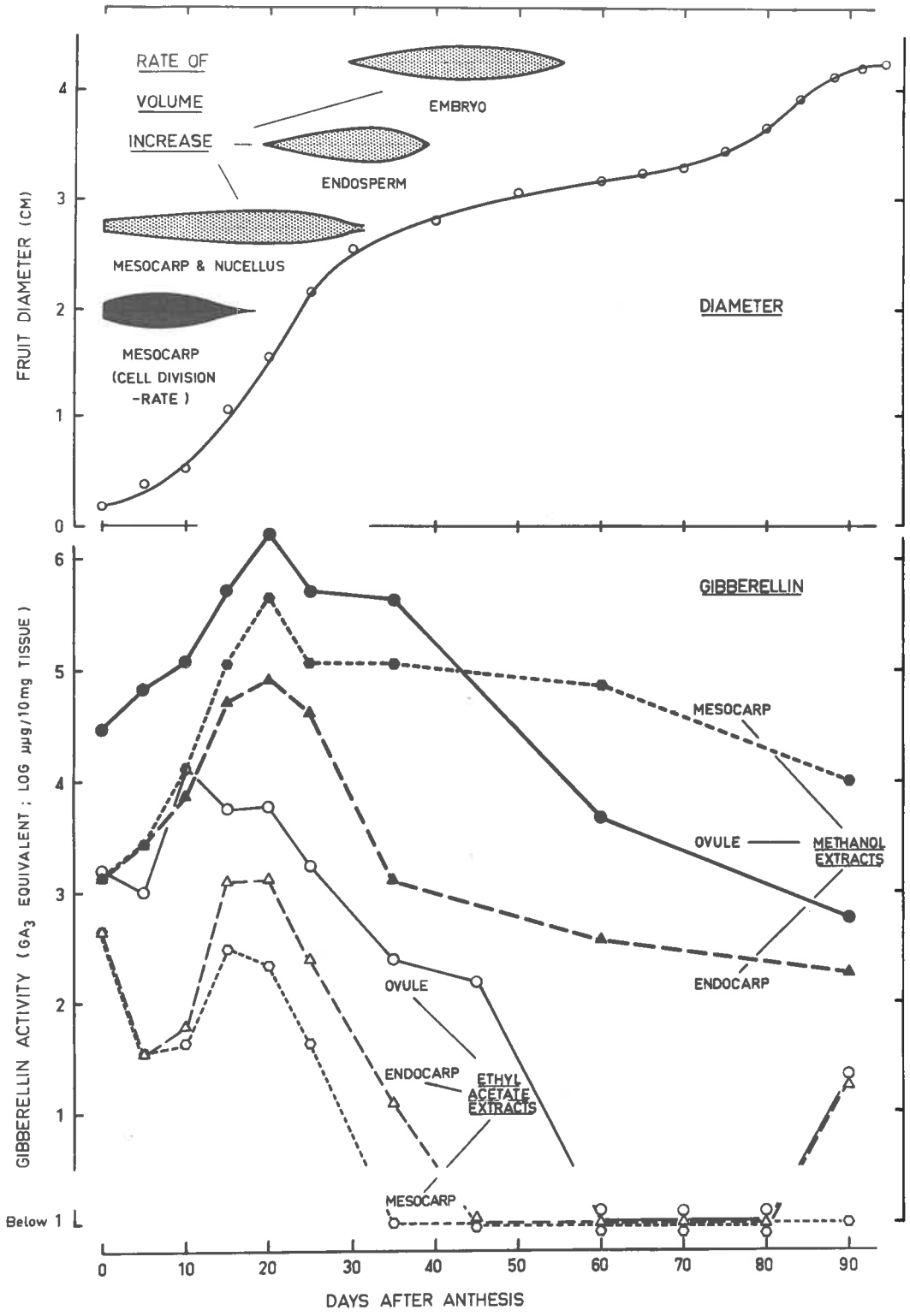
GIBBERELLIN ACTIVITY (GA_3 EQUIVALENTS - LOG µµg) FROM SEED AND PERICARP 5 AND 15 DAYS AFTER ANTHESIS USING TWO EXTRACTANTS

Days after FB	Tissue	Extractant		Total activity	L. S. D. (Total activity only)
		Ethyl Acetate	Methanol		
5	Seed	2.95	4.91	4.96	P below 0.05 = 0.56
	Pericarp	3.13	3.70	3.90	P below 0.01 = 0.74
15	Seed	4.45	5.40	5.51	P below 0.001 = 1.00
	Pericarp	2.75	3.11	3.39	

Results are presented below of two barley endosperm tests for gibberellin activity in seed, endocarp, and mesocarp of apricots throughout the growing season. The first tested activity in ethyl acetate extracts, and the second, activity in methanol extracts of the residue after ethyl acetate extraction. 100 mg of tissue was extracted, aliquots equivalent to 10 mg being placed in each of six vials. After evaporation 1 ml of water was added to three vials and 1 ml containing 10^4 µµg GA_3 to the other three. The results for both extracts are shown in Fig. 12 as GA_3 equivalents per 10 mg dry weight. Each point is a mean from three vials containing extract without GA_3. No extract when included with 10^4 µµg GA_3 significantly lowered its response, thus no evidence was gained of the presence of inhibitor in any extract.

Above the points on the graph diagrams are shown representing the growth of the fruit and growth rate of various fruit tissues, the purpose being to relate gibberellin activity to growth and development of the fruit; this will be discussed later. The figure demonstrates again the greater effectiveness of methanol as an extractant (cf Experiment 8 vi). It is apparent that the seed has greater activity than endocarp or mesocarp at all stages before pit-hardening, but that mesocarp is the only tissue which retains appreciable activity later in the season. Peaks occurred in all tissues at the same time, i.e. 20 days after anthesis.

Fig. 12.- Gibberellin activity in crude ethyl acetate extracts of apricot tissues, and methanol extracts of the residue after ethyl acetate extraction. Above, and for comparison, are shown diagrammatic representations of growth and growth rate of various tissues, and cell division rate in the mesocarp.


RATE OF
VOLUME
INCREASE
EMBRYO
ENDOSPERM
MESOCARP & NUCELLUS
MESOCARP
(CELL DIVISION
-RATE)
DIAMETER
FRUIT DIAMETER (CM)
GIBBERELLIN
MESOCARP
OVULE
METHANOL
EXTRACTS
ENDOCARP
OVULE
ENDOCARP
ETHYL
ACETATE
EXTRACTS
MESOCARP
GIBBERELLIN ACTIVITY (GA3 EQUIVALENT ; LOG µµg/10mg TISSUE)
Below 1
DAYS AFTER ANTHESIS

2. Gibberellin activity in apricot tissue and its behaviour in a partitioning procedure and in paper chromatography

(a) Activity of ethyl acetate extracts after partitioning

One hundred mg of seed and pericarp five days after full-bloom were partitioned as previously described. From each fraction - crude ethyl acetate (S1), ethyl acetate non-acid fraction (s2), aqueous acidic fraction (S3) and ethyl acetate acidic fraction (S4), 10 mg tissue equivalents were placed in each of three vials and 1 mg equivalent in a further three vials. Gibberellin activity was measured using the barley endosperm test. The slope between the values obtained from the extracts containing 10 mg and 1 mg tissue was parallel to the standard curve. The results in Table 13, therefore, could have been computed from either value; results from the former were in fact used.

TABLE 13

GIBBERELLIN ACTIVITY (GA_3 EQUIVALENT, LOG μμg) PER 10 mg APRICOT TISSUE EXTRACTED AND FARTITIONED FROM ETHYL ACETATE

Extract	5 Days after Full-bloom		20 Days after Full-Bloom		
	Seed	Pericarp	Seed	Endocarp	Mesocarp
Crude ethyl acetate extract, S1	2.50 (2.50)	0.55	3.97	1.64	1.23
Non-acid ethyl acetate fraction, S2	4.50 (2.30)	0.10	1.12	1.04	<0.1
Acidic aqueous fraction, S3	4.07	2.93	3.00	3.26	2.15
Acidic ethyl acetate fraction S4	1.92	2.78	2.40	1.61	0.25

These results are from the second of two identical tests. The calculation of GA_3 equivalents in the first test was made difficult by rather erratic values in the standard curve. Extracts however appeared to behave in a similar manner to this test, with one noticeable exception, that S2 was below S1 rather than greatly above, in seed at five days after full-bloom. These two values are shown in brackets in Table 13. With this one exception it is apparent that the non-acid fraction has recovered virtually no activity. Most was contained in the acidic fractions S3 and S4, particularly S3. In all cases, except seed at 20 days, there has been a greater recovery of activity from the sum of these two fractions than in crude extracts.

It is difficult to reconcile the results from purified extracts here, with those from crude ethyl acetate extracts shown in Fig. 12 where, in contrast, activity rises from 5 to 20 days in all tissues. Even activity in crude extracts from both experiments is at variance. Both experiments C1 and C2 (a) were repeated and the results presented in Fig. 12 and Table 13 are the second of two identical tests. Whereas the two results from Experiment C1 gave almost equivalent activity at all stages, the first test in Experiment C2 (a) was mostly a failure and these results obtained were sometimes at variance with the second test. Unless results are reproducible it is obvious that a true comparison between two experiments cannot be satisfactory.

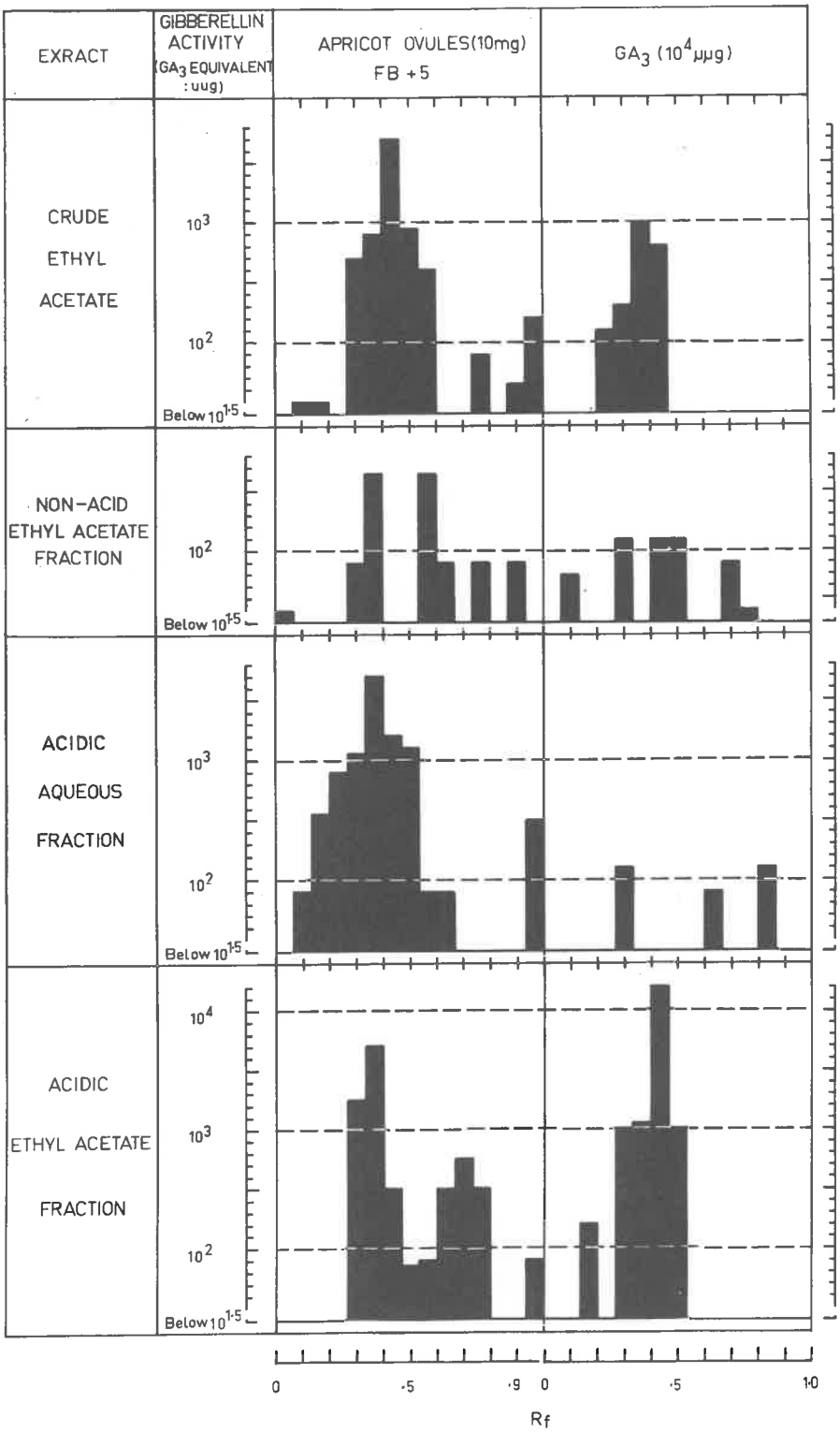
Experiment C2 (a) will clearly have to be repeated at some future date before the findings can be related to those from crude extracts.

(b) Activity of ethyl acetate extracts after partitioning and chromatography

Fractions S1, S2, S3, and S4 of seed five days after full-bloom were prepared by partitioning, chromatographed, and tested for gibberellin activity. Another test was conducted to show the behaviour of 10^4 µµg GA_3 under partitioning and chromatography, and results from both tests are shown as histograms in Fig. 13.

Contrary to results of experiment C2(a), fraction S2 has not retained any appreciable activity. This and the results in the first test in Experiment C2(a), shown in brackets in Table 13, suggest the high value for S2 obtained in the second test may have been erroneous. Unlike GA_3, the activity from the extracts partitions equally between S3 and S4. Without further experimentation it is impossible to tell whether this is due to the presence of two compounds in the extract, or to incomplete recovery of one compound by ethyl acetate. Had the aqueous fraction been partitioned again with ethyl acetate, leading to a similar distribution of activity, the presence of one compound would have been inferred. Both fractions, however, had activity which ran at an almost identical Rf and

Fig. 13.- Gibberellin activity from an ethyl acetate extract of apricot seeds, 5 days after full-bloom, and from gibberellic acid (GA_3) after partitioning and chromatography. Ordinates on log scale.


EXRACT
GIBBERELLIN ACTIVITY (GA_3 EQUIVALENT : uug)
APRICOT OVULES(10mg) FB +5
GA_3 (10^4 µµg)
CRUDE ETHYL ACETATE
NON-ACID ETHYL ACETATE FRACTION
ACIDIC AQUEOUS FRACTION
ACIDIC ETHYL ACETATE FRACTION
10^4
10^3
10^2
Below $10^{1.5}$
0
·5
·9
1·0
Rf

this favours the interpretation that only one compound is involved. This gibberellin may be difficult to extract into ethyl acetate from an aqueous solution because of the presence of several hydroxyl groups in the molecule. Its low Rf shows that it is more polar than GA_3; this may be due to its content of more than two hydroxyls, the number present in GA_3.

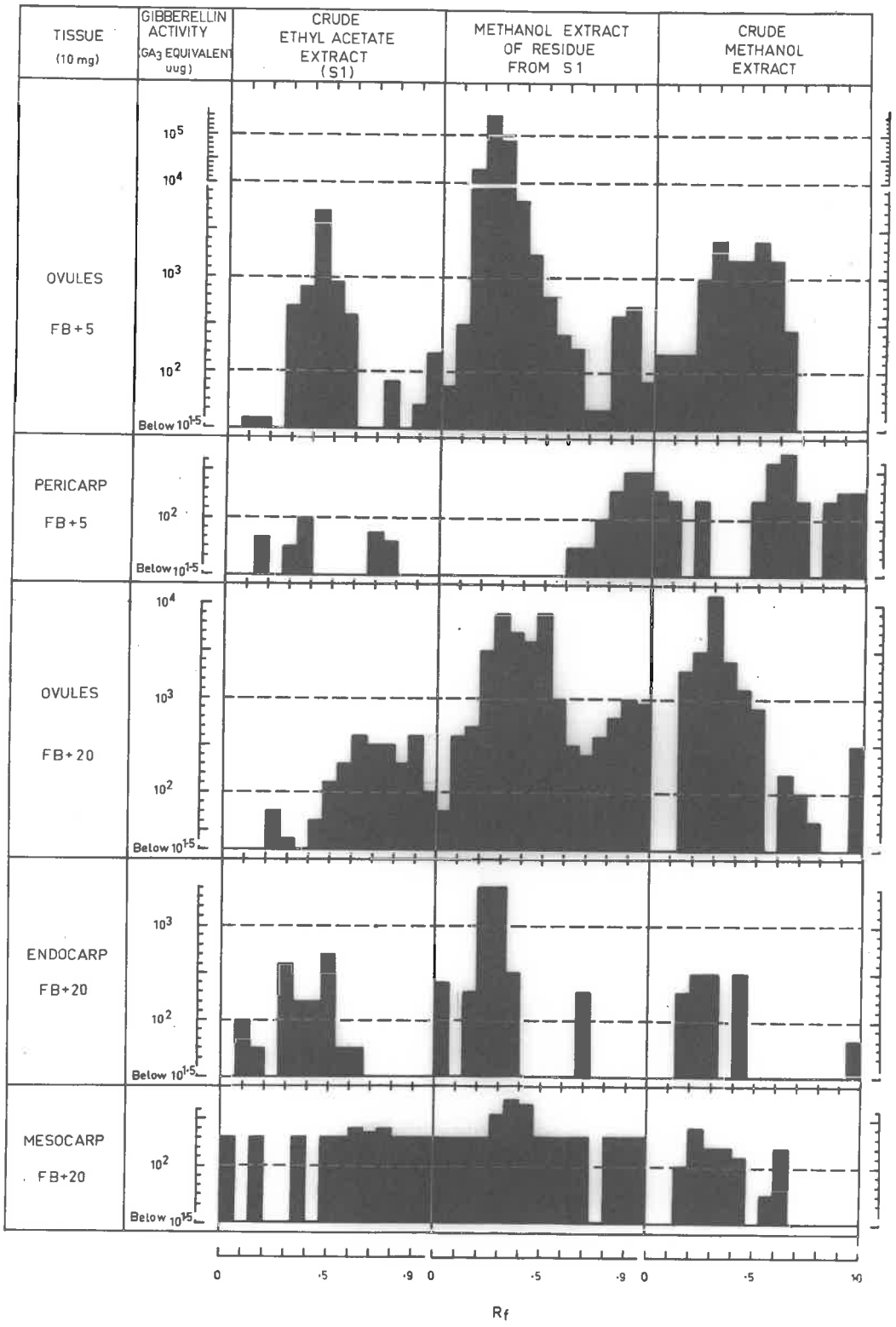
GA_3, unpartitioned, has a lower activity than expected. Unlike Experiment C8(c)(vii) there is no definate difference in Rf between seed gibberellin and GA_3.

Included in this test were three chromatogram strips equiliberated and run in the normal way. The first was unspotted, the second and third were spotted with the residue after evaporation of 30 ml ethyl acetate and 30 ml methanol respectively. None showed activity.

(c) Activity of ethyl acetate and methanol extracts after chromatography

Seed and pericarp five days after anthesis and seed , endocarp, and mesocarp twenty days after anthesis were extracted as follows. Crude ethyl acetate and methanol extracts, each of 10 mg tissue, were prepared and chromatographed. The crude ethyl acetate extract of seed at five days, having been previously tested (Fig. 13), was not repeated. Results are shown in Fig. 14. Most activity is recovered from the seeds, and methanol has proved itself the better extractant for this tissue than ethyl acetate. Rf values are somewhat erratic when the activity is

Fig. 14.- Gibberellin activity from chromatographs of ethyl acetate and methanol extracts of apricot tissue, 5 and 15 days after anthesis. Ordinates on log scale.


TISSUE
(10 mg)
GIBBERELLIN ACTIVITY
GA3 EQUIVALENT
uug)
CRUDE ETHYL ACETATE EXTRACT (S1)
METHANOL EXTRACT OF RESIDUE FROM S1
CRUDE METHANOL EXTRACT
OVULES
FB+5
PERICARP
FB+5
OVULES
FB+20
ENDOCARP
FB+20
MESOCARP
FB+20
Below 10^1·5
0
·5
·9
Rf

below 10^3 µµg GA_3 equivalent. Above 10^3 µµg GA_3 peaks occur between the Rf values 0.27 and 0.43, the mean being 0.34 which is higher than the mean in Experiment C8 (c) (vii).

(d) Activity in methanol extract recovered by ethyl acetate

To test whether native sugars in the extract were contributing to the response in the barley endosperm test, seed at 20 days was extracted with ethyl acetate and methanol. Both were chromatographed and subjected to the barley endosperm test without the addition of endosperm pieces. No activity was recorded.

Seed at 20 days (10 mg) was extracted with ethyl acetate and after evaporation re-extracted with methanol. These two extracts were chromatographed and tested for gibberellin activity. A further 10 mg was identically extracted and the methanol extract (SM) was evaporated and extracted with ethyl acetate. This extract was chromatographed and tested for activity. Results are shown in Fig. 15. It is apparent that once the activity has been taken from the tissue with methanol, ethyl acetate will recover at least the majority of this activity. Ethyl acetate is obviously unreliable as an extractant for apricot tissue (see Figs. 12, 14, 15 and Table 13).

3. The dwarf corn test - a comparison with the barley endosperm test

Three 33 mg samples of apricot seeds, 15 days after

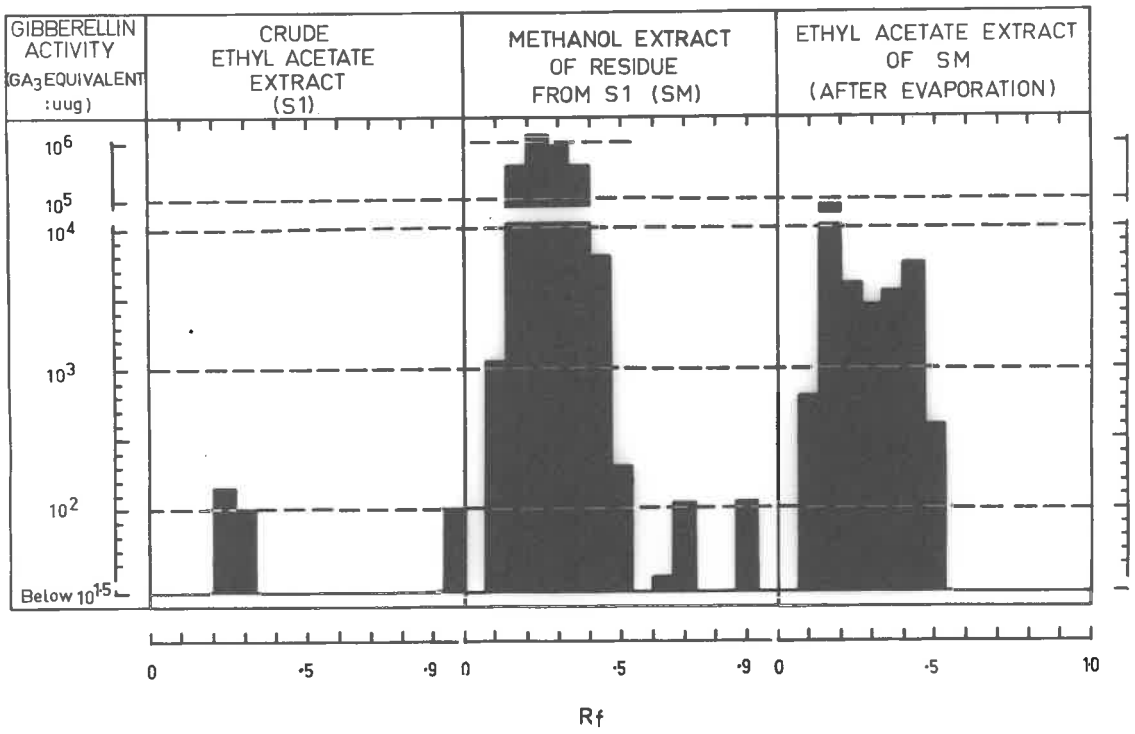


Fig. 15.- Gibberellin activity, as revealed by three extraction methods and chromatography, in apricot seeds (10mg) 20 days after anthesis. Ordinates on log scale.

anthesis, were extracted with ethyl acetate and the residue re-extracted with methanol. The volumes of the six extracts were reduced and spotted on chromatograms. After developing, chromatograms were cut into six sections covering the Rf values 0 - 0.15, 0.15 - 0.25, 0.25 - 0.35, 0.35 - 0.50, 0.50 - 0.70, and 0.70 - 1.00; each section was shaken for two hours with 4 ml ethyl acetate or methanol. Extracts were evaporated to dryness and activity taken up by shaking in 0.4 ml water for a further two hours. Three d - 5 dwarf corn seedlings were treated with 0.1 ml of this solution; thus from three chromatograms nine plants were treated, at each Rf value, with 8.25 mg tissue. 8.25 mg of tissue was extracted with ethyl acetate followed by methanol, chromatographed and subjected to the barley endosperm test. The results and the respective standard curves are shown in Fig. 16.

Methanol extracts in both tests have similar activity, and this runs at the same Rf. The dwarf corn test apparently is not sensitive enough to show activity in ethyl acetate extracts.

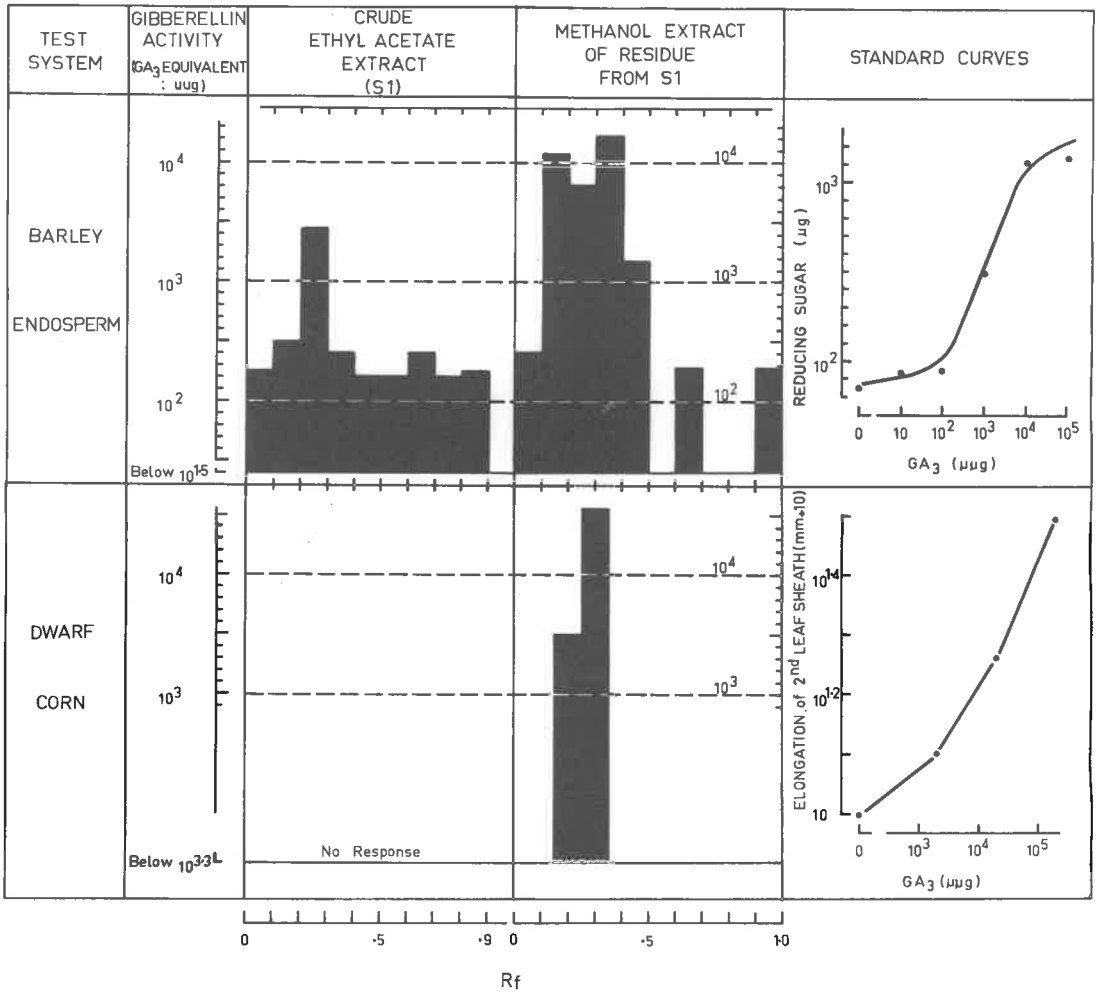


Fig. 16.- Gibberellin activity, as revealed by two test systems, in apricot seeds (8.25mg) 15 days after anthesis.
Ordinates on log scale.

VIII. DISCUSSION

A. Morphological Changes during Development of the Apricot Fruit

This examination of growth of Moorpark apricot fruit in Adelaide supports, in general, the sequence shown in seed and fruit growth of many stone fruit (eg Idilleland 1930; Ragland 1934; Tukey 1933, 1934; Tukey and Young 1939). The seed shows the usual developmental sequence of nucellus and integuments, followed by endosperm, then embryo (Maheshwari 1950). The endosperm was unusual in that it was 'digested' by the embryo by the time it had attained only half the volume within the integuments (Fig. 5). The shape of the fruit was shown to change from oval (each of the three right-angle sections a, b, and c, being oval, see Fig. 1A) near full-bloom, to an almost spherical structure at maturity (Tables 9 and 10). The whole fruit showed the well-proven double-sigmoid curve (Fig. 12).

Growth of the mesocarp was a product of cell expansion from anthesis onwards and cell division till approximately 15 days after anthesis. It was therefore similar in this respect to most cultivated fruit varieties (Crane 1964). The difference between cells close to either the epidermis or the endocarp at maturity (Fig. 11) was shown to be one of shape rather than volume. Similar differences in shape were shown by Sterling

(1953) for Prunus domestica. They are no doubt due to the fact that cell division ceases long before the fruit stops expanding. To accomodate size increase in such a fruit, constructed initially of equally-sized cells, cells near the circumference would mainly expand tangentially while those nearer the centre would tend to expand radially.

B. Factors affecting the Growth of Apricot Fruit

1. Within- and between-tree factors

In fruit, which are genetically identical, differences in size or rate of growth must be due to factors, occuring within or between trees, which favour the growth of some fruit at the expense of others. These experiments showed that trees differed significantly in fruit size at pit-hardening and at maturity, and in rate of growth between these stages. Fruits within trees showed considerable variation in size at any stage of growth.

Since fruit size at pit-hardening was correlated significantly with mesocarp volume at this stage, and with fruit size at maturity (both with P below 0.001), differences in fruit size can be related to the mesocarp components, cell size and cell number. (Intercellular spaces were not observed in the apricot, and the stone does not expand after the commencement of pit-

hardening.)

Martin and Lewis (1952) showed that differences in fruit size between similar apple trees bearing either light or heavy crops were not related to cell number, and, on this evidence, concluded that differences were due to cell size. The present investigations suggest that both cell number and cell size contribute to between-tree size differences, but that in fruit from early and late flowers opposing mechanisms are operative. Thus, on the one hand, there was a very definite correlation between fruit size and cell volume in "early" fruit, although the same correlation in "late" fruit was not significant, while, on the other hand, cell numbers correlated with fruit size in "late", but not "early" fruit (see page 43). To the author's knowledge no other workers have reported such differences and it is felt that confirmatory evidence is needed before a puzzling result such as this can be discussed with confidence. The present discussion will therefore be limited to the overall within- and between-tree effects.

These experiments confirmed the results of Bain and Robertson (1951) for apples and Bradley (1959) for peaches that variation in fruit size within a tree is mainly a function of cell number.

The present experiments proved that a significant positive correlation existed in four trees individually, and over these four trees collectively, between size of fruit at pit-hardening and size at maturity. There was a similar but less significant correlation between this early size and subsequent growth rate and

earliness of ripening. A relationship between the size of peaches at early pit-hardening and that at maturity has been shown to exist when the data of several of several seasons are correlated (e.g. Davis and Davis 1948; Proebsting 1962). Lilleland (1930) was unable to demonstrate a similar relationship with apricots; his results did suggest, however, that in the same season a tree with larger fruit at pit-hardening had larger fruit at maturity than a similar tree with smaller fruit at pit-hardening.

Correlations of fruit growth with fruit number, leaf/fruit ratio etc. (Experiment B1) are not considered definite enough to discuss in relation to within- and between-tree differences. Denne (1963) however, was able to show that such factors as the position of the fruit on the spur, the position of the spur on the branch, etc., did influence apple fruit growth. Martin et al. (1964), concluding experiments on natural and induced variation in cell numbers of the Jonathan apple, suggest that the major factors controlling cell division are reserves available from the previous season. Reserves are probably mainly carbohydrates, but may, in addition, contain minerals, hormones, and other organic constituents. Although not proven in apricots, indirect evidence for the importance of reserves has been gained by Martin et al. (1964) and by other workers (see Review of Literature) who have shown that thinning at or near full-bloom will increase cell numbers, presumably by reducing competition for reserves. The results of Abbott (1960), investigating the relationship between growth and the bourse shoot in apples, suggested that competition between leaves and fruit reduced

early fruit growth and limited cell division; later the presence of leaves stimulated fruit growth, chiefly by increasing cell size.

In accordance with the above speculations two factor groups can be suggested as affecting growth: (a) position of the fruit in relation to reserves, and the degree of competition from developing leaves for these reserves, (b) leaf/fruit ratio, nutrition and water availability. It is suggested that, within a tree, movement of nutrients, both organic and inorganic, is greater later in the season than early, so that factors of group (b) would be distributed more evenly to all fruit than factors of group (a). This might suggest why between-tree differences are more affected by cell size than within-tree differences. The results would further suggest that position of fruit in relation to reserves varies more within trees than between trees.

2. Effect of temperature on apricot fruit growth

The effect of flowering date on apricot growth was explained by considering the different temperature conditions under which fruit grew. Thus, in 1961, when the conditions during development in the first few weeks after anthesis were much warmer for fruit from late flowers than from early flowers, the former grew at a more rapid rate (Fig. 6) and ripened in fewer days.

The major experiments on temperature effect upon fruit

growth considered three temperature regimes, with and without gibberellic acid, upon fruit growth, cell enlargement, and cell division. Except in effect upon cell size, there was no real evidence for interaction between GA_3 and temperature; neither was there any observable effect of temperature (or GA_3) on endogenous gibberellins. It is possible therefore to consider temperature effects, for the most part, independently from gibberellic acid effects.

Several workers (Tufts 1929; Lilleland 1935; Tukey 1952, 1956, 1958, 1960) have shown that temperature affects the growth of fruit, but none have demonstrated what effect this has had on the growth of cells. Under the experimental conditions of this work, the effect of increasing night temperature has been to compress the whole process of growth into a fewer number of days. Thus fruit grew more rapidly and matured earlier (Fig. 9) and the change in fruit shape from approximately ovoid to spherical was hastened by high temperatures (Tables 9 and 10). No evidence that temperature altered final size or shape was gained. The unequal growth in radial diameter of cells close to the endocarp and skin respectively was accentuated early in the season, but no effect on final cell size or shape was found (Fig. 11). The rate of cell division was increased but the final cell number was the same (Fig. 10). Rather similar results were obtained by Humphries and Wheeler (1964) with leaves of Phaseolus vulgaris; growth and cell division were stimulated but final leaf size and cell number were not affected.

3. Effects of gibberellic acid on apricot fruit growth

Although auxins, kinins, and gibberellic acid were tested for their effect upon apricots, only the latter effectively altered fruit growth.

Plate 3A shows the general effect of gibberellic acid applied pre-bloom in elongating the pedicels of apricot flowers, and this is shown in longitudinal section in Plate 3B. Also shown in plate 3A, is an example typical of very late flowers (untreated) showing the elongated pedicel and modified flower parts. One wonders whether this elongated pedicel is due to a greater availability of endogenous gibberellins at this time. In Plate 3B, the elongated pedicel of GA_3-treated apricots is compared with a flower of Prunus cerasifera (cherry plum). It is interesting to note that all cases of parthenocarpically-induced growth in Prunus by GA_3, have been in species with nearly-sessile fruits, eg almond, apricot, and peach (Crane et al. 1960). Plum has not responded to GA3 and cherry has done so only in the presence of an auxin (Rebeis and Crane 1961). The length of the pedicel may perhaps be an indication of the gibberellin status in the fruit. Lack of parthenocarpic response to GA_3 shown by species with long pedicels could be related to their already-high gibberellin content, perhaps inferring a different hormonal mechanism controlling fruit growth.

Gibberellic acid applied to apricots before blossoming induced elongated vegetative growth, tended to hasten leaf and shoot production in the spring, and caused many lateral shoots

to develop into spines. These are not uncommon manifestations of GA_3 treatment and have been shown to occur with other fruit species (see Review of Literature). One result which conflicts with that of other workers is the effect of GA_3 on bud production. Bradley and Crane (1960) sprayed apricot trees at full-bloom, and one week later, with GA_3 at 50 and 250 p.p.m. The lower concentration inhibited flower bud production, while the higher inhibited both flower- and leaf-bud initiation. Marcelle and Sironval (1962) found that in an apple tree GA_3 reduced percent flower buds in relation to vegetative buds, but increased the total number of flower buds. Guttridge (1962), also with apple, showed that on single branches vegetative spurs were increased and flowering spurs were reduced in number. The above results and those of Hull and Lewis (1959) with cherry, peach, and apple, indicate a general depressive effect of GA_3 on flower bud initiation. By contrast, in the present experiments, the proportion of flower buds to leaf buds was increased, especially towards the tip of actively-growing branches (Plate 4), and, although no counts were made during the dormant season, or at flowering the following season, it seemed probable that the total production of flower buds was increased.

Several conclusions can be made on the effects of GA_3 on flower-bud drop and upon fruit growth. Gibberellic acid, whether applied early, in the middle, or late in winter, tends to cause bud drop before anthesis, and fruit drop after this, roughly in direct proportion to the concentration applied (Table 5, and

compare Table 7). The general effect of GA_3 injection is to increase early growth rate of fruit but to depress it subsequently (Fig. 7). The extent of this is dependent on the date of application; June injection increases early growth more, and size remains greater for longer, than April or August treatments (Table 6). It was not determined whether this was reflected in differences in cell size, or cell number, or both, but the general result of GA_3 application is to reduce growth by its effect on cell number rather than size (cf. Figs. 10 and 11). The final major effect of GA_3 was on fruit shape: GA_3-treated fruits were longer and possibly somewhat more laterally (dimension a, Fig. 1A) flattened than control in the early part of the season, but this was lost by maturity (Tables 8 and 9).

C. Endogenous Gibberellin in Apricot Fruit

1. Partitioning and chromatography of apricot gibberellins

The single peak of gibberellin activity in extracts of apricot tissue found in two test systems (the barley endosperm test and the dwarf-corn test) suggested the presence of only one gibberellin with an Rf in isopropanol:ammonia:water (10:1:1) of 0.28. Macmillan (1964) has summarised the Rf values on paper in four different solvent systems of the then-known gibberellins (A_1 - A_9). In a similar system to the present one no gibberellin

runs at such a low Rf , though GA_8 has a mobility, relative to GA_3, of 70%, which is similar to the apricot gibberellin. Paleg et al. (1964), however, found GA_8 was probably inactive in the barley endosperm test, and it seems unlikely that this is the gibberellin present in apricot fruit.

The results of partitioning when compared with results of other workers using similar extraction procedures (e.g. Wheeler 1960; Radley 1958, 1959; Hayashi and Rappaport 1962) do not greatly assist in the identification of the compound in apricot. It has been concluded from these results and those of chromatography, that the gibberellin activity in apricot fruit is probably due to one compound, which is not basic, and is more polar than gibberellic acid (Experiment C2(b)).

While the Rf of peak activity in most tissues tested did not differ significantly from the mean of 0.28, pericarp at five days after anthesis, with a mean Rf value of 0.33, was significantly greater (P below 0.001) (Experiment 8 (c) (vii)). This, coupled with a marked increase in total activity of this tissue from chromatographed extracts (Table 11) compared with crude extracts(Fig. 12), and the suggested increase after partitioning (Table 13), might indicate that the properties or the type of gibberellin in the pericarp might vary with time. This is perhaps supported by the fact that absolute values for seed in Table 11 and Fig. 12 are similar, but pericarp gibberellin drops between 5 and 15 days in chromatographed extracts, while in crude extracts

it shows a big increase.

The presence of inhibitors was investigated, but not confirmed, in crude extracts (Experiment C 1), and no other experiments were conducted to test the possibility that inhibitors, impurities, or synergism could be responsible for the above differences. These differences, although interesting, need to be substantiated by further research into changes of the purified extracts with time and tissue, and by a more comprehensive survey of inhibitors etc. in the tissue. Results with crude extracts will therefore be examined assuming, in the absence of adequate evidence to the contrary, that values there approximate to actual levels in the tissues.

2. Changes in concentration in relation to fruit growth

This work is unusual in obtaining a fairly good correlation between hormone level and growth rate; possibly for the reason that, whereas most workers have correlated hormone content of the whole fruit, or one part (eg. seeds), with growth as a whole, tissues here have been related to their individual hormone content. Thus in Fig. 12 it can be seen that the level of gibberellin in the seed remains high over the period in which nucellus, endosperm, and embryo are rapidly expanding. The peak of activity occurs when the nucellus expands at its most rapid rate and endosperm becomes cellular at the beginning of its period of rapid growth. Endocarp gibberellin reaches its peak during the maximum growth rate of this tissue (which is equivalent to that of

the mesocarp in Stage I - Fig. 12) but drops suddenly at the beginning of pit-hardening. The level in the mesocarp reaches a peak at the same time, but remains high throughout growth of the apricot. Apart from there being no apparent positive relationship between mesocarp gibberellin and Stage II and III of growth, the overall impression is that, whenever growth is active in any tissue, gibberellin level is high in that tissue.

Although a good correlation has been obtained it should not be assumed that the level of gibberellin has a causal relationship with growth; it could conceivably be a by-product of growth and have no causal role whatsoever. This difficulty in interpretation is a major problem of hormone research and some reference to results and conclusions of other workers, although they concern a different tissue and hormone might be profitable.

In 1937 Went and Thimann showed that auxin diffusing from the tip of Avena coleoptiles exhibited quite a good correlation with coleoptile growth; they therefore concluded that auxin produced in the tip was responsible for growth. Later, Went (1942) found growth rate of individual segments from decapitated coleoptiles correlated better with ether-extractable auxin than diffusible auxin from that segment; it was suggested that since the normal supply of diffusible auxin was removed, extractable auxin was a better measure of hormone physiologically active in the cell. Dattary and Mer (1964), who extracted the whole coleoptile with ether and related this to growth, found no definite correlation; they postulated that extractable auxin

is a measure of the difference between rate of synthesis and the rate of consumption, and, as such, is not necessarily related to growth. It is suggested here that Dattamy and Mer's proposition would apply when the tissue extracted contains both the site of production and the site of consumption; by separating these sites Went and Thimann (1937) and Went (1942) probably overcame this problem and therefore obtained a better correlation.

In apricots no investigation of diffusible gibberellin was made, and since all tissues contained high extractable gibberellin at one stage or another, no indication that one tissue might be the site of production and another the site of utilisation was gained. Thus, although seed, which is often thought to supply auxin for growth in other regions of the fruit (see Crane 1964), contained more gibberellin than any other tissue in Stage I, gibberellin in the mesocarp remained high after the level in the seed had been reduced to a low level. It is felt that each tissue, at least after pollination, manufactures its own gibberellin, and if production exceeds utilisation a surplus exists which is largely responsible for the level of hormone measured as extractable gibberellin. The possible consequences of such an idea will be discussed below.

There are several reports of GA_3 increasing diffusible auxin in plants (e.g. Kuraishi and Muir 1962, 1963), and it is possible that one mechanism whereby gibberellin exerts its influence on growth is via auxin. Whereas untreated and unpollinated plants contained no diffusible auxin, Sastry and

Muir (1963) showed that pollination, or the application of GA_3 to tomatoes at anthesis, stimulated the production of diffusible auxin. Since pollen was shown to contain high levels of gibberellin, it may be suggested that, apart from fertilisation, the effects of pollination and GA_3 application are equivalent in initiating auxin production via gibberellin. In other words the stimulation of auxin production by an external supply of gibberellin is the prerequisite for fruit growth following pollination. An auxin applied to the fruit may perhaps obviate the need for pollination or GA_3 application if it can successfully replace the natural auxin. This hypothesis may explain why GA_3 and auxin are sometimes equally effective in initiating parthenocarpy (see Review of Literature). It is further suggested that the applied hormone (auxin or gibberellin), which most closely resembles the naturally-occurring compound is the most successful in producing parthenocarpy.

Since prevention of pollination will normally inhibit fruit growth, most workers have concluded that hormone supplied by the seed has a causal effect on growth of the other fruit tissues; yet a hormone will generally induce parthenocarpy when applied at or near full-bloom only (Crane 1964) and indeed, in the apple, seeds may be removed later in the season with no adverse effects on growth (Abbott 1959; Southwick et al. 1962). It is suggested here that after pollination tissues are self-sufficient in growth hormones, and, in particular, the level of gibberellin extracted from apricots may, as was previously postulated, be a measure

of excess and have other physiological functions. A clue to the action of gibberellin resulting from this hypothesis may be found in the effect of GA_3 on growth in seeded fruit. It will be remembered that, at full-bloom, GA_3 stimulated growth in pollinated apricots but later depressed it (Figs. 7, 9), and similar effects were noted for other fruits in the Review of Literature. Comparative levels of auxin and gibberellin were studied in seedless and seeded grapes by Coombe (1960). Gibberellin activity was found in seedless but not seeded grapes, seeded grapes, however, contained a higher concentration of auxin which appeared to parallel meristematic activity in the seed. Prosser and Jackson (1959) found auxin activity only in pollinated fruit of Rosa arvensis; those produced parthenocarpically with GA_3 had no auxin, yet their growth pattern was similar. It might be suggested that once auxin production has been stimulated by pollination, high gibberellin levels may become inhibitory.

Confirmatory evidence for the above hypothesis is available from tissue-culture work. Bergmann (1958) showed that when auxin levels were depleted, GA_3 stimulated callus production, while when auxin levels were high, GA_3 acted in the reverse way. Other workers have shown that low levels of gibberellic acid will stimulate and high levels depress growth in tissue culture (Schroeder and Spector 1957; Straus and Epp 1960; Nickell and Tulecke 1961). It is perhaps surprising that GA_3 applied four days before anthesis did not affect endogenous gibberellin 5

and 15 days after anthesis (Experiment 8(c)(vii)) but still affected growth (Fig. 9). If, however, the GA_3 was utilised for auxin production at full-bloom, a higher level of auxin, together with the increasing endogenous gibberellin level in the fruit, could have a limiting effect upon growth.

The obvious sequel to the above speculations is that the high levels of extractable gibberellins, found at the periods of most rapid growth in each tissue, are responsible for the reduction or cessation of growth shortly afterwards. That applied GA_3 reduces cell numbers (Fig. 10) but not cell size (Fig. 11), might suggest that cell division is more sensitive to high gibberellin levels than cell expansion, and therefore, in a normal fruit, cell division would be arrested before gibberellin levels were sufficiently high to reduce cell expansion. If the slow growth phase of the mesocarp (Stage II) is due to its high gibberellin content, it is possible that the gradual lowering of gibberellin over this stage may enable the mesocarp to enter its final flush of growth; seed and endocarp are probably prevented from further growth by the mechanical restriction of the stony endocarp.

The effect of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in increasing growth over Stage II for apricots, and thereby hastening maturity and increasing size (Crane and Brooks 1952), might be cited as evidence against the above scheme; yet the increase in growth induced by 2,4,5-T, seems to be related to a much higher degree of polyploidy in the mesocarp cells

of treated fruit than normal fruit (Bradley and Crane 1955). This may indicate that a mechanism of growth has been induced by 2,4,5-T which is different to that normally operative within the fruit. If so the objection may not be a very serious one, though clearly more research on this and other aspects will be needed before the above hypothesis of gibberellin action can be regarded as unequivocal.

Gibberellin has been discussed in the role of growth regulator, rather than as a growth stimulant. There is no evidence, however, that gibberellin always acts this way, or only stimulates growth via its effect upon auxin; it may itself be required as a stimulant, although, if the location of the site of synthesis cannot be differentiated from the site of action, this will be difficult to prove. The extent of this work has necessarily been limited, and the possible role of other hormones or of inhibitors has been omitted, or inferred from reports of other workers with different fruit. No one satisfactory system has been found for all fruit, and the above scheme has been suggested as one possible method whereby gibberellin might exert its control over growth of one fruit, the apricot.

D. Conclusions

This project has investigated some of the changes which take place during the growth of apricot fruit, and has attempted to relate these to factors which were shown to influence growth.

Fruit were shown to vary in size and in the number and volume of cells within the mesocarp both within and between trees. Size variation within a tree depends more on differences in cell number than cell volume, and it is suggested that this is due to the restricted movement of reserves in the tree at blossom time and the competition for these reserves from other flowers and developing leaves. Later, when leaves are no longer competing and there is a greater movement of solutes within the tree, differences are likely to be due to factors such as leaf/fruit ratio and water or nutrient availability. These probably vary more between than within trees, and, since cell division ceases within two weeks of full-bloom, may account for the relatively greater contribution of cell size to between-tree differences than within-tree differences. Relative fruit sizes at maturity were probably determined early in the season, since size at the beginning of pit-hardening was positively correlated with size at maturity.

It was possible to influence growth by modifying the night temperature in which the fruit grew. Higher temperature increased early growth of the fruit by increasing rate of mesocarp cell division and cell expansion; it also hastened fruit maturity. Since the final size and cell number were similar at maturity in all temperature treatments, it is concluded that high temperatures merely compress the growth period into a shorter number of days. Gibberellic acid applied to apricots also increased the early growth rate and hastened maturity of the fruit. One might therefore hypothesize that the effects of GA_3 and temperature on fruit

growth are connected; perhaps increased temperature acts via increased gibberellin. Gibberellic acid, however, reduced cell number and cell size, and, furthermore, neither GA_3 nor temperature affected endogenous gibberellin; on both counts therefore, this hypothesis seems untenable.

Several interesting aspects of gibberellic acid application were discovered, but further research will be needed to understand their relationship to growth phenomena. Thus GA_3 applied mid-winter gave more pronounced effects on fruit growth than the same concentration applied early or late in winter. Gibberellic acid initiated a higher proportion of flower buds in the season following application, an effect which is contrary to most reported work involving GA_3. Treated fruit tended to have elongated pedicels. It is noted that a connection seems to exist between length of the pedicel in Prunus species and its likelihood to grow parthenocarpically after GA_3 treatment.

Investigations into endogenous gibberellin in apricots suggest the presence of only one active compound; this is non-basic and more polar than gibberellic acid, but it could not be identified as any known gibberellin. Levels of gibberellin from crude extracts of seed, endocarp, and mesocarp during growth from anthesis to maturity were discussed in relation to growth of these tissues. The amount of gibberellin in partially-purified extracts did not always agree with that in crude extracts; unfortunately insufficient data was available to decide what significance should be attached to this finding. More work on

this aspect is required, particularly to discover what effect different purification methods have on the recorded level of gibberellin over the growth period.

In spite of these uncertainties, this work is unusual in obtaining a good positive relationship between growth and an endogenous level of hormone in a fruit. A theory is propounded which, it is felt, accounts for several aspects of gibberellin in fruit, recorded both here and elsewhere. Gibberellin estimated from extracted tissue is considered a measure of that which is surplus to normal requirements for growth in the particular tissue. After the stimulation of pollination, or GA_3 application, gibberellin is probably manufactured by the tissues themselves, where, in addition to supplying their own needs for further growth, it may act as a regulator of other functions. Thus high levels are postulated as being inhibitory to growth, and may be responsible for the plateau in Stage II; lower levels may act by stimulating production of diffusible auxin, especially in seedless fruit. The hypothesis is tendered that pollination itself stimulates ovary growth by the effects of pollen gibberellin initiating auxin production.

IX. REFERENCES

Abbott, D. L. 1959. The effects of seed removal on the growth of apple fruitlets. Ann. Rep. Long Ashton Res. Sta. for 1958: 52-56.

Abbott, D. L. 1960. The bourse shoot as a factor in the growth of apple fruits. Ann. Appl. Biol. 48: 434-438.

Addoms, R. M., Nightingale, G. T., and Blake, M. A. 1930. Development and ripening of peaches as correlated with physical characteristics, chemical composition and histological structure of the fresh fruit: II Histology and microchemistry. N. J. Agr. Exp. Sta. Bul. 507.

Anon. 1958. Fruit investigations. Rep. CSIRO for 1957-1958: 28-29.

Bain, J. M. and Robertson, R. N. 1951. The physiology of growth in apple fruits. I. Cell size, cell number, and fruit development. Aust. J. Sci. Res. 4: 75-91.

Bergmann, L. 1958. Der Einfluss von Gibberellin auf das Wachstum von Gewebekulturen des Callus von Daucas carota. Planta. 51: 70-73.

Bradley, M. V. 1959. Mean cell size in the mesocarp of mature peaches of different sizes. Proc. Amer. Soc. Hort. Sci. 73: 120-124.

Bradley, M. V. and Crane, J. C. 1955. The effect of 2,4,5-trichlorophenoxyacetic acid on cell and nuclear size and endopolyploidy in parenchyma of apricot fruits. Amer. J. Bot. 42: 273-281.

Bradley, M. V. and Crane, J. C. 1960. Gibberellin-induced inhibition of bud development in some species of

Prunus. Science. 131: 825-826.

Carlone, R. 1962. The effect of gibberellic acid and 2,4,5-trichlorophenoxyacetic acid on the growth of J. H. Hale peach fruits. Fruttiqultura. 24: 267-273.

Coggins, C. W., Hield, H. Z., and Garber, M. J. 1960. The influence of potassium gibberellate on Valencia orange trees and fruit. Proc. Amer. Soc. Hort. Sci. 76: 193-198.

Coombe, B. G. 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of Vitis vinifera. Plant Physiology. 35: 241-250.

Cornwall, J. B. and Davison, R. M. 1960. Rapid counter for small particles in suspension. J. Sci. Instruments. 37: 414-417.

Crane, J. C. 1964. Growth substances in fruit setting and development. Ann. Rev. Plant Physiol. 15: 303-326.

Crane, J. C. and Brooks, R. M. 1932. Growth of apricot fruits as influenced by 2,4,5-trichlorophenoxyacetic acid application. Proc. Amer. Soc. Hort. Sci. 59: 218-224.

Dattaray, P. and Mer, C. L. 1964. Auxin metabolism and the growth of etiolated oat seedlings. Proc. Regulateurs Naturels de la Croissance Vegetale. Gif s/Yvette 1963 : 475-488.

Davis, L. D. and Davis, M. M. 1948. Size in canning peaches. The relation between the diameter of cling peaches early

in the season and at harvest. Proc. Amer. Soc. Hort. Sci. 51: 225-230.

Denne, M.P. 1960. The growth of apple fruitlets, and the effect of early thinning on fruit development. Ann. Bot., Lond. 24: 397-406.

Denne, M. P. 1961. Observations on cell size in apples. Rep. E. Malling Res. Sta. for 1960: 120-122.

Denne, M. P. 1963. Fruit development and some factors affecting it. N. Z. J. Bot. 1: 265-294.

Frankland, B. and Wareing, P. F. 1962. Changes in endogenous gibberellins in relation to chilling of dormant seeds. Nature. 194: 313-314.

Friend, D. J. C. 1961. A simple method of measuring integrated light values in the field. Ecology. 42: 577-580

Griggs, W. H. and Iwakiri, B. T. 1961. Effects of gibberellin and 2,4,5-trichlorophenoxypropionic acid sprays on Bartlett pear trees. Proc. Amer Soc. Hort. Sci. 77: 73-89.

Guttridge, C. G. 1962. Inhibition of fruit-bud formation in apple with gibberellic acid. Nature. 196: 1008.

Havis, A. L. 1962. Effects of time of fruit thinning of Redhaven peaches. Proc Amer. Soc. Hort. Sci. 80: 172-176.

Hayashi, F. and Rappeport, L. 1962. Gibberellin-like activity of neutral and acidic substances in the potato tuber. Nature. 195: 617-618.

Houghtaling, H. B. 1935. A developmental analysis of size and

shape in tomato fruits. Bull. Torrey Bot. Club. 62: 243-252.

Hull, J. and Lewis, L. N. 1959. Response of one-year-old cherry and mature bearing cherry, peach and apple trees to gibberellin. Proc. Amer. Soc. Hort. Sci. 74: 93-100.

Humphries, E. C. and Wheeler, A. W. 1964. Cell division and growth substances in leaves. Proc. Regulateurs Naturels de la Croissance Vegetale. Gif s/Yvette 1963: 507-515.

Johansen, D. A. 1940. Plant Microtechnique. (McGraw-Hill, London).

Jones, D. F. 1964. Examination of the gibberellins of Zea mays and Phaseolus multiflorus using thin-layer chromatography. Nature. 202: 1309-1310.

Kuraishi, S. and Muir, R. M. 1962. Increase in diffusible auxin after treatment with gibberellin. Science. 137: 760-761.

Kuraishi, S. and Muir, R. M. 1963. Diffusible auxin increase in a rosette plant treated with gibberellin. Naturwissenschaften. 50: 337-338.

Lavee, S. 1960. Effect of gibberellic acid on seedol grapes. Nature. 185: 395.

Letham, D. S. 1960. The separation of plant cells with ethylenediaminetetraacetic acid. Exp. Cell Res. 21: 353-360.

Letham, D. S. 1962. Separation of plant cells with hexametaphosphate and the nature of intercellular bonding. Exp. Cell Res. 27: 352-355.

Lilleland, O. 1930. Growth study of the apricot fruit. Proc. Amer. Soc. Hort. Sci. 27: 237-245.

Lilleland, O. 1935. Growth study of the apricot fruit II: the effect of temperature. Proc. Amer. Soc. Hort. Sci. 33: 269-279.

Luckwill, L. C. 1957. Hormonal aspects of fruit development in higher plants. Symposia of the Society for Experimental Biology. No. XI : 63-85.

McCombs, C. L. 1955. An anatomical study of the developing tomato fruit following application of certain chemicals to the ovulary. Diss. Abstr. 16: 640-641.

MacDaniels, L. H. 1940. The morphology of the apple and other pome fruits. Mem. Cornell Agric. Exp. Sta. 230.

MacMillan, J., Seaton, J. C., and Suter, P. J. 1961. Isolation and structures of gibberellins from higher plants. Amer. Chem. Soc. Sym. The Gibberellins. 28: 18-25.

Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. 1 st. Ed. 453 pp (McGraw-Hill Book Company, Inc: New York, Toronto, London).

Marcelle, R. and Sironval, C. 1962. Effect of gibberellic acid on flowering of apple trees. Nature. 197: 405.

Martin, D. and Lewis, T. L. 1952. The physiology of growth in apple fruits. III. Cell characteristics and respiratory activity of light and heavy crop fruits. Aust. J. Sci. Res., B. 5: 315-327.

Martin, D., Lewis, T. L., and Corny, J. 1954. Apple fruit cell numbers in relation to cropping alternation and certain treatments. Aust. J. Agric. Res. 15: 905-919.

Nicholls, P. B. and Paleg, L. G. 1963. A barley endosperm bioassay for gibberellins. Nature. 199: 823-824.

Nickell, L. G. and Tulecke, W. R. 1961. Plant Growth Regulation (Ed. R. M. Klein) (Iowa State University Press: Ames, Iowa) : 675-685.

Paleg, L. G. 1959. Citric acid interference in the estimation of reducing sugars with alkaline copper reagents. Analyt. Chem. 31: 1902-1904.

Paleg, L., Aspinall, D., Coombe, B. and Nicholls, P. 1964. Physiological effects of gibberellic acid. VI. Other gibberellins in three test systems. Plant Physiol. 39: 286-290.

Pearson, J. A. and Robertson, R. N. 1953. The physiology of growth in apple fruits. IV. Seasonal variation in cell size, nitrogen metabolism, and respiration in developing Granny Smith apple fruits. Aust. J. Biol. Sci. 6: 1-20.

Proebsting, E. L. 1962. Factors influencing the relationship of harvest diameter to reference date diameter of Elberta peaches. Proc. Amer. Soc. Hort. Sci. 80: 154-162

Prosser, M. V. and Jackson, G.A.D. 1959. Induction of parthenocarpy in Rosa arvensis Huds. with gibberellic acid. Nature 184: 168.

Radley, M. 1958. The distribution of substances similar to gibberellic acid in higher plants. Ann. Bot., Lond.

N.S. 22: 297-307.

Radley, M. 1959. The occurrence of gibberellin-like substances in barley and malt. Chem. and Indust. July 4, No. 27, 1252 : 877-878.

Eagland, C. H. 1934. The development of the peach fruit, with special reference to split-pit and gumming. Proc. Amer. Soc. Hort. Sci. 31: 1-21.

Rebeiz, C. A. and Crane, J. C. 1961. Growth regulator-induced parthenocarpy in the Bing cherry. Proc. Amer. Soc. Hort. Sci. 78: 69-75.

Sastry, K. K. S. and Muir, R. M. 1963. Gibberellin: effect on diffusible auxin in fruit development. Science. 140: 494-495.

Schroeder, C. A. and Spector, C. 1957. Effect of gibberellic acid and indoleacetic acid on growth of excised fruit tissue. Science. 126: 701-702.

Simons, D. H. 1960. A study of morphological changes and free auxin content during the post-flowering development of tomato and almond fruits. Hons. B. Ag. Sc. thesis, University of Adelaide. 67pp.

Singh, J. P., Randhawa, G. S., and Jain, M. L. 1960. Response of strawberry to gibberellic acid. Indian J. Hort. 17: 21-30.

Smith, C. R., Scossek, Z., and Collins, W. B. 1961. Flowering and fruiting of strawberries in relation to gibberellins. Amer. Chem. Soc. Sym. The Gibberellins. 28: 109-115.

Smith, W. H. 1940. The histological structure of the flesh of the apple in relation to growth and senescence. J. Pomol. 18: 249-260.

Smith, W. H. 1950. Cell-multiplication and cell-enlargement in the development of the flesh of the apple fruit. Ann. Bot., Lond. N.S. 14: 23-38.

Southwick, F. W., Weeks, W. D., Sawada, K., and Anderson, J. F. 1962. The influence of chemical thinners and seeds on the growth rate of apples. Proc. Amer Soc. Hort. Sci. 80: 33-42.

Sterling, C. 1953. Developmental anatomy of the fruit of Prunus domestica L. Bull. Torrey Bot. Club. 80: 457-477.

Straus, J. and Epp, R. R. 1960. Response of Cupressus funebris tissue cultures to gibberellins. Science. 131: 1806-1807.

Tufts, W. P. 1929. Seasonal temperatures and fruit ripening: a preliminary report. Proc. Amer. Soc. Hort. Sci. 26: 163-166.

Tukey, H. B. 1933. Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. 30: 209-218.

Tukey, H. B. 1934. Growth of the embryo, seed, and pericarp of the sour cherry (Prunus cerasus) in relation to season of fruit ripening. Proc. Amer. Soc. Hort. Sci. 31: 125-144.

Tukey, H. B. and Young, J. O. 1939. Histological study of the developing fruit of the sour cherry. Bot. Gaz. 100: 723-749.

Tukey, L. D. 1952. Effect of night temperature on growth of the fruit of sour cherry. Bot. Gaz. 114: 155-165.

Tukey, L. D. 1956. Some effects of night temperature on the growth of McIntosh apples, I. Proc. Amer Soc. Hort. Sci. 68: 32-43.

Tukey, L. D. 1958. Effects of controlled temperatures following bloom on berry development of the Concord grape (Vitis labrusca). Proc. Amer. Soc. Hort. Sci. 71: 157-166.

Tukey, L. D. 1960. Some effects of night temperature on the growth of McIntosh apples, II. Proc. Amer. Soc. Hort. Sci. 75: 39-46.

Turner, J. N. 1963. Application of gibberellic acid to strawberry plants at different stages of development. Nature. 197: 95-96.

Walker, D. R. and Donoho, C. W. 1959. Further studies of the effect of gibberellic acid on breaking the rest period of young peach and apple trees. Proc. Amer. Soc. Hort. Sci. 74: 87-92.

Weaver, R. J. and McCune, S. B. 1959. Response of certain varieties of Vitis vinifera to gibberellin. Hilgardia. 28: 297-350.

Weaver, R. J., McCune, S. B., and Hale, C. R. 1962. Effect of plant regulators on set and berry development in certain seedless varieties of Vitis vinifera L. Vitis. 3: 84-96.

Went, F. W. 1942. Growth, auxin, and tropisms in decapitated Avena coleoptiles. Plant Physiol. 17: 236-249.

Went, F. W. and Thimann, K. V. 1937. Phytohormones. (The Macmillan Company, New York.)

West, C. A. and Phinney, B. O. 1959. Gibberellins from flowering plants. X. Isolation and properties of a gibberellin from Phaseolus vulgaris L. J. Amer. Chem. Soc. 81: 2424-2427.

Wheeler, A. W. 1960. Changes in a leaf-growth substance in cotyledons and primary leaves during the growth of dwarf corn seedlings. J. Exp. Bot. 11: 217-226.

White, P. R. 1943. A Handbook of Plant Tissue Culture. (The Jaques Cattell Press, Lancaster.)