MORPHOLOGICAL AND HISTOLOGICAL RESPONSES OF THE CHERRY (PRUNUS AVIUM L. AND PRUNUS CERASUS L.) TO FALL APPLICATION OF PLANT GROWTH REGULATORS

By

SUNDARAM KRISHNAMURTHI

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Department of Horticulture

1949

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C O N T E N T S

A. Growth Regulator Studies

1. Beans

Among the noteworthy investigations on the effects of plant growth regulators, have been the studies of their histological effects on bean plants. Kraus, Brown and Hamner (51) found the endodermis and cambium the most responsive to application of indoleacetic acid, the endodermis proliferating and differentiating into vascular strands, and the cambium cells proliferating rapidly but with delayed differentiation of derivatives. The primary phloem was also highly sensitive with abundant proliferation and frequent radial elongation. The epidermis and pericycle were not very responsive while the pith proliferated slowly but profusely. Hamner and Kraus (43) extended their observations over Kraus, Brown and Hamner (51) to indolebutyric acid and naphthaleneacetic acid with similar results. Kraus and Mitchell (52) reported the histological responses of the bean to naphthaleneacetamide. There was less extensive proliferation than with indoleacetic acid and the most evident feature was the great increment of secondary xylem, the decided thickening of the walls of cells of most of the tissues composing the stem and the partial suppression of elongation of axillary buds. The

INTRODUCTION

Tukey and Hamner (96) reported in 1947 from field observations that fall application of a spray mixture of 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid to cherry trees, resulted in delayed bloom and in changes in form and composition of the fruit. They noted among gross effects, injury to trees, delayed abscission of abortive flowers and abortive fruits, elongate shape of fruits with pointed apex and development of a strong vascular system within the fruits. Their data have been summarised under "Review of Literature". These observations seemed to indicate the value of a more detailed study of the responses of the cherry (*Prunus avium*, L. and *Prunus cerasus*, L.) to the fall application of growth regulators. This thesis is a report of such a study of the morphological and histological responses, made at the experimental orchard of the Michigan State College.

REVIEW OF LITERATURE

For a decade and more, morphological and histological effects of plant growth regulators have been rather intensively studied. This review describes the responses of plants that are generally characteristic of the particular substances used, for example the formative effects of phenoxy compounds and unless the differential effects of concentrations are a factor, they will not be mentioned.

The literature has been classified under the following groups:

A. Growth Regulator Studies
   1. On beans
   2. On tomatoes
   3. On other herbaceous plants
   4. On herbicidal effects
   5. Effects of soil and seed treatments
   6. Special studies of formative effects
   7. Studies with fruits other than cherries
   8. Studies with cherries

B. Studies on morphology, growing habits and development of cherries.

A. Growth Regulator Studies

1. Beans

Among the noteworthy investigations on the effects of plant growth regulators, have been the studies of their histological effects on bean plants. Kraus, Brown and Hamner (51) found the endodermis and cambium the most responsive to application of indoleacetic acid, the endodermis proliferating and differentiating into vascular strands, and the cambium cells proliferating rapidly but with delayed differentiation of derivatives. The primary phloem was also highly sensitive with abundant proliferation and frequent radial elongation. The epidermis and pericycle were not very responsive while the pith proliferated slowly but profusely. Hamner and Kraus (43) extended their observations over Kraus, Brown and Hamner (51) to indolebutyric acid and naphthaleneacetic acid with similar results. Kraus and Mitchell (52) reported the histological responses of the bean to naphthaleneacetamide. There was less extensive proliferation than with indoleacetic acid and the most evident feature was the great increment of secondary xylem, the decided thickening of the walls of cells of most of the tissues composing the stem and the partial suppression of elongation of axillary buds. The effect was similar in the African Marigold and in Mirabilis. Another most obvious characteristic was the increased firmness of the treated internodes, resulting in prevention of abscission, when decapitated and treated. Among other effects were the reduction in the height and total leaf area, and reduction in size of primary leaves.

Mitchell and Stewart (68) compared the effects of naphthaleneacetic acid and naphthaleneacetamide upon the bean. They showed again the tendency of the acetamide to increase the wall material, especially of the xylem and the pericycle fibers, and not to affect the cortex. They made other observations that the treated leaves were lighter green in color and thicker, that the abscission of cotyledons was delayed by several days, and that treatment inhibited bud development. Mullison (71) observed that application of tetrahydrofurfuryl butyrate induced certain different responses from those obtained from other growth substances, among which were the more localized response, the highest activity of the xylem and moderate activity of the endodermis with no differentiation of vascular tissue.

Hamner (38) found that A-naphthaleneacetamide when applied to the bean in nutrient solution reduced top growth and increased root growth, and induced earlier maturity and greater extension of xylem and phloem in the roots. Palser (79) observed that *Vicia faba* responded to indoleacetic acid histologically with activity to some extent in every parenchymatous tissue, and notably with somewhat extensive vascularisation of derived tissues. Beal (12) applied 4 substituted phenoxy compounds and found that in the second internode nearer the point of application, with the exception of the epidermis, possibly the pericycle and limited portions of outer cortex, all the other tissues responded actively, whereas in the first internode and hypocotyl, farther from the point of application, only the endodermis, cambium, phloem and ray parenchyma were activated. Whiting and Murray (106) reported that histological reactions of bean to phenylacetic acid consisted of marked proliferation of inner cortical parenchyma, endodermis and primary phloem parenchyma, also of the activity of the outer cortical parenchyma, the rays, and the peripheral pith, and the cambium, with slight response in the pericycle and secondary phloem, and no activity of the epidermis and the central pith.

Swanson (86) studied the histological responses of the Red Kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid and noted the activity in the endodermis, pericycle (if undifferentiated or embryonic), phloem parenchyma, cambium and rays (the latter two zones being very active). He found that the formation of xylary elements was greatly inhibited and when occurring, much disorganized; that cortex showed little response; and epidermis and pith no response. He observed that the effect of 2,4-D was systemic in nature, even at relatively low concentrations and in this respect, it differed from other growth regulating substances. Murray and Whiting (76) applied 2,4-D acid and four of its salts to decapitated stem of kidney bean plants and found similar histological responses in the early stages, with initiation, soon after treatment, of proliferation of the parenchymatous tissues of the inner cortex including the endodermis, of the pericycle, phloem, cambial zone and rays. Weaver (100) in studying the subsequent growth of various parts of red kidney bean and soya bean plants sprayed with 2,4-dichlorophenoxyacetic acid observed that the primary leaves of treated plants were much heavier and that those of red kidney beans remained green and attached to the plants long after those of the controls had withered and fallen, that the growth regulator inhibited the growth in length and width of leaflets which developed after treatment, and delayed the onset and decreased the amount of pod-formation.

Murray and Whiting (75) studied the histological responses of bean stems to low concentrations of indoleacetic acid and compared with those described by Kraus (50) for tryptophane. The important differences between the two substances were that with tryptophane, the cortical parenchyma was more reactive, vascularisation in proliferated endodermis greater, primary phloem less active, pith relatively inactive and the course of vascularisation in other tissues different for the two substances.

Laibach and Fishnich (54) observed the histological effects of indoleacetic acid upon coleus, Vicia faba and the tomato and noted in coleus that new vascular bundles appeared among the three originally present on each side.

2. Tomatoes

Tomato is another plant which has received attention from workers not only in connection with effects of growth regulators on fruit-set but also as an experimental material for morphological and histological studies. Borthwick, Hamner and Parker (18) studied the histological response of tomato stem to indoleacetic acid. The results were quite similar to those reported previously for the bean. Cortical enlargement occurred, accompanied by some division; the endodermis divided actively; the phloem and the wood parenchyma proliferated, while the epidermis and pericycle were not very responsive.

Bausor, Reinhart and Tice (9) reported the response of tomato stem to treatment with B-naphthoxyacetic acid. In the young stem, there was enlargement of the cortical cells and great activity of cambium and differentiation of secondary xylem. In older stems the cortex was also activated but the greatest change occurred in the tissues from cambium to endodermis inclusive in which proliferation took place rapidly.

Hamner, Schomer and Marth (40) in using growth-regulating substances in aerosol form for fruit-set in tomato found among other observations that as a result of the use of B-naphthoxyacetic acid, the style persisted longer and was enlarged and the treated tomatoes ripened earlier, were larger in diameter and heavier than controls and some fruits were "mis-shapen", and there was bud-inhibition and leaf "malformation".

Murneek, Wittwer and Hemphill (74) found in their experiments with greenhouse tomatoes that sprays of p-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid at certain concentrations had a strong morphogenetic effect on meristematic tissues resulting in elongation of internodes, modification of newly formed leaves and abnormal growth of pericarps. In the case of 2,4-D, and chlorophenoxyacetic acid as a whole, notable was the influence on the development of fruits which at maturity became unusually large, mostly pointed, softer, with an orange color. The authors concluded that some of these chemicals stimulated directly or indirectly the growth of the outer region of the pericarp. Murneek (72) confirmed in further investigations the formative influences of p-chlorophenoxyacetic acid and cautioned that in spraying of a whole plant for fruit-set, the spray should avoid the growing points to prevent undesirable formative effects.

3. Other Herbaceous Plants

Ennis, Swanson and others (31) reported that 2,4,5-trichlorophenoxyacetate significantly reduced the yield of potato tubers, stunted the vegetative growth, showed swelling of the pulvini and thickening of the stem immediately adjacent to the petiole. Goldberg (36) applied indoleacetic acid to cabbage stem and found that all tissues responded to some extent; most generally responsive were those of phloem, rays and pith. The cambium, cortex, endodermis, and xylem were moderately stimulated, while epidermis and pericycle reacted weakly. Harrison (45) treated Iresine lindenii with indoleacetic acid. The endodermis and cortical parenchyma were not very reactive, while the phloem, pericycle, rays and extra-fascicular cambium proliferated. Thinning of the walls of the collenchyma was also noted. The fascicular cambium was not very responsive. Of rather unusual response was the proliferation of cutinised epidermal cells with thickened walls. The general degree of activity of Iresine resembled more closely that of the tomato than of the bean.

Beal (10) worked on three species of Lilium with indoleacetic acid. The rate of response was much slower than that reported for bean, tomato and Iresine. In Lilium philippinense and Lilium longiflorum, there was proliferation of the parenchyma cells centripetal to the bundles, which later became organised into roots. In these, the outer cortex and the epidermis were only slightly affected. In Lilium harrisii, however, the epidermis and the cortex in the axil of the leaf were activated to divide and become organised as buds.

Hamner (43) worked on Mirabilis jalapa with indoleacetic acid and found wide variation in histological responses of the various tissues. The pericycle, interfascicular parenchyma just inside it and the interfascicular cambium (in the older stems) were the most reactive, while the vascular tissues were generally unresponsive, the epidermis and cortical parenchyma showed little response and the endodermis considerable. The pith was slow to start activity but once it started, proliferated rapidly, differentiating as internal roots or as strands of vascular tissue.

Blum (16) worked on sunflower stem and found that naphthaleneacetic acid caused marked epinasty, completely inhibited bud development, caused extreme development and differentiation of secondary xylem and marked development of activity of the horizontal cambium.

Beal (11) treated sweet pea with 4-chlorophenoxyacetic acid and observed stem curvature, epinasty, inhibition of buds, swollen root-tips with most proliferation of pericycle. Pfeiffer (80) worked on the aerial roots of Cissus sicyoides L. with indolebutyric acid and found that the cells of the phloem and pericycle proved most reactive, enlarging and dividing, with derivatives of the pericycle opposite the protoxylem forming the lateral roots.

4. Herbicidal Effects

Tukey, Hamner and Imhofe (97) were the first to study histological effects of herbicidal concentrations of 2,4-D and the experimental plant materials were bindweed and sow-thistle. In the bindweed, the pollengrains were plasmolysed and disorganised, flowers arrested in development, chlorophyll formation checked and cell division activited in the large vascular bundles of the leaves. The majority of the cells in the leaves were plasmolysed. Cell division was greatly increased in all cambial zones and phloem regions of the stem and rhizomes. Enlargement and rupture of cortical cells were conspicuous. In the rhizome of sow-thistle, the reactions of cortex, cambium and phloem zones were similar. Hamner and Tukey (41) in studying the herbicidal action of 2,4-D on several shrbus, vines and trees, noted marked proliferation of the region outside cambium leading to splitting of the bark, curvature of new shoots and downward bending of petioles. Young trees were more responsive than old ones and in general, plants which had just broken dormancy in the spring and were making new growth were more sensitive to treatment than plants with fully developed foliage.

Pridham (81) studied the delayed action of 2,4-D on trees, shrubs, and perennials, sprayed in the fall and observed delayed bud-break in spring and production of distorted foliage, excessive serrations, exaggeration of length/width ratio of leaves, prominence of veins and thickening of leaf.

5. Effects of Soil and Seed Treatments

While upto this stage, the literature has dealt with direct application of growth regulators to plants or trees, it is relevant to state that some workers have experimented with treatment of soil or seed with growth regulating substances and noted the subsequent effects on plants. Allard, DeRose and Swanson (1) found that treatment of seeds of several crop plants with 2,4-dichlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid in general inhibited germination, decreased the growth of young seedlings, and caused abnormalities in the anatomy of the seedlings. As examples of the latter in the hypocotyl of kidney beans were the delay in the maturation of the primary conductive tissues, failure of the pith to split to give rise to a hollow stem, enlargement of cortical and pith cells and slight stimulation of cell-proliferation in regions of the primary phloem and the rays. Hamner, Moulton and Tukey (39) treated in one case soils with 2,4-D prior to sowing and in another soaked the seeds in solution of 2,4-D and in both cases, seedlings of beans exhibited characteristic formative effects and "virus-like" symptoms.

6. Special Studies of Formative Effects

Of particular importance to the subject of this thesis are the series of papers on formative effects of growth regulating substances presented by Zimmerman and Hitchcock (110, 111, 112), and Zimmerman (109, 114). Zimmerman and Hitchcock (110) treated several species of plants with solutions and vapors of B-naphthoxyacetic acid and its derivatives and found that in the new leaves that were formed after treatment, there was modification of the venation pattern and form. They varied from frenched or fern leaf types to simple leaves. The leaflets often failed to separate on one side of the mid-rib giving a different appearance to the two sides of the leaf. In many respects, the leaves resembled those of virus-diseased or mite-infested plants. There was pronounced clearing of the veins. With treatment of soil with the solutions of naphthoxyacetic acid, the effects were even more pronounced and more lasting. The flower-buds of tomato plants treated by way of soil were abnormally long with a decided calyx tube, separating only at the tip. The floral parts -- petals, stamens and styles -- persisted for an abnormally long time during parthenocarpic development. Zimmerman (109) in further experiments with B-naphthoxy compounds, not only confirmed the findings above but also found that they modified the simple tendrils of cucumbers into branched and leaf-like structures, and the size and shape of flower-buds and fruits. Zimmerman and Hitchcock (112) reported that under the influence of tri-iodobenzoic acid, a hormone-like substance, axillary buds which normally produced leafy shoots were induced to grow flower-clusters. The main shoot of the plant also lost the shoot producing bud and terminated in flower-clusters.

Zimmerman and Hitchcock (111) emphasized the more lasting nature of the responses of dichlorophenoxyacetic acid with low concentrations compared to other hormone-like compounds and also showed that in tomato, cell division of the cambium and various parts of the cortex was accelerated within 24 hours, by chlorophenoxy compounds. Other observations of the effect of chlorophenoxyacetic acid on tomato were bud-inhibition, persistence of floral parts, and formative influences on the leaves. Zimmerman (114) reported that plants treated with active xelenoxy acids grew modified leaves differing from the normal in size, shape, pattern, texture and venation, and appearing like those of virus diseased plants. Burton (26) studied the formative effects of substituted chlorophenoxy compounds and found that treatment of young plants with 0.5 per cent concentration of 2-chlorophenoxyacetic acid in carbowax inhibited the formation of intercellular spaces in the mesophyll of bean leaflets. Similar application of 0.5 per cent 4-chlorophenoxyacetic acid in lanolin inhibited the activity of plate meristem in the laminae of bean leaflets. Similar treatment with 0.5 per cent concentrations of 2,4-dichlorophenoxyacetic acid, in either lanolin or carbowax as the carrier induced progressive modification of bean leaves. The earliest leaves were similar to those induced by treatment with 2-chlorophenoxyacetic acid. In later leaves, the external form and internal structure resembled that found in leaflets of plants treated with 4-chlorophenoxyacetic acid.

7. Fruits Other Than Cherries

Marth and Meader (58) used four compounds, indoleacetic, indolebutyric, B-naphthoxyacetic and 4-chlorophenoxyacetic acids on somewhat unfruitful blackberry selections at the time of flowering and obtained significant increase in berry weight and reported that the treatments stimulated the development of the receptacle and of the pulp of the individual drupelets and increased the number of drupelets and seeds per fruit but many of the seeds were small and undeveloped so that the ratio of seedweight to fresh berry weight was actually less in treated fruits than in control.

Clark and Kerns (28) reported data obtained from pineapple plants treated with naphthaleneacetic acid after completion of normal floral differentiation but prior to actual blossoming. Higher concentrations increased the weight of the fruit and delayed ripening a week or more, and no treatments induced anatomical effect within the flesh of the fruit, but the concentration of the juice of treated fruits was about 2° Brix lower than that of the controls. Treatments did not also affect the number of fruitlets per spiral. High concentrations induced longer and thicker peduncles and difficulty in separating the fruits from the peduncles.

Stewart and Klotz (88) brought to attention some unusual effects of 2,4-D on the morphology of oranges. Young Navel orange fruits sprayed with concentrations varying from 25 to 225 p.p.m. of 2,4-D developed seeds or seed-like structures. The highest concentrations induced a thick rind and excessively large, protruding navels, in some fruits and cylindrical shape and small navels in others. When applications were made during the period of full bloom, all treatments induced a downward and inward rolling of the margins of the young, expanding leaves, the response decreasing with lower concentrations; later prevented flower-drop but reduced fruit-set. It was found that the blossom-retaining effect was not transmitted from the sprayed portion of the tree to the unsprayed area. In Valencia, the high concentrations caused a curious pebbling of the peel of the green fruit due to outward and inward elongation of the oil glands, and highly marked thickness of rind of the sprayed fruits. Some fruits became cylindrical in shape and developed a small navel "complete with juice-vesicle, and a persistent style". Lower concentrations increased fruit size by 25 per cent, but at the expense of commercial quality.

Nixon and Gardner (77) reported that application of naphthaleneacetic acid to dates, in higher concentrations of aqueous solutions of 0.1 and 0.01 per cent inhibited development of carpels but caused the enveloping perianths to persist and to show apparent enlargement in some instances. The strand with its persisting perianths was still alive and normally colored long after they were dead and dry in the untreated ones. Naphthaleneacetic acid at 0.01 per cent aqueous spray applied ten days after pollination followed by 0.02 per cent six days later reduced set by 50 per cent and induced a considerable number of fruits with varying degrees of "malformation". The dates ripened a little earlier.

Moon, Regeimbal and Harley (70) airplane sprayed a small block of apple trees with 8100 p.p.m. 2,4-D in an oil-emulsion, on November 9, 1946. Stayman Winesap and Winesap trees so treated held their leaves well into the winter after all leaves were dead and long after untreated trees were completely defoliated, and in the spring of 1947, numerous leaf petioles were still attached to the branches, even though the blades had been broken off during the winter. Subsequently during the growing season, there were typical signs of delayed foliation, badly deformed primary leaves, death of lateral buds on terminal shoots and of weaker spurs on older branches. Similar response, even more widespread, was obtained with 10 p.p.m. of 2,4-D butyl ester as groundspray of Stayman Winesap applied on August 29 and September 11, as also tight adherence of all subsequent blossoms and fruits killed by frost on May 9 to 11. These authors observed in addition the residual effect of 2,4-D at 10 p.p.m. applied in August 29, 1946, for a full year later, in reducing harvest drop.

Bryant, Vincent and Schafer (22) observed that applications of 2,4-D made to bindweed in 1945 in late summer in an apple orchard resulted in abnormalities in the apples harvested in 1946. Some of the fruits showed only rudimentary or no seed development. McIntosh apples, normally round oblate, had become oblong in shape with a more open core, while Jonathan had changed from round conic to oblong conic. A considerable number of Jonathan and Delicious apples were "doubles". The authors considered that "these malformations may have come from the 2,4-D being transmitted through the roots of the trees, from the material drifting at the time the bindweed was sprayed, or from volatile materials in the spray itself".

Marsh and Taylor (56) applied 2,4-D on August 22, 1945, as an eradicant spray for poison ivy in an apple orchard. After cleaning and rinsing the spray equipment with water as much as to remove all traces of odor and visible evidence of the 2,4-D residue, applied an oil spray one week later to control orchard mites. In fall, fruit and foliage in this Winesap and Stayman block adhered tenaciously to the trees. Through the winter, the foliage hung on those trees "dry and brown like the leaves on shingle oaks". In 1946, the blossoming of this block was 7 to 10 days later. The leaves were narrowed, deeply serrated, epinastic, frequently mottled. There was a very low amount of total foliage area. The adherence of undeveloped, dried apple blossom parts was very noticeable. The fruit-set was very much reduced. The apples were prematurely ripened and not suitable for storage.

8. Cherries

Hitchcock and Zimmerman (46) have reported effects of application of summer sprays of potassium naphthaleneacetate to Montmorency cherry trees, in retarding opening of buds. The degree of inhibition varied with the concentration of the substance, and the time the spray was applied. The vegetative buds were generally delayed to a greater extent than flower buds. The sprays of July had the greatest retarding and those in September the least. The increased delay resulting from an increase in concentration was relatively the same as that due to the difference in time of application. Flower buds located on spurs were delayed to a greater extent than those on one year old shoots. The opening of flower buds was delayed from a few days to 14 days depending on the concentration and time of applying the spray, and that of vegetative buds similarly up to 19 days. Terminal vegetative buds were retarded to a lesser extent than side buds. The fruit-set on treated branches was delayed slightly and appeared to be spread over a longer period as compared to the controls. Ripe fruit had the same general appearance on the treated and control branches. The experiments of the author also included those on varieties of apples, peaches, pears and plums, with similar results but with a variation in resistance to the material, the apple was slightly more resistant than cherries, and peach and plum more sensitive than cherry.

Tukey and Hamner (48, 96) gave an account of observations of the effects of fall application of 2,4-D and naphthaleneacetic acid to sweet and sour cherries and brought out the facts of injury to twigs, buds and spurs, delayed blossoming in the following spring, delayed abscission of abortive flowers and abortive fruits, and above all the elongate fruit with pointed apex, and strong vascular development within both the fruit and the pedicel.

B. Studies on Morphology, Growing Habits and Development of Cherries

Bradbury (20, 21) in making a study of the developing and aborting fruits of sour cherries came to the conclusion that lack of nutrition was the principal cause of the abortion, particularly of the first drop fruits. Chandler (27) described the nature and position of fruit-buds in cherries and stated that differentiation of buds in cherries is discernible in early July.

Tukey (94) has described the growing and fruiting habits of the sour cherry, making the distinction between the shoot and the spur, and mentioning the correlation between the positions where the flower-buds are formed and the continued fruitfulness of the tree. In describing the distinctive growing habits of the three major varieties of sour cherry in the Hudson River Valley, he sums up the growing habit of Montmorency cherry thus:- "Shoots over 6 inches in length tend to form leaf-buds in contrast with blossom buds which are formed largely by shoots less than 6 inches in length. The leaf-buds develop into spurs or strong lateral shoots -- mostly spurs in the case of shoots between 6 and 15 inches in length which bear repeatedly in subsequent years. The blossom-buds on one year old wood, however, fruit and leave barren wood behind".

Tukey (95) reported three stages of pericarp development in sour cherry; namely, I - a period of rapid increase following fertilization, II - a period of delayed increase during mid-season, and III - a second period of rapid increase to fruit ripening. He found the duration of stages I and III nearly identical in the three varieties tested and the duration of period of delayed increase (Stage II) correlated directly with the season of fruit-ripening.

Tukey and Young (98) made detailed histological study of the development of the different tissues of the sour cherry fruit (Montmorency variety), step by step through the three stages mentioned above, and correlated the internal histological changes with the external evidences of growth.

MATERIALS AND METHODS

Bearing trees of the Montmorency sour cherry (*Prunus cerasus* L.) and of the Schmidt sweet cherry (*Prunus avium* L.) were selected in the experimental orchard of Michigan State College, East Lansing, for treatment and study. The trees at the time of treatment were fifteen to sixteen years of age. One sour cherry tree and one sweet cherry tree were sprayed and one of each left untreated for comparison. The spraying was done on September 22, 1947, between 6 and 7 P.M. with a Hydraulic Bean Sprayer, 20 gallon capacity, operating at 500 pounds pressure. The discharge equipment included a single nozzle bean gun equipped with No. 10 disc. The spray consisted of 100 p.p.m. of 2,4-dichlorophenoxyacetic acid in the form of "Dow 70% Wettable", a commercial product of the Dow Chemical Company and 30 p.p.m. of naphthaleneacetic acid in the form of "Endrop", a commercial product of the Shell Oil Company. The method of spraying consisted of spraying the whole tree thoroughly from top to bottom until the spray material dripped from the leaves.

Collection of Material

Samples of buds were collected beginning October 1, 1947, at intervals of three weeks upto the 20th of March, 1948, and at intervals of two weeks until the 22nd of

April, 1948. From then on, the required plant materials for examination or preservation were collected as frequently as the needs arose.

Fixation, Sectioning and Staining

Subsequent to spraying, till the harvest of fruits, the materials collected were all fixed in killing solution of the following proportions:-

500 ml.	95 per cent alcohol
450 ml.	distilled water
21 ml.	glacial acetic acid
60 ml.	formalin

In the case of materials like stem, pedicel, leaf, and certain stages of the fruit, it was easy to make suitable sections with the table microtome. In many cases, the tissues of such sections were clear enough without staining so that semipermanent slides were conveniently made with glycerin as explained by Sass (82). In some sections, however, to make a proper distinction between lignified and cellulose walls, methyl green and Bismarck brown were used. Certain stages of the winter buds were utilized for making sections, for the sake of some confirmatory observations, by running up to paraffin, then sectioning with a rotary microtome and staining with Delafields hematoxylin.

In advanced stages of fruit development when the stony pericarp had begun to harden, freehand sections for detailed study were made separately in the different regions of the fruit outside the stony pericarp.

The epidermis of the leaves and fruits was examined in surface view by stripping thin pieces with razor and in both cases, no stain was needed to show good contrast.

Measurements and Drawings

Outline drawings, where no cell-details were desired, were made with the use of a Bausch and Lomb triple purpose micro-projector, using the same objective and a constant set-up for the same set of comparative materials. Detailed drawings were made with a camera lucida, to one scale for the same set of comparative materials, to give identical enlargements.

Many detailed measurements became unnecessary, because of the obviously large differences resulting from treatment. In an experiment of this kind, it is the comparative rather than the original measurements that stress the nature of responses obtained by treatment. Where, however, to make a point clear, an original measurement was necessary, it has been provided.

Besides this, the behavior-pattern of the sour cherry and the sweet cherry has been identical in so many respects that to separate them in the treatment of subject would be merely repetitious. To avoid this, where there was no need to refer to them separately, the expression "cherry" has been used or no particular mention is made of any of the cherries.

For histological studies, descriptions and drawings, sour cherry has been more freely used than sweet cherry because of the advantage of detailed information on normal development of sour cherry available from Tukey (95) and Tukey and Young (98) and because of incompleteness of materials from sweet cherry, due to injury. An examination of the available materials from sweet cherry, however, showed no significant difference in general, except for a tendency for a relatively greater accentuation of response to treatment.

Even in the case of sour cherry, to avoid the interfering factor of injury, materials from the healthier portions of the tree were utilised for observations of direct responses to treatment. The reasons for this become clear from the description under "Tree-Injury".

RESULTS

1. Tree Injury

Injury to trees as a result of application of high concentrations of plant growth substances, particularly of naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid, has frequently been reported (66, 57, 93, 87, 56). Such injury was also found in our experiments. The following is an account of the nature and extent of injury.

Reduction of Leaf Area

The most severe injury was to the leaf buds. A large proportion of them on the tree as a whole were killed. This was reflected in the ultimate reduction of leaf area. Plates 2 and 4 show this condition of sour and sweet cherry trees respectively, on July 16, 1948, in contrast to the normal leaf area of the trees, as shown in Plates 1 and 3. The framework of the treated trees in the photographs, because of lack of foliage, can be easily traced to its intimate details, while it is completely hidden from view by the foliage in the control trees.

The malformed and stunted leaves that emerged from the inhibited leaf-buds as shown in Plates 5 and 7 also contributed to the decrease in leaf area.

Reduction of Fruit-set

Compared to the yield of fruits from the untreated trees, there was a great reduction, by 90 and 95 per cent in the sour and sweet cherry respectively, as a result of treatment. Damage to fruit-buds was severe. A great number of them were completely dead. A smaller proportion of them were inhibited in varying degrees and while opening subsequently, proved abortive later. The ultimate result of reduced fruit-set is, however, attributable to three causes, decrease in leaf area, damage to fruit-buds and injury to the tree as a whole.

Factors in Injury

While injury itself was a direct result of the treatment, there were certain factors, chiefly light, vigor of branches and the method of spraying adopted, that seemed either to accentuate injury or to resist it. The terminal portions of branches that had more light tended to develop greater amount of foliage, whereas the inner portions of branches were almost bare. This is clearly indicated in Plates 2 and 4. The less vigorous branches showed greater injury to buds and spurs on them than the more vigorous ones.

Above all the very method of application of the spray, viz., spraying the whole tree till the material dripped from the leaves seemed to have created a situation in which the drip from the topmost portions added on to the amount of material on the lower portions of the tree, creating a differential concentration with relatively harmless lower concentrations in the upper levels and relatively toxic higher concentrations in the lower levels. This is evidenced also by the difference in intensity of responses that have been noted, which have been correlated with difference in concentration by other workers such as Hitchcock and Zimmerman (46).

Plate 2 shows clear indications of such a demarcation, with better development of foliage at higher levels compared to the relative bareness of lower branches. This distinction has been made use of in our experiments in selection of materials for observations of direct responses to treatment, to avoid as much as possible an intervening factor like injury.

Nature of Injury

A closer examination of the twigs that were injured showed small necrotic areas and brown fleckings either in or under the bark. Pronounced browning was noticeable in the pith and xylem of spurs. A few lower branches of sweet cherry that were dead resembled winter injury from an oil-spray.

Varietal Differences

In comparing the total effect of injury, it was clear that the sweet cherry tree suffered more than the sour. This difference is very clear from the appearance of trees in Plates 2 and 4. Varietal differences in resistance to injury have been pointed out by Hitchcock and Zimmerman (46).

2. Observations Connected with Abscission

From the time that the application of spray was made to the cherry trees to the time of harvest of fruits, one of the most conspicuous responses observed successively was the noticeable delay or even complete prevention of normal abscission of plant parts.

The Leaves in the Fall

While on the untreated trees, the leaves started to fall by the 10th of November, the leaves on the treated trees continued to be green and held onto the twigs. These latter turned brown through the winter, but nevertheless most of the leaves with their petioles stayed firm, with the result that as late as the 20th of March, the treated trees could be easily distinguished from all the rest by the brown leaves sticking out all over the tree. While subsequently, due to winds, most of the blades were torn off it was still possible to see here and there full brown leaves, and portions of brown leaves holding on with the petioles, as shown in the photograph taken on the 24th of April, 1948 (Plate 8). Still later, almost all through the succeeding summer, one could observe most of the petiole-stubs tight on the twigs, a specimen of which is shown in the photograph (Plate 6). Thus the formation of an abscission layer was entirely prevented by the spray.

Previous-season Fruits

The abscission of the few stray fruits that were ripening late on the tree, at the time of application of the spray, was affected also and long after the fleshy portion of the fruit decayed and dried, the pedicel with the stone attached continued to stay firmly on the twigs. Here also the normal process of abscission was thus completely prevented as a result of treatment.

Flowers and Flower-parts

In the case of flowers that opened in spring, where fertilisation had taken place, the petals were slow in dropping off. In the case of flowers where pollination had not taken place, the flowers stayed on the twigs. They subsequently dried up but still held on. Plate 6 shows the photograph of such an example, taken on the 18th of July. It is evident from this photograph that every part of the flower - the calyx, the petals, the stamens and the pistil - were still intact. When the trees were observed on the 15th of December, 1948, the dried-up flowers were still there. Here again the formation of an abscission layer was completely prevented. In the case of fruits, with aborted ovules, they took on color early and continued to stay on the twigs.

Mature Fruit

The abscission of the fruit was also noticeably delayed, and the fruit held on for weeks after the harvest time, indicating a continuity of effect of the plant growth regulators for more than ten months from the time of application.

The Style

The style of the developing fruit on the treated trees persisted not only for an abnormally long period, but a small portion of it remained attached to the mature fruit, thus accentuating the pointedness of the fruit. Delayed abscission of style as a result of treatment with plant growth regulators has been reported by Elmsweller and Stewart (30), Hamner, Schomer and Marth (40), Zimmerman and Hitchcock (110) and Stewart and Klotz (88), among others.

Discussion

The ability of plant "hormones" in varying degrees of effectiveness to prevent or delay abscission of flowers, fruits and leaves has been demonstrated by numerous workers (44, 34, 110, 111, 88, 70). But what deserves particular attention in this respect in our experiments is the fact that there was no plant organ, which normally undergoes abscission in some stage or other, that did not show effective response to the treatment. That there are anatomical differences in the process of abscission of different plant parts has been conceded (59, 60, 55) and it may be pointed out that under conditions of this experiment, the difference in method of abscission has not stood in the way of effective action of the plant growth substances.

Literature reveals that plant growth regulators have acted consistently in this respect not only on different parts of the same plant, but on a variety of plants involving different types of abscission. To mention a few, Gardner, Marth and Batjer (33) reported delayed abscission of mature fruits of apples; Milbrach and Hartman (61) that of leaves of holly; Kraus and Mitchell (52) that of treated internodes of decapitated beans; Beal and Whiting (13) that of treated internode of decapitated Mirabilis jalapa; Mitchell and Stewart (68) that of cotyledons from treated bean plants; Weaver (100) that of primary leaves of red kidney beans; Zimmerman and Hitchcock (110) that of petals, stamens and styles of tomatoes; Stewart and Klotz (88) that of leaves and blossoms of oranges; Nixon and Gardner (77) that of perianth and fruits of dates.

In spite of all this evidence of the potential capacity of plant growth regulators to delay or prevent abscission, or in other words, to arrest senescence, a certain opinion has been held popularly but on insufficient data, by some workers (33, 23, 32) that when a plant growth regulator has not proved effective in preventing or delaying abscission, it is because of a difference in type of abscission. But a review of a large amount of literature in the field of plant growth regulators, indicates that while there may be some truth in it, the problem is not as simple of explanation, but that it is allied to the problem that is faced in the whole field of plant "hormones", in attempting to explain the differential reaction of the various tissues of the same organ, the differential varietal responses, the differential responses on account of changes in external environment or the selectivity of 2,4-dichlorophenoxyacetic acid as a herbicide. It seems to be more related to the fundamental problem of the variable and unknown state of protoplasm, the substrate, at the time of application of the causal factor, the plant growth regulator.

3. Effects on the Opening of Floral and Vegetative Buds

Floral Buds of Sour Cherry

Some general but continuous observations from the time the tree was sprayed, on this aspect were possible with the sour cherry but was not possible with the sweet cherry, because of the fact that the buds in treated sweet cherry tree were very severely injured and during the winter, almost every bud that was examined from this tree was discolored and showed signs of injury.

Growth of Buds up to the Bloom Stage.

The observations on sour cherry buds at frequent intervals showed that as far as external evidence of size was concerned, the untreated buds showed slightly larger size as the first few weeks went by and they were somewhat noticeably larger when examined on the 31st of December, 1947. Between this date and the 2nd of April, 1948, the treated buds had almost caught up and very little difference in size could be noticed between the treated and the control.

But when a subsequent examination was made on the 17th of April, it was evident that while the control buds had advanced farther towards subsequent opening, though still closed, actually as far as the size of the buds was concerned, many of the treated buds were definitely larger than the control, both in length and width. Seven days later, i.e. on the 24th of April, 1948, such a size comparison was no longer possible, since in the untreated tree, the pedicels had elongated and the individual flowers still closed, had emerged out of the bracts while in the case of the treated tree, many buds had swollen further but no such elongation of the pedicels had taken place. Please see Plate 8 for comparison.

After another interval of six days, i.e. on the 30th of April, 1948, in many flower-buds of the treated tree, the pedicels had elongated with the flowers still closed, a stage comparable to the buds of the control, 6 days earlier. In making observations of the flower-buds of this comparable stage, it was noted that those of the treated tree were again much longer and broader than the buds of the untreated. At this stage, in both the treated and the untreated, the length of pedicels was variable and was not a factor for comparison.

Opening of Buds.

Day by day observations showed that the untreated tree attained its full bloom on the 30th of April. On this date, flowers in the treated tree were still closed without exception. This was evidence by itself of the effect of treatment in delaying bloom. Further daily observations revealed that the upper levels of the tree were the earliest to bloom, and in a relatively uniform manner. The maximum flowering in these relatively healthy portions of the tree was attained on the 7th of May, indicating a definite delay of 7 days, as a direct response to treatment.

In the rest of the tree, the story was different. The behavior of blossoming was erratic. The flowers were opening here and there at irregular intervals. There was definitely an interfering factor of injury but there were evidences, however, that the delay in these cases was due to a combination of factors of effect of injury and effect traceable directly to the treatment, as the formative pattern of the flowers subsequent to the opening proved in almost all the flowers. In these portions of the tree, flowers were seen opening, as late as the 13th of June, 44 days after the control had recorded full bloom.

Floral Buds of Sweet Cherry

The untreated sweet cherry tree attained full bloom on the 28th of April. Since, as mentioned before, the treated tree was severely injured, there was no possibility of demarcation between the injured and the uninjured portions, with the result that all that could be said was that there was definitely a delay in opening of flowers, and that the first few flowers opened 9 days later than the untreated and subsequently, the flowers continued to open at irregular intervals here and there. On the 4th of June, 1948, 37 days after the date of full bloom of the untreated tree, a few flowers were still opening. As late as the 19th of July, two flowers were noted opening out. In sweet cherries as in portions of the sour cherry tree, the delay in blossoming was due to a combination of effects of injury and treatment, as the formative patterns of flowers and fruits produced subsequently showed.

Leaf-buds

General Observations.

The effect of the treatment in retarding the opening of the vegetative buds was even more severe. There was not only delay in the opening of the vegetative buds; there were buds that were killed completely as mentioned under "Injury". There was curvature and twisting of leaves as they emerged from the buds (Plate 5). In some severe cases of affection, the leaves as they emerged were chlorotic to some extent and had mottled appearance. On account of these differences between the untreated and treated trees, the comparable appearances of the trees were also different.

Sour Cherry.

On the 3rd of May, 1948, the untreated sour cherry was almost fully clothed with foliage and in contrast, the treated tree was almost bare of leaves, with a very negligible number of opened vegetative buds. It was only on the 28th of May, that the tips of branches and the upper levels of the treated tree were noticed to be covered with leaves, indicating a delay of at least 25 days in the opening of vegetative buds of the sour cherry. From this date onwards, the opening of the buds in the other portions of the treated tree was by fits and starts and was prolonged by an indefinite period. For instance, as late as the 10th of October, 1948, 3 buds were noticed opening.

Sweet Cherry.

In the treated sweet cherry, the retardation effect and the irregularity of opening of vegetative buds was even more severe. The maximum foliation had occurred by the 30th of April, 1948, in the untreated tree, and it was not until the 28th of May that the tips of branches and the topmost levels of the treated tree showed foliation. The opening of vegetative buds on this tree was noticed as late as the 19th of July, 1948, and the reason why no further opening was noticed was obviously due to the fact that the rest of the buds had been killed on the treated tree.

Thus, in comparison to the floral buds, the retardation effect and the irregularity of opening of vegetative buds was even more severe.

Discussion

The whole question of delayed foliation and delayed blossoming is related to the subject of bud-inhibition, the relation between which and the growth regulators has been a subject of inquiry by several workers (for example, 90, 103, 52, 68). Observations on the extent of delay of flowering have been made by Krone and Hamner (53) who reported a delay in gladiolii of about two weeks as a result of the soil application of 2,4-D at 10 to 20 pounds per acre, by Winklepeck (107) who reported a delay of 11 days in peach as a result of aqueous spray of naphthaleneacetic acid at the rate of 125 mg. per liter prior to blossoming and by Hitchcock and Zimmerman (46) who reported a delay of a few days to fourteen days in sour cherry depending on the concentration and time of applying potassium naphthaleneacetate summer sprays. The inhibition effect on vegetative buds has been studied extensively (19, 37, 91).

While in all this literature quoted, a direct response of delay in opening of buds was reported, there are a few workers (57, 63) who considered any delay obtained as being associated with injury and not a direct response to treatment. In our experiments, the delay in almost all cases was to some extent at least due to the treatment as shown by formative responses but the greater the injury, the greater was the additional delay.

4. Formative Effects and Histological Studies

The Fruit

Under these studies, the fruit is taken up first, because of convenience, its economic importance and because the most notable modifications are found in it, as a result of treatment.

Comparative Description of the Treated Fruit.

Since the pattern of changes was almost identical in both cases of sweet cherry and sour cherry, the description applies to both unless there is a distinctive point to be made or a distinctive measurement to be stated. The original measurements of the treated and untreated fruits are mentioned in Tables 1 and 2. The description given here is to emphasize the changes in the morphology of the fruit, also from a horticultural point of view, compared to the untreated fruits. Plates 11 and 12 show the photographs of comparative appearances of the fruits of the untreated and treated sour cherry and sweet cherry respectively.

Hanging Quality:- Fruits of the treated cherry held tenaciously to the tree. In many cases, it was not possible to harvest the fruit by stripping it from the tree without the stem. If the fruit was mature enough at the time of harvest, attempts to pick the fruit would

Table 1. Measurements of Fruit of Sour Cherry*

	Untreated	Treated
Size - length in mm.	16.9	20.1
diameter in mm.	19.4	19.0
Weight in gm.	3.7	4.3
Volume in ml. water displacement	4.1	4.2
Width of receptacle in mm.	3.2	6.5
Stem - length in mm.	25.9	22.1
thickness in mm.	0.8	2.1
Stone - length in mm.	9.4	11.7
width in mm.	8.7	8.2
thickness in mm.	6.7	7.0
Volume in ml. water displacement	0.27	0.32
Weight in gm.	0.4	0.9
Thickness of stony pericarp in mm.	0.8	1.6

*Values given are from average of 100 fruits.

ordinarily result in the soft flesh tearing away, leaving the pit and stem on the tree. A closer examination revealed that it was because the pit and the vascular system were firmly attached to each other. Observations on the vascular system will be mentioned later. In fact, even after the fruits were over-ripe and rotting, it would be hanging on to the tree in contrast to the normally easier dropping of the fruits of the control trees.

Size:- By water displacement method, the average volume of the treated fruits was almost identical with

Table 2. Measurements of Fruit of Sweet Cherry*

	Untreated	Treated
Size - length in mm.	18.4	22.1
diameter in mm.	21.9	19.8
Weight in gm.	4.9	5.8
Volume in ml. water displacement	4.8	4.7
Width of receptacle in mm.	3.5	9.1
Stem - length in mm.	33.0	45.1
thickness in mm.	1.2	2.1
Stone - length in mm.	9.6	11.3
width in mm.	8.8	9.8
thickness in mm.	6.9	8.9
Volume in ml. water displacement	0.50	0.67
Weight in gm.	0.2	0.4
Thickness of stony pericarp in mm.	1.1	2.3

*Values given are from average of 100 fruits.

that of the untreated fruits, within about 2 per cent. This came as a surprise, since the differing shapes of the treated and untreated fruits gave no indication of that being the case.

Weight:- When the same set of fruits that were used for measurement of size above were used for weight measurement, it revealed that the treated fruits showed definite increase in weight, over the untreated. The sour cherry showed 16.2 per cent increase and the sweet cherry 18.4

per cent. Increase of weight of fruit as a result of application of plant growth regulators has been reported for example, in tomatoes (40), in blackberry fruit (58) and pineapple (28).

Shape:- One of the most evident effects of the treatment was on the shape of the fruit. The fruits of the treated trees were elongate and broader near the stem end and tapering down to a point at the apex, usually with a small persisting portion of the style. This description was broadly true of almost all the fruits irrespective of from which portion of the treated tree they came. The only variation in shape that was noticeable was the more regular or symmetrical shape of the fruits coming from the healthier portions of the tree and the lack of that regularity and symmetry, increasing with the injury to the tree. All this is in contrast to the fairly round and regular shape of the fruits from the untreated tree.

Some measurements of length and diameter of the fruits were indicative of the pattern. The length of the treated fruits was 18.9 and 20.1 per cent higher in the sour and sweet cherry respectively, over the untreated. The diameter was 2.1 and 9.6 per cent less respectively, over the untreated. A length/diameter ratio is even more indicative of the elongation aspect of the fruit and such a ratio is higher in the treated fruits.

Suture:- The suture of the treated fruits was more distinct.

Apex:- The treated fruit had a well-pointed apex, sometimes with a small nipple-like protuberance as previously mentioned. In contrast, in the untreated fruit, it was almost absent, but with a very indistinct blunt point at the tip.

Receptacle:- In the untreated fruits, the receptacle is almost dry and shrivelled to the size of the pedicel, but in the treated fruit, the receptacle is often green, fleshy and spread out in more or less a circle with an increase in diameter of 103.1 and 160.0 per cent in the sour and sweet cherry respectively, over the untreated.

Marth and Meader (42) reported that the receptacle of blackberry was stimulated by application of growth regulators at time of flowering.

Stem or Pedicel:- The treated pedicels of sour and sweet cherry showed an increase in thickness of 162.3 and 75.0 per cent respectively over the untreated. They were also firmer to the touch and this firmness was most noticeable at the time of taking freehand sections for histological studies. Increased development of the pedicel has been reported by others. Among them, Stewart and Ebeling (87) reported increased thickness of pedicel and secure attachment of lemon fruit when 5-year-old lemon trees were sprayed with 2,4-dichlorophenoxyacetic acid in September. Clark and Kerns (43) found that high concentrations of naphthalene-acetic acid resulted in larger and thicker peduncles and difficulty was experienced in separating the fruits from the peduncles at the time of picking.

Color of the Fruit:- The treated fruits were definitely lighter in color than the untreated fruits, and this lightness of color was more accentuated in the case of the sweet cherry. Decrease in color as a result of application of growth regulators has been reported in tomato (21).

The Skin:- The skin of the treated fruit was pronouncedly thicker, tougher, more astringent and more adherent. Stewart and Klotz(44) reported that as a result of 2,4-dichlorophenoxyacetic acid application, the rind of oranges became several times thicker than the normal.

The Flesh:- In the treated fruits the flesh was lighter colored and the resulting juice was also lighter colored. Regarding texture, since in the treated fruit, there was lack of extension of the vascular system into the flesh, the flesh seemed finer to the touch than in the control fruit where, because of the greater extension of the vascular system into the flesh, it seemed to be coarser to touch.

Taste and Flavor:- The flavor of the treated fruit was relatively poor and taste relatively insipid and less sweet.

The Stone:- The stone in the treated cherry had elongate and pointed shape like the fruit and the surface rough and variously ridged, compared to a roundish shape and an almost smooth surface of the stone in untreated fruit.

As far as adherence of the stone to the flesh was concerned, as explained previously, it was easy to slip off the flesh from the stone with the vascular system sticking to the stone in the case of the treated fruits, whereas this was not possible with the untreated fruits.

Comparative measurements of the three dimensions of the stone revealed that there was a closer correlation between the shape of the fruit and the shape of the stone in the case of the treated fruits than in the case of the untreated fruits.

The size of the stone as a result of treatment showed by water displacement method 18.5 and 34.0 per cent increase; the weight 125.0 and 100.0 per cent increase; and the thickness of the stony pericarp 100.0 and 109.1 per cent increase, respectively over the untreated.

These figures are indicative of an undue development of the stone, in relation to the rest of the fruit.

Development of the Fruit.

A clear picture of what we have seen of the large number of modifications in the mature fruit as a result of the application of spray of 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid can only be obtained by going through the development of the fruit at the different stages of growth.

The observations made are detailed below:

The Shape of the Fruit (some developmental observations):- At the time when the flowers are just open, regarding the shape of the ovary, close observation reveals no very significant differences between the treated and the untreated. But there is a factor which creates an impression of a difference at first glance. The style of the treated pistil is much enlarged in size, and often it tapers at its base indistinctly into the tip of the ovary and the ovary itself is not much thicker at this stage than the base of the enlarged style and all this contributes to a different appearance of the ovary. In fact, however, the ovaries in the flowers of both the treated and untreated are elongate and more or less cylindrical. The shape is very distinct in the case of the ovary of the untreated because of the distinctness with which the style separates off from the outline of the ovary. At this stage, there is no relationship to the ultimate shape of the mature fruit, particularly in the case of the untreated cherry where normally the shape is more or less round. In this respect, perhaps, the ovary of the treated cherry is slightly more akin to the final shape of its mature fruit, which is elongate, but in other respects the proportions of dimensions are different. When observations were made of the fruits of untreated sour cherry on May 21, 1948, they were still cylindrical, with the tip rounded off and with a little portion of the style attached. Before 7 days more elapsed, i.e. by the 28th of May, the fruits had slightly enlarged breadthwise. From then onward, every external evidence of growth was more and more towards the ultimate rounded shape of the fruit. In the case of the fruit of the treated sour cherry, within about 15 days of fruit-set, the fruits were more like the ultimate shape of the fruit. Here again, the persistent style and the original elongate nature which does not change much is responsible for this distinguishing feature.

The pattern of behavior in the matter of shape was the same in both the cherries.

A discussion of what factors contribute to the modification of shape will be made later.

Size:- Here again, when the flower is just open, there is very little distinction between the ovaries of the treated and untreated trees. We have already noted the little difference in size of the mature fruits also. A discussion of the factors that contribute to the maintenance of size, irrespective of treatment will be made later.

Histological Observations of the Developing Fruit.

General Observations:- The observations made here and the data quoted in their support relate to sour cherry. This is because, for comparison and proper interpretation, the work of Tukey and Young (98) is available. But examination of sweet cherry revealed broadly the same pattern.

According to Tukey and Young (98), the ovary wall consists of three principal tissues, the inner and outer epidermis, inner and outer stony pericarp, and the fleshy pericarp. The fleshy pericarp in turn consists of an innermost layer of small thin-walled parenchyma, a middle region of large thin-walled parenchyma and an outer or hypodermal layer of collenchyma. The fleshy and stony pericarp are derived each from distinct group of cells. The developmental behavior of the fleshy and stony pericarps is somewhat different. In the three characteristic stages of development mentioned under Tukey (95) in "Review of Literature", while in Stage I both the pericarps multiply and enlarge, in Stage II greater activity is in the stony pericarp, where there is continued thickening of cell-walls and hardening and there is less activity in the fleshy pericarp, where the enlargement of cells is very slight, and in Stage III there is almost cessation of activity in the stony pericarp but in the fleshy pericarp, there is greatest activity with increase in size of cells, radial elongation of cells towards the inner regions of fleshy pericarp and tangential elongation of the hypodermal cells.

Examination of series of cross-sections of the developing fruit from the full bloom stage revealed that in the broad pattern of development, the treated fruit behaves just in the same manner as described by Tukey and Young (98). But there are certain features which stand distinct as a consistent result of the treatment. To indicate these features, detailed diagrams have been made at certain relevant stages (Plates 14, 15, 16).

Figure 1 of Plate 14 is a cross-section of the fruits in the early part of Stage II, when the first rapid development of pericarp has been completed and thickening and hardening of the cell walls of stony pericarp has commenced. There are certain conspicuous differences which become immediately evident, particularly in the vascular system and the stony pericarp.

The Vascular System:- As shown in the diagram, the relative position of the vascular system has completely changed. According to Tukey and Young (98) and as shown in the drawing (Plate 14, Fig. 1B), the vascular system normally exists as a ring of vascular bundles, generally 18 to 20 in number and extend through the fleshy pericarp. In the words of Tukey and Young (98), "the vascular system, moreover, is extended progressively throughout the tissue as the fruit develops, largely by transverse divergence of small bundles from the main bundles...... At maturity the vascular bundles ramify throughout the fleshy pericarp to give a skeleton network of conductive tissue."

In contrast to this, in the case of fruit of the treated sour cherry, the relative position of the vascular system has shifted from about the center of the fleshy pericarp to close to the outer layer of the stony pericarp. But for a few interruptions, it hugs close to the outer edge of the stony pericarp. There has also been a great amount of proliferation of the vascular tissues resulting in an undue thickness and coalescence of bundles giving an appearance of one solid mass of vascular tissue with very few gaps in-between, in contrast to the "ring of vascular bundles" in the fruit of the untreated cherry. Examination of the ovary, just after the flower opened from the bud, revealed that this pattern of vascular system had already been established prior to opening. Moreover, this pattern appeared even more accentuated when mature fruits were examined. Plate 14, Fig. 2 shows a part of cross-section through epidermis and fleshy pericarp extending to almost the outside of stony pericarp. The remarkable proliferation of the vascular tissue into a thick mass of network is shown out against the moderate size of the vascular tissues in the untreated mature fruit. The condition and extent of the vascular system is most impressive in opening up the fruit by hand. The outer portion of the flesh comes off free from the stony pericarp and its strong vascular tissues, adhering to and enveloping the stony pericarp.

Stony Pericarp:- The next important difference lies in the stony pericarp. There are evidences of proliferation of the tissues of the stony pericarp resulting in a greater thickness of the stony pericarp. Its ultimate thickness being more than twice the normal has already been mentioned. The proliferation is so irregular that the margin of the outer layer of stony pericarp takes on an uneven, wavy, and rough outline. The appearance remains so throughout the development of the fruit so that finally when a mature fruit is opened, and the clinging vascular tissues are removed, and the stone is wiped dry, the difference in appearance of the stone of treated cherry from that of the untreated is remarkable, the surface of the untreated being relatively smooth, in contrast to the roughness of surface of that of the treated, as shown in Plate 13. The accentuation of these rough markings on the sweet cherry stone is well seen in the photograph. It is obvious that the irregularly outlining vascular system clinging to the stony pericarp region even prior to opening of the flower-bud has exerted also a retarding influence on the expansion of the cells of the stony pericarp at the points in contact and has thus contributed to the irregular markings on the stone of the treated cherry. Part of the markings may also represent the impression left by the clinging vascular tissues early in the development of the stony pericarp, when the cells were still pliable.

Cellular Details.

Stage II:- Tukey and Young (98) have also reported enough information on the cell-size, shape and thickness of cell-walls, etc., of the different tissues of the sour cherry at different stages of growth of the fruit. Early part of Stage II marks an important stage in development and due to the suppleness of the stony pericarp for taking sections of the fruit, it has advantage in detailed observations of the cells. Drawings in Plate 15 represent portions of epidermis, hypodermis, fleshy pericarp, and outer and inner stony pericarp. As shown in the diagram at this stage, the cells of the epidermis and hypodermal layer do not show much difference in size or shape, between the treated and the untreated. In the case of the cells of the thin-walled parenchyma of the fleshy pericarp, there is very little difference as far as size is concerned, but regarding shape, the cells of the untreated are more regular than those of the treated. It appears as though, in the treated, the enlargement of these cells prior to Stage II has been rather irregular, resulting in some amount of intercellular spaces.

In the case of the outer layer of the stony pericarp, the individual cells of the treated are slightly larger, but the amount of thickening at this stage appears to be more or less the same, and there is very little difference in shape.

Regarding the inner layer of the stony pericarp, the shape of the majority of cells, and the kind of juxtaposition of individual cells, dovetailing into each other, are the same in both treated and untreated. But there again seems to be certain irregularity in expansion of individual cells of the treated.

Stage III:- In this connection, it must be remembered that

Stage II of which we have reported above, represents, according to Tukey and Young (98), the culmination of a period in which the cell-enlargement of the fleshy pericarp had not progressed much. It is Stage III in which a rapid enlargement occurs with certain changes in shape of cells in the different regions of the fleshy pericarp. To ascertain, therefore, in what manner this aspect of development has been affected by treatment, tissues were examined from the cross-section of the mature fruit and the results are shown in Plate 16. It shows the following features:

While the tangential enlargement of the epidermal cells does not differ much, that of the hypodermal cells has been conspicuously greater than that of the untreated. In fact, during Stage III, the growth of the individual cells of the hypodermis in the treated fruit has all around been greater than that of the untreated fruit. The result of all this with regard to the hypodermis is reflected in the additional thickness of the skin noticed in the treated fruit.

In examining the size and shape of cells involved in the final swell of fleshy pericarp in radial direction, the pattern is similar to that mentioned by Tukey and Young (98), irrespective of treatment, viz. that the cells next to the hypodermal layer are roundish, followed by oval shape indicating beginning of radial elongation followed subsequently by a decided radial elongation in the inner region. As far as the individual size of cells in cross-section is concerned, there are only slight differences.

The Pedicel

General Observations.

A study of certain histological aspects of the pedicel is essential for the following reasons. It is important as an intermediary between the branch and the fruit, whose nature of conductive tissues to a large extent decides the type of nutrition to the fruit. It plays an important role in the abscission phenomena connected with the fruit. Besides all this, we have previouly described certain modifications in the pedicel as a result of treatment, which need correlation with a study of the internal structure. Plate 17 shows the cross-sections of the treated and untreated pedicel at the mature stage of the fruit. In examining the different zones, there has been increase in thickness of every zone, the epidermis, the cortex, the fiber-sheath, the vascular cylinder, and the pith.

Fiber Cells:- The fiber-sheath, which by virtue of its position may be called as pericycle fibers, is shown as continuous in the diagram for the sake of convenience, but it is actually discontinuous, and consists of groups of fiber cells with gaps in between. The fiber-sheath of the treated is more continuous, or in other words, the gap between groups of cells is relatively smaller and each group is individually larger and more extensive. Plate 19 shows the differences in individual cells. In the untreated, the cell-wall is so thick that there is hardly any vacuole left, while in the treated there is relatively less amount of thickening, but fair enough to contribute to the strength of the individual cells. The main purpose of fibers cells is to contribute to strength of the tissue, and this difference in thickening of cell-wall is of minor importance from this point of view, since in the case of the treated pedicel, the largeness of individual cells, the largeness of individual groups and a greater continuity of fiber-sheath all contribute to greater strength and firmness of the pedicel, which has been observed in handling the pedicel, even without examining the tissues inside.

The Vascular System:- Of the other tissues in general, the vascular system again, as in the fruit, is of striking importance. If the radial width of the vascular region is taken into account, (See Plate 17, E and F) that of the treated pedicel is more than twice that of the control. What this means in actual total area of the vascular tissues in cross-section and in the ultimate amount of vascular tissues inside the pedicel is of great significance, in the conduction of nutrients from the stem into the fruit.

In a closer examination of the phloem region and the xylem region, in several sections, it was consistently clear that the phloem zone exceeded in its radial width over the xylem, in the case of the treated, while it was the reverse in the case of the untreated pedicel. Besides this, a detailed examination showed that not only has there been during the development of the pedicel, a greater activity of the phloem but also to some extent an inhibition of the activity of xylem, of which we will mention in a little more detail in dealing with the shoot growth in spring and summer, during the period of our experiment.

While this was the condition in the vascular system of the mature pedicel, examination of the pedicel just when the flower was open revealed generally a fluted shape of the vascular cylinder in cross-section, which developed later into a more uniform thickness, as shown in the mature pedicel. At the time of flower opening, in the treated cherry, the number of ridges in the fluted shape of the vascular cylinder generally exceeded those of the untreated, the former being 10 to 12 and the latter

6 to 8. It was sometimes noticed that, at this stage, the bundles might be distinguished as individual bundles with very little gap particularly towards the center.

At this stage of flower-opening, there already has arisen a remarkable difference in size of the vascular tissue. Plate 18 shows a camera lucida drawing of cross-sections of comparable portions of the vascular system. In the treated, the radial width of the vascular area is twice that of the untreated and the tangential width about one and one-half times. Calculations of relative areas in cross-section indicate the vascular system being 4 to 5 times larger at this stage due to treatment.

A comparison also at this stage between the relative proportions of phloem and xylem indicate that in both treated and untreated, the xylem elements seem to be greater in content than the phloem, but the greater activity of the cambium layer is already evident, as represented by a wider band as indicated in Plate 18.

The comparatively greater development of the vascular system as a result of treatment, accompanied with certain other important differences in details becomes even more impressive, in watching subsequent development of the pedicel. For instance, Plate 19 represents camera lucida drawings of the same comparable portions of vascular tissue as seen at the end of 3 weeks after full bloom. In comparing these drawings with those of Plate 18, certain points stand out clearly. In the untreated, during this period, the vascular tissue has doubled itself, both radially and tangentially. But even after three weeks of development, it has not caught up, however, with the radial development that the tissue of the treated had attained three weeks earlier.

Besides this, the measurement of the radial width shows that in the untreated, the development has been more towards greater development of xylem than of phloem.

In the case of the treated, the tendency of development is clear. While the radial width of the xylem has changed very little, the average width of the phloem has almost doubled. What was a ridged cylinder is being straightened out into a more uniformly thickened cylinder and in doing so, the phloem is tending to expand farther than the xylem. The ultimate result, however, of this relatively higher development of the phloem over the xylem in the development of the vascular tissue, as a result of treatment is most clearly brought out in the examination of the cross-section of the pedicel, when the fruit is mature, about which we have already spoken before.

Thus, as a result of treatment, a more expansive development of the vascular system takes place in the pedicel, as we have already seen in the fruit. We have also gone one step further in noticing that there is a relatively higher activity in the phloem than in the xylem. From all this, it would be reasonable to assume that the most important contributory cause for the strength of the pedicel of the sprayed tree is its well developed vascular system and among other important causes is the more extensive pericycle fibers encircling the pedicel. The importance also of the development of phloem in its nutrition role cannot be overlooked. Plate 19 also shows a conspicuously wide band of cambium zone in the pedicel of the treated. In fact, under the microscope, it was in the nature of a multilayered band, giving the impression of a ladder-like structure and giving indications of delay in differentiation of derivatives.

Other Tissues:- Plate 20 shows the camera lucida drawings of representative cells of the tissues other than the vascular and fiber. The drawings show the obvious differences and similarities. There were clear differences in the cuticular structure. It seems as though because of a greater length of the epidermis, forming the circumference of the treated pedicel, the cuticle gets flattened out (See Plate 20).

In the cells of the epidermis, the collenchyma and the parenchyma cells of the cortex, in the treated, there has been cell-enlargement by about 50 per cent, on the average, over the untreated, but no change in shape from the untreated. Further development of the pedicel showed even more increased enlargement of the cells of these tissues. It was evident from observations throughout the development of the pedicel that the increase in size of these regions, epidermis, collenchyma, and the cortex in the case of the treated, was almost solely on account of the increase in size of the cells.

Examination of the cells of the pith did not show much difference in size at the time of opening of the flower but in further development showed enlargement in size, contributing its quota to increased thickness of the pith, in the treated pedicel.

In calculating the increase in size of the cells due to treatment, it was found to vary over the untreated from 60 to 100 per cent in the case of pith and cortex and 50 per cent in the case of epidermal and collenchymacells, during the whole course of development.

Other Parts of the Flower

Having dealt with the ovary, its development to a mature fruit and with the pedicel, it is relevant at this juncture, to note observations on the other accessary parts of the flower.

The Receptacle.

The larger size of the flower buds of the treated cherry, noticed prior to opening and mentioned before in the studies of retardation of opening of floral buds, was mostly on account of a fleshier receptacle. Thus from the very early stages upto fruit maturity, the more prominent receptacle is one of the consistent responses to treatment. Its spread in the mature fruit compared to the untreated has been described under the comparative description of the treated fruit.

The Calyx.

The first difference that is noticed between open flowers of treated and untreated cherry is the difference in angle at which the calyx lobes are bent. Normally in an open flower, the lobes are completely reflexed, so that the lobes are bent parallel to the axis while in the flower of the treated cherry, it was very usual to find varying degrees of bending, some of the flowers having the lobes either at right angles to the axis or even bent slightly in the opposite direction to the normal or, in other words, tending to bend towards the axis. (See Plate 9)

This aspect in the calyx is emphasized, because it is in consonance with a tendency observed for certain types of organs of the plant to bend in the same direction.

Besides this feature, the lobes of the calyx in the treated were thicker and narrower with a characteristic triangular shape, a relatively acute tip, more prominent, more numerous and deeper serrations, and more conspicuous venation, than in the untreated.

The Corolla.

A little more study of corolla than of the calyx was involved, because of the strikingly different appearances of the petals as a result of treatment, even at first glance.

The change in shape was the most easily noticeable. The petal had become elongate and narrow compared to an almost roundish shape of the petal. (See Plates 9 and 10). It was usual that the increase in length was by 20 per cent and the decrease in width by 33 per cent compared to the dimensions of the untreated flower.

The thick, leathery texture with a stiffness of the petal was again most striking. The increased thickness consisted mostly of the enlargement of cells, of inter-cellular spaces and of the vascular tissues, which latter contributed also to the stiffness of the petal. In dissection of the flower, consequently, it was easier to handle compared to the petal of the untreated cherry which was comparatively delicate, thin and easily folded on itself. It was also not so easy to pull out the petal of the treated cherry.

The very thick and prominent venation of the petal of the treated cherry was even more striking. A detailed drawing of the venation has been made in Plate 21. This is in many ways comparable to the character of the vascular system in the fruit, particularly in its massiness, and its lack of tendency to ramify. The finer ramifications as seen in the flower of the untreated cherry are almost lacking in the treated.

A surface view of the epidermal cells indicated certain differences and a group of such cells have been drawn (Plate 21). The waviness of the margin of the cells of the untreated petal becomes more or less levelled up as a result of treatment and the distinct increase in size is also indicated. It is a case of longitudinal stretching.

Lastly, another important feature with regard to the petal is again the tendency to incurve or to bend towards the axis, so clearly seen in Plates 9 and 10.

The Stamens.

The following features were noticed as a result of treatment.

The length of the stamens was variable just as in the case of the normal flower, but the average length was often higher, particularly in the case of the sweet cherry, by 20 per cent (Plate 10).

There was a decided increase in thickness of the filaments, often being twice thicker than the normal filament. This was most prominent in the sweet cherry (Plate 10). The increased thickness was mostly on account of enlargement of individual cells and increased vascular material. This was reflected also in handling of the stamens in dissection, being firmer and stiffer and easier to handle.

The anthers were noticeably larger (Plate 10) and they had a characteristic heart-shape, pointed at tip, compared to the roundish shape of the anthers of the normal flower.

The stamens, again like the petals, had a tendency to incurve or bend towards the axis.

The viability of pollen of the flowers was tested by germinating it in sugar and agar media and treatment was not found to affect the normal viability of the pollen.

The Pistil.

The ovary and the development of fruit have been dealt with above.

The increased thickness of style, as well as its persisting quality as a result of treatment, has also been mentioned.

The stigma was also slightly larger, but more irregular in shape, as a result of treatment.

Enlargement of style and its unusual persistence as a result of application of growth regulators has been recorded in tomatoes (40) and in oranges (88).

The Leaf

External Modifications.

Under delayed foliation has been mentioned the fact that in many cases of leaf emergence, the leaves were twisted (Plate 5). Here again, as in certain cases of flower parts mentioned before, the twisting or curving was a consistently regular pattern, i.e. tending towards the axis.

This was not characteristic, however, of the leaves in portions of the tree which were relatively uninjured. In such cases, the emergence was fairly normal, but often with a slightly noticeable twist and the leaf expansion was also normal. But consistently the leaf was narrower, the tip acutely pointed, the serrations more numerous particularly at the tip, much thicker, not as pliable as the normal leaf, and with a stronger and thicker petiole, and with a very prominent and thick midrib and venation. Most of these characteristics are evident from Plate 9.

But distributed over the rest of the tree were all degrees of twisting at emergence, later showing varying degrees of expansion, all depending on the extent of injury.

One of the most striking features was the number of modifications of size and shape of leaves that could be collected at random from the tree, as a whole when the leaves had reached maturity. A representative sample is shown in Plate 7.

But from whatever portion, the leaves might be gathered, there were certain common features for all the leaves from the treated trees. They were a tendency towards narrowing and elongation of the leaf, the thickness of the leaf, the prominence of the veins and the midrib, greater number of serrations, and the apex of the leaf being acute and pointed.

In other respects, there were differences. Some of the leaves were chlorotic or had a mottled appearance, and in some, the veins were translucent and on the whole had the appearance described by Zimmerman and Hitchcock (109) as resembling virus infected leaves.

But in the case of leaves coming from relatively uninjured portions, it was usual to find the leaves actually looking greener than the normal leaves, indicating stimulation to chlorophyll formation. Such stimulation by plant growth regulators has been indicated by workers (73, 74).

Internal Structure.

A study of the internal structure of the leaves reveals a few important features as indicated in the diagrams (Plate 22).

The cross-section of the midrib shows the increased size of almost every region in it, particularly the cortex, the vascular tissues, and the fiber-sheath. Here again the fiber-sheath is not continuous, though shown in the diagram for convenience but consists of groups of fiber-cells which are discontinuous. But just as in the pedicel, the individual fiber-cells are larger, the individual groups are bigger, and the gaps between two adjacent groups smaller as a result of treatment.

Here again must be noted the proportional larger phloem zone as compared to the xylem, as was pointed out in the pedicel.

The cross-section of a segment of the lamina shows the following features as a result of treatment:

The number of layers of cells that form the thickness of the lamina are more or less the same. But there is a greater increase in size of the cells, particularly the length, and there is a marked increase of intercellular spaces both in the palisade and spongy parenchyma.

In fact, the increased thickness of the treated leaf is almost solely made up by the elongation of cells in cross-section and by the increase of intercellular spaces. There has also been increase in the size of the epidermal cells.

A surface view of the epidermal cells reveals that the individual stomata are larger in the treated leaf. A longitudinal stretching of the epdiermal cells is also indicated.

Discussion.

The treatment in our experiments thus resulted in increase in growth of the midrib and veins, and also increase in growth of the mesophyll. Some previous work relating to this is of an interesting nature. Avery (2) working with leaves of Nicotiana was the first to correlate growth of the midrib and probably of the larger lateral veins with auxin concentration. He indicated that auxin is not directly concerned in the growth of the lamina, it giving no response to applied auxin. Went and Thimann (105) and Went (102) also suggested distinction between the growth of the vein which can be increased by auxin application and growth of mesophyll which is independent of auxin. Bonner (17) went further and obtained gross enlargement in area and thickness of the leaf sections of tobacco and radish grown in pea diffusate and the internal structure of such treated leaves showed that there was cell enlargement and marked increase of intercellular spaces of the spongy parenchyma, a result that is identical to ours. He concluded that the growth of leaves is also controlled by a hormone. It is therefore of deep interest to consider here in our experiment that a mixture of 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid, in the case of the leaf, have not only substituted for the auxin in increasing the thickness of the veins and midrib but has also substituted for or stimulated the production of hormone for the growth of mesophyll.

The Shoot-growth in Spring and Summer

Among the observations made on the spring growth was the fact that there was a tendency for reduction in length of the shoot, varying in degrees. But invariably, the stem was much thicker, firmer and stronger as a result of treatment.

Plate 17 shows the various regions as seen in cross-section. There may be noted certain simularity in features between the cross-sections of the annual growth and the pedicel. Even the characteristic shape of the vascular cylinder is similar. The points explained in connection with pedicel with regard to size, shape, and character of cells in several tissues as well as broad features of development, are almost generally applicable to the spring growth, as far as histological observations are concerned.

It is here, however, that the points regarding the comparative development of phloem and xylem can be even more emphatically mentioned.

In the cross-section of the untreated stem, the radial width of the phloem zone is only one-third to one-half of the xylem region, whereas in the cross-section of the treated, the position is almost the reverse; at any rate the radial width of the phloem region is greater than that of the xylem.

Besides this, the difference in size of the xylem vessels between the treated and the untreated is very remarkable. The individual vessels of the normal stem are in many cases more than double that of the treated stem (Plate 17) in spite of the fact that the cross-section of the treated pedicel itself is so much larger. This indicates that not only has there been increased activity of phloem as a result of treatment but that there has been relative inactivity or even inhibition of the xylem. Inhibition of xylem as a result of treatment with growth regulators has been reported by Swanson (86).

5. Other Observations

Influence on Period of Maturity of Fruit

No detailed studies of this had been planned. But during the last days of ripening of fruit on the tree, it became evident that some of the fruits on treated trees were catching up with those on the untreated, with regard to ripening. Even then, when on the 28th of July, it was found that most of the sour cherry fruits were ready to be picked and samples were taken for measurements and detailed observations, it was not expected that enough fruits of the same stage could be obtained from the treated sour cherry for such examinations, because of the initial delay in opening of flowers, But on close examination of the tree, particularly of the higher levels, it showed that many of the fruits had attained that stage and could be picked on the same date. Considering that there was a delay in bloom by about 7 days, this was a fairly definite proof of earlier maturity as a result of treatment.

It is of interest to note here that in varietal differences in period of maturity in sour cherry, Tukey (95) has reported that the difference in duration is reflected only in the duration of the period of delayed increase (Stage II) and the durations of the other stages are not affected.

In trying to trace indications of the stage in which the treated sour cherry made up in the duration, the preserved specimens were examined step by step, for external evidences of the size, color, and firmness of fruit. The evidences tended more towards accelerated ripening occuring in the last stage. The indications are that after the completion of hardening of the stony pericarp in stage II, the stimulus is transferred from that area to the tissues of the fleshy pericarp and accelerates there the chemical and physical processes associated with ripening. Further work on the modifying influence of growth regulators on the normal pattern of development and maturity of cherry fruits as described by Tukey (95) would be of utmost interest.

The most remarkable effect on the period of maturity of fruits upto date is the report of Blondeau and Crane (15) that the period of maturity of figs was reduced from 120 days to 60 days by the use of 2,4,5-trichlorophenoxyacetic acid. Earlier ripening by growth regulators has been reported also by other workers (67, 35, 3).

In this connection it may be stated that there are certain factors that are likely to mislead in the matter of determining the date of ripening of the treated sour cherry. The color of the fruit never attains the deep color of the normal fruit, and this difference is particularly accentuated in the sweet cherry. Again, the firmness of the pedicel helps in holding the fruit on the tree much longer on the treated tree, even though the fruit has reached its maturity earlier, giving delayed observations an impression of delayed ripeness. Moreover, even a month after the normal date of harvest there would be a few ripe fruits on the tree because of the unduly delayed opening of many buds.

Influence on the Storage Quality

When the fruits of sour cherry were harvested on July 28th and the observations and measurements required, were made, the rest of the fruits were kept away in the cold storage room, with the idea that they might later be required for any further checks, if needed, on some of those already made. When 10 days later, these samples were taken out of the cold storage roon, the treated fruits were well on their way towards decay, whereas the fruits of the control tree continued to be firm. No detailed studies were possible, as at that time not many fruits were available for comparative study. Depreciation of storage quality of fruits of plants or trees treated with plant growth regulators has been reported by others (35, 3).

Influence on Composition of Fruit

It seemed evident from the taste and flavor of the treated fruit that some changes in its composition must have occurred. The fruits were therefore analysed. The determinations as shown in the table below (Table 3) were made for sour cherry. They were averaged values of the results from replicate determinations, expressed on the weight of the pitted fruit except for the pits which are expressed on the weight of the whole fruit.

Table 3.

	Per Cent			Ml. 0.1 N acid/gram
	Dry Matter	Total Sugar	Pits	
Control	13.67	7.39	11.62	2.03
Treated	11.15	5.04	20.92	1.85

From this analysis, the following points are clear:-

1. The weight of pits has been almost doubled as a result of treatment. The increased weight, thickness and size of the pit have previously been mentioned.

2. The normal fruit shows increased percentage of dry matter, total sugar and acidity. This is the composition of the pitted fruit and not of the whole fruit. As such it gives an idea of the composition as it affects the quality of the edible portion of the fruit.

The decreased contents of sugar and acid in the pulp are reflected in the flavor and taste, the poorer quality of which has been noted previously under the description of the treated fruit.

However, to find out how the dry matter and sugar are affected, as percentages of the whole fruit including the pit, since the pit in the case of the treated sour cherry fruit forms a much larger proportion, than the untreated, this time samples of sweet cherry were tested on this basis. The following determinations in Table 4 are the averaged values of the results from replicate determinations expressed on the weight of the whole fruit.

Table 4.

	Per Cent		
	Dry Matter	Total Sugar	Ml. of 0.1 N acid/gram
Control	24.5	8.85	1.07
Treated	25.1	9.25	1.40

In connection with this data, it may be mentioned that, the sweet cherry fruits that were used were not as uniform as in the case of sour cherry, for the obvious reasons that fruits of very uniform size and stage were not available. Even so, as a total content in the fruit including the pit, the dry matter and sugar have increased, as anticipated.

Stewart and Klotz (88) reported that in oranges, as a result of application of 2,4-D sprays, the total acids and soluble acids were decreased in the fruit juice and the percentage of rind and sap were increased. Clark and Kerns (28) observed that in pineapples, spray-applications of A-naphthaleneacetic acid resulted in decrease of 2° Brix in the juice.

GENERAL DISCUSSION

Translocation of Stimulus

Among the most significant observations in this study is the remarkable influence of the treatment on every plant organ and every aspect of annual growth. This indicates transport of the "spray-stimulus" throughout the plant as a whole. Some workers (6, 81) have observed indications that the absorption of such spray materials is first through the leaf and petiole into stem. That it was clearly so under conditions of our experiment is indicated by the high physiological activity that was first shown by the leaves and the petioles where this activity led to a prevention of abscission of the leaves.

In trying to decide through which of the tissues, the stimulus is translocated, Bausor, Reinhart and Tice (9), by observing distant effects of application of a growth regulator to tomato stem suggested that "the distant movements were probably confined to the xylem and especially to the external phloem". Mitchell and Brown (62) observed that the movement of 2,4-dichlorophenoxyacetic acid stimulus in plants was associated with the translocation of organic materials from the leaves into other parts of the plant and concluded that the translocation was "apparently confined to living tissues, which indicates that it travels mainly in the phloem and parenchyma cells". We have very definite indications in our experiments that the phloem in general seems to be the most important tissue for such translocation. We have shown relatively higher activity in phloem in almost every organ of the plant, the leaf, the stem, the pedicel, the fruit. We have particularly shown indications that the xylem is not activated so highly and in fact have given instances of their inhibition, and as far as the evidence of these experiments go, the xylem probably does not play an important role in this respect. Besides all this, one fact has particularly struck us, viz., the fact that curvature towards axis has been shown by the calyx, the petals, the stamens and leaves -- all organs in which normally the phloem is on the side away from the axis, indicating that curvature has been caused because of elongation of the cells on the side of the phloem. In the case of the fruit, mobilisation of food was greatly increased, as evidenced by increased dry matter mentioned under composition and that this mobilisation and translocation of stimulus was directed to the nearest tissue of the phloem, viz., the stony pericarp, adds up to the indications of the importance of phloem in translocation of stimulus.

The Order in Succession of Responses

An orderly succession of responses is a striking feature among the observations:-

Firstly, the action on the petiole and leaf almost immediately following treatment, causing inhibition of flower and leaf-buds.

Secondly, a release of the inhibition of flower-buds, leading to opening of flowers and their normal subsequent functioning.

Thirdly, a release of the inhibition of leaf-buds leading to emergence of the leaves and their normal subsequent functioning.

It is obvious that this orderly succession of events is tied up with the state of growth of the tissues at the time of application of stimulus, the mobilisation of food materials to the points of activity at the expense of other growing portions of the plant and the movement of that activity from one set of tissues to another, releasing the inhibitions and activating them, in succession. To explain this clearly, there is earliest activity in the leaves and petioles, because they are the first points of absorption, probably because they are relatively the most tender at the time of spraying. Now, the high physiological activity caused at these portions of the plant mobilises the necessary food materials from other parts of the plant, causing inhibition particularly of both the flower-buds and the leaf-buds. After having set up the stimulus there, the stimulus now seems to move to the next set of tissues which are in a growing condition. Of the flower-buds and the leaf-buds, according to Chandler (27), leaf-buds are differentiated much earlier than the flower-buds, indicating that in the end of September, it would be reasonable to assume that the tissues are more tender in the flower-buds than in the leaf-buds so that the stimulus acts more readily on the flower-buds than on the leaf-buds. This releases inhibition at those points and at the same time accelerates their activity and by the mobilisation process continues to cause inhibition in the remaining growing portions of the plant, which are particularly the leaf-buds. This explains the reason for delay in releasing inhibition of the leaf-buds, ultimately causing delay in emergence of leaves. These conclusions are supported by the findings of workers on the nature of bud-inhibitions, the mobilisation of food factors, and the kind of tissues most readily acted upon, all as a result of application of growth regulators. Regarding the mobilisation of food factors at the region of activity of growth regulators, a consistent point of view as a result of experimental data have been presented by many workers (89, 69, 65, 97, 8, 7, 30, 52). Regarding the fact that the readiness with which plant growth regulators act on a tissue depends on its growing condition, it has been confirmed by many workers (101, 87, 41, 1).

Regarding the nature of inhibition itself, a large amount of literature has been accumulated (for example, 90, 92, 104, 103, 53, 68). Went (104) explained clearly that "the inhibition is due to removal of growth factors (in the avena coleoptile, the food factor) from the zone of inhibition, which are then accumulated near the place of application of the active substances". The findings of Went in another report (103) are of particular interest to our experiments, where as a result of application of growth regulators, for two to four or more days, the buds were completely inhibited and that the inhibition was then "almost instantaneously and completely released" and "it even seems that the initial growth of the buds was slightly more rapid than when they had first been inhibited". This is in accord with our observations that in spite of initial inhibition, the growth was highly accelerated during the subsequent period prior to opening of the bud.

The Vascular System

The histological studies have revealed that among the most significant responses of treatment has been the development of the vascular system, almost out of all comparison to the normal. There was no organ arising out of the subsequent growth to the treatment that did not show this response, and we have described its gross development in the annual shoot, in the leaf, in the pedicel, in the different parts of the flower, and finally in the mature fruit. In almost all of the literature concerning histological responses of growth regulators, activity of the vascular tissues has been recorded but it seems to the author that only a few have recorded it with the emphasis that it needs. For, in going through details of the responses in literature, one is struck with the fairly close correlation between the growth regulators and the vascular system. Among those that have laid emphasis on this aspect, Overbreek (78) reported that "auxin is most effective in inhibiting lateral buds when introduced into the vascular system of the stele.... This indicates that the place of action from which auxin controls development of the lateral buds must be the vascular system". He stated also that "any treatment leading to the development of lateral buds is followed by an increase in the vascular supply to these buds". The whole trend of our observations in this respect seem to amply bear this out.

But in dealing with the relationship of plant growth regulators and the vascular system, one of the most striking effects of these substances that is noticed when going through literature is the consistent tendency for vascularization of derived tissues even outside the normal regions of existence of vascular strands, for examples in such unusual places as the pith, the endodermis, etc. (51, 52, 79, 42). Laibach and Fishnich (54) noted in coleus that new vascular bundles appeared among the three originally present on each side.

There is no doubt that the vascular system has played an important role as a conducting tissue in producing all the responses which we have recorded, such growth responses as the increased thickness of organs - the stem, the leaf, the pedicel, the receptacle and almost every other part of the flower - and ultimately, in the increased weight of the fruit itself. There is also no doubt that it has played its part in all the formative responses that we have noticed, particularly in the fruit, some aspects of which we will reserve in dealing with the discussion of the size and shape of fruit. It has also played its part in strengthening plant organs and making abscission phenomena difficult.

Other Histological Responses

In other histological responses, it has been in consonance with the general feature of behavior of the growth regulators to the effect as expressed by Kraus, Brown and Hamner (51) that none of the types of cells was fundamentally different from cells occurring in the plant grown under the usual conditions of culture and environment.

In our experiments, the activity of the growth regulators has been greatest in the vascular region and has been mostly confined to enlargement of cells in the others.

The Size

It has been previously mentioned that the size has largely been unaffected by treatment. This leads one to suspect whether size in the cherry is a genetic factor, not easily affected by either internal or external environmental changes. A discussion of this aspect is beyond the scope of this paper. But one would have, however, expected an increase in size as a result of all the factors for accelerated growth, particularly the increased conductive tissues and the increased mobilisation of food materials. In many experiments of inducing fruit-set with plant growth regulators, significant increase in size has very often been obtained (113, 108, 99, 73, 40, 74, 72, 88). Even in our experiments, the increase in size of organs such as the stem, the pedicel, and the stamens has been the rule but the fruit has increased in weight and has been modified in shape almost out of recognition, but still the size has not increased. But to us, the greatest factor that has been responsible for this end result is the role played by the stony pericarp, a structure similar to which is not found in most of the fruits in which increase in size has been reported by the use of plant growth regulators. Sinnott (83) in discussing the differences in fruit sizes of the cucurbits reported evidence that in the case of smaller size, "a more important factor in limiting the amount of expansion is the deposition of the secondary cell-wall, which soon becomes so strong that further enlargement is impossible. The fact that it is commonly those types (notably the small fruited forms) with the least amount of cell-expansion which become the most hard and woody at maturity, and the ones with relatively great cell expansion which tend to remain soft (like the pumpkins and watermelons) seems to indicate the importance of secondary wall formation in limiting growth". Such a limitation of size has occurred in the treated cherries on account of the secondary wall thickenings of the stony pericarp. Reference to the table on page 45 will show that the weight of the treated stony pericarp has been more than doubled by treatment. This gives an indication regarding the extent of physiological activity that has gone towards the formation of a highly compact tissue, which has controlled the ultimate size of the fruit, from expanding.

All this also explains as to why the size of the pedicel which has increased even as far as nearly three times the normal, as a result of response, has not produced an increase in size of fruit, whereas in some experiments the size of fruit has been to some extent influenced by the size of the pedicel (84, 28). The pedicel, however, through its increased size of conducting tissue has contributed towards increase of the total weight of the fruit and has therefore played its expected role.

Shape or Form

One of the most puzzling questions that are involved in these experiments is regarding the consistent and definite change of shape of the fruit as a result of treatment. It has long been a fundamental problem to biologists as to what factors contribute to differences in form. Talking broadly about form in animals, Huxley (47) spoke of the growth gradients which were "either directly or indirectly correlated with the morphogenetic gradients or fields of child, Weiss and others and in general with the various polarised and field effects in the animal body". He also talked of the two phases of development which together ultimately decide the form and called them the "auxano-differentiation" and the "histo-differentiation". Coming down, however, to differences in fruit form within the varieties of the same plant species, Burr (24) in a study with the cucurbits suggested a significant relationship between the field forces in the fruit and the form which it ultimately assumes. But as Burr and Sinnott (25) have stated, "the morphogenetic implications of the problem of electrical correlates of organic form are not well understood" and a discussion of this in relation to the change in shape of the cherry fruit under these experiments is beyond the scope of this paper, except to state the application of plant growth regulators might result in modification of the field forces in the fruit.

But there are a few points which need to be stressed in this connection. First of all, it is the elongation aspect of the fruit. That the fruits are elongate in shape, Tukey and Hamner (96) emphasized with regard to cherry, under almost identical treatment to ours and it was so in our experiments as previously described. These are not the only reports of tendency of fruits to elongate as a result of treatment with growth regulators. Stewart and Klotz (88) reported Washington Navel oranges and Valencia oranges both of which became cylindrical in shape as a result of spraying trees with 2,4-D. Bryant, Vincent and Schafer (22) reported that as a result of 2,4-D spray to the apple trees, McIntosh apples, normally round oblate, had become oblong in shape and Jonathan had changed from round conic to oblong conic. In some experiments, where changes in shape have been noticed as a result of treatment with growth regulators, they have not been definitely described since they were not presumably important to their main subject of research and have therefore been mentioned merely as "abnormal", "misshapen" or "malformed". But where there was a possibility of looking at the photographs of such fruits, there seemed to be no mistaking the tendency for elongation, at least in most of the fruits photographed (109, 77). It is not only the fruits that are elongated; the leaves, and parts of the flower, like the petal and the stamen, have also shown this characteristic. The surface view of the epidermal cells in the petal (Plate 21) and in the leaf (Plate 22) show definite longitudinal stretching. Particularly in the petal, this stretching has resulted in the leveling up of the wavy outline of the cells. An examination of the longitudinal section of the fruit also shows the elongation of the individual cells parallel to the axis. All this means that the basic pattern that results in an elongation of the fruit is nothing else than that characteristic of auxins -- cell elongation. This seems to explain the first main characteristic of the fruit around which the principle modification of form has been built up.

The next thing that needs explaining is the modification of the shape of the fruit as a whole from a roundish shape to oblong conic. As previously explained under comparative description of treated fruits, commencing with a maximum diameter near the stem end, there is a tapering down to a point at the apex. In this respect, there seems to be more or less a definite correlation between the shape of the fruit and an approximate outline of the stone. If we go back to the differentiation of the zone destined to become the stony pericarp, Tukey and Young (96) have mentioned that it had happened when the ovary was examined 18 days prior to full bloom. The same thing applies to the pattern of vascular system.

When the cherry ovary was examined by us, at the time of opening of the flower, this vascular system was closely woven around the stony pericarp zone. We have also explained that this was so throughout the life of the fruit. We have also indicated that the nearness of the stony pericarp area to the vascular system seems to have been responsible for a greater stimulus to the growth of this particular tissue and the proliferation of the vascular tissues themselves has resulted in an undue concentration of the vascular system in one place, with little of that ramification, which is characteristic of a normal fruit, as per Tukey and Young (96). The vascular system after all is akin to a skeleton to which flesh of the fruit is the "clothing" and what form or appearance would be finally taken by the fruit also depends to some extent on the vascular skeleton. Here in the case of the cherry fruit, the vascular skeleton happens to follow the outline of the stony pericarp and that outline is more or less followed by the final shape of the fruit. Further, the points of reach of nutrition or even of the stimulus from a plant growth regulator depend on the distance from the vascular strands and these strands in our experiment are close to the stony pericarp and it seems as though the resulting outline of the fruit envelops points approximately equidistant from the vascular tissues and hence of the stony pericarp.

Lastly, the fairly thick short portion of the style that persists with the mature fruit adds up to the final characteristic shape of the fruit of the treated cherry.

The Preponderent Effect of 2,4-dichlorophenoxyacetic Acid.

Mixtures of growth substances have been now and then used with the hope of increased effectiveness (85, 44, 29, 4). In some instances competitive action has been noticed as in the combination of phenylbutyric acid and indoleacetic acid reported by Skoog, Schneider and Peter (85). There are specific instances, however, when 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid have been combined as a stop-drop material for apples but no more effectiveness was found than their individual action (44, 29, 4). Nevertheless, the relatively shorter period of effect of naphthaleneacetic acid has been definitely observed by several workers (44, 33, 93). Thompson and Batjer (93) have emphasized also on the greater intensity of effect of 2,4-dichlorophenoxyacetic acid. Besides this, Hitchcock and Zimmerman (47) while obtaining delayed bloom in spring in cherries as a result of summer application of potassium naphthaleneacetate, reported development of only normal fruit at a normal rate. In fact, almost all workers in this field are agreed that naphthaleneacetic acid has no formative effects, whereas the formative effects of 2,4-dichlorophenoxyacetic acid have been well-known (22, 41, 39).

With this background, if we compare the duration of effect of treatment in this experiment lasting for ten months, its intensity in growth responses and the strong formative effects, there seems to be no doubt that 2,4-dichlorophenoxyacetic acid has shown a preponderent effect over naphthaleneacetic acid at the proportion of concentrations used. It also leads to the supposition that the use of 2,4-dichlorophenoxyacetic acid by itself, at adequate concentrations, is likely to lead to the same type of responses as observed in this experiment.

Practical Applications

Importance to the Fruit Grower

From the point of view of a fruit grower who is concerned with fruit as the economic product, and with continued maintenance of the health of the tree, the chemicals used, at the proportions and concentrations mentioned, have not in our experiments led us to encourage their use, considering the extent of injury to the tree, reduction of yield, and lowering of the marketable quality of the fruit. But the fact that there were certain portions of the tree which were relatively healthy, indicates that injury to the tree and consequent reduction in yield may perhaps be lessened by lowering the concentrations. Such lower concentrations, if effective may also lessen the extent of harmful effects on quality. It is even conceivable that by further trials with several concentrations, it is possible to arrive at an optimum in which the stony pericarp is not unduly stimulated but the stimulus will be directed towards increasing the sugar content and dry matter of the pulp. After all, the final result as we have noticed has been to increase the sugar content and dry matter of the fruit as a whole.

As mentioned by Hitchcock and Zimmerman (46), the definite effects of delay in opening of flower and leaf buds through the use of plant growth regulators can be utilised in a variety of ways, in protecting the fruit trees from spring frost injury, regulating the growth and development of plants in the nursery, and extending the total period of flowering in some ornamental plants.

Importance to the Taxonomist or Systematic Pomologist

To the taxonomist and the systematic pomologist the findings present a problem, because of the ever-increasing use of plant growth regulators, particularly of 2,4-dichlorophenoxyacetic acid, for various purposes. For instance, there have been reports of the formative effects on apple fruits in orchards, where 2,4-D has been used as herbicide (22, 56). The modifications as we have seen in the cherry are thorough with regard to shape, thickness of skin, color of skin, flesh and juice, taste, flavor and, in fact, in almost all characters on which a systematic pomologist bases his classification of varieties.

Often stone markings and patterns in a drupe such as the cherry have been described as valuable evidence for identification of a variety, as for example by Blake and Edgerton (14). One of the responses of treatment in our experiment has been the entire alteration of the surface character of the stone.

Under such modifications as we have reported in the experiment, of also the leaf, floral and growth characters, the taxonomist does not have even the hope of identification of a variety by tree characters, the identification of fruit having failed. Such findings, however, open up to the taxonomist the vista of possibilities of growth regulators in this direction and impress on him the fact that plant descriptions do not stay static but that they need modification with the progress of such application of science.

Importance to the Horticulturist

In connection with the diagnostic problems that a horticulturist meets with tree behavior, it is interesting to note that Kinman in 1930 (49), in studying the problem of unproductive cherry trees in California, reported certain characteristic symptoms in the way of morphological modifications observed as a result of treatment in our experiment. According to him "the shape and color of leaves on the unproductive trees differ from those of productive trees, the blossoms are defective, and many blossom buds fail to open. In affected leaves the margins are very irregular." There was also mottling due to less chlorophyll. There were changes in shape of the fruit. A glance at the photographs of leaf modifications showed them to be similar in many respects to those in our experiments. The author at that time, 18 years ago, considered such trees a result of propagation of sporting branches. But a similarity of modifications to those described by us leads one to suspect the probabilities of hormonal maladjustments within a tree and of the role that the knowledge of such responses as we have described might play in the field of diagnosis.

Importance to the Geneticist

To the geneticist, who considers such characters as the shape and size of the leaf, the shape of the floral parts, the size and shape of the fruit as functions of the gene, it gives an idea of the hormonal complexities that might be allied with the genetic complex, in the reproduction and maintenance of characters.

SUMMARY

1. Cherry trees (<u>Prunus avium</u> and <u>Prunus cerasus</u>) were sprayed in the fall (September 22, 1947) with a mixture of 100 p.p.m. of 2,4-dichlorophenoxyacetic acid and 30 p.p.m. of naphthaleneacetic acid and the subsequent morphological and histological responses studies the next growing season.

2. There was injury to both the vegetative and floral buds, spurs and twigs, resulting in reduction of leaf area and fruit-set. Light, vigor and the method of spraying itself influenced the degree of injury in the various parts of the tree. The sweet cherry showed more injury than the sour cherry.

3. There was delay or even complete prevention of abscission of all plant parts that normally absciss, beginning with the leaf-fall to the dropping of mature fruits. Evidences were cited for questioning the contention that the effectiveness of a plant growth regulator depended on the anatomical type of abscission.

4. The opening of floral and vegetative buds was delayed and very irregularly distributed. In no case was the delay solely due to injury, where injury was severe but was to some extent direct result of treatment, as subsequent formative patterns revealed.

5. The modifications in the fruit as a result of treatment were so thorough that the fruit was not recognizable as belonging to the specific variety. The shape became elongate and pointed, broader at stem end and tapering to a point at the apex, instead of the roundish shape of the normal fruit. The skin was lighter in color and thicker; the flesh and juice also lighter colored, with poorer taste and flavor. The fruit was heavier by as much as 18 per cent. There was an abnormal development of the stone. It showed a close correlation to the fruit in shape, had a rough, variously ridged surface compared to a relatively smooth surface of the normal stone; its size increased by as much as 34.0 per cent, and the weight by 125.0 per cent and the thickness of the stony pericarp by 109.0 per cent.

The pedicel increased in thickness by nearly twice that of the normal, as also the receptacle. The pedicel was also much firmer to the touch and adhered strongly to the tree and to the fruit, enhancing the hanging quality of the fruit.

Against all the rest of the modifications, there was one aspect of the normal fruit that did not change -- the size.

6. A study of the histological development of the

fruit revealed distinct effects of the treatment. The vascular system proliferated abnormally and shifted its position from the center of the fleshy pericarp and adhered and almost clinged to the outer layer of the stony pericarp, forming a massy network, and ramifying very little. The tissues of the stony pericarp also showed undue activity and proliferation.

In the other tissues of the pericarp, while the pattern of shape of cells broadly resembled the normal, the cells of the hypodermal layer showed increased enlargement and relatively higher degree of tangential elongation, compared to the normal.

7. Histological study of the pedicel, the petiole and midrib of leaves, and the annual growth showed an increase in size of almost every tissue inside due to enlargement of cells, and increased activity of the vascular system, but of more of the phloem, than of the xylem. There were, in fact, evidences of inhibition of the activity of xylem.

The pericycle fiber cells became larger, both individually and as groups, and formed a more continuous sheath around the vascular cylinder, contributing to the strength of the pedicel, stem, and petiole and midrib of the leaf.

8. The accessory parts of the flower were also

affected. The calyx lobes, the petals, and stamens all had a definite tendency to bend towards the axis. Both the calyx lobes and the petals were narrower, longer, and thicker and with a much stronger development of the vascular system than the normal. The filaments of the stamens were thicker and longer and the anthers larger and more pointed at the end. The style was thicker and a portion of it persisted with the mature fruit.

9. The leaf showed bending at emergence, in the same direction as parts of the flower mentioned above. The expanded leaves were generally narrower, longer, thicker, with acutely pointed tip, deeper and more numerous serrations, with a very strong and thick petiole and very prominent midrib and venation. There were, however, several modifications in size and shape, though a higher length/breadth ratio compared to the normal was always consistent.

The internal structure of leaves showed enlargement of cells and marked increase of intercellular spaces. The treatment was shown to substitute for the auxin in increasing thickness of veins and midrib and for the hormone for growth of mesophyll, both specifically different according to published literature.

10. There were indications of earlier ripening of

the fruits due to treatment and of the transfer of stimulus after the completion of hardening of the stony pericarp to accelerate the activities of the fleshy pericarp towards ripening.

11. There were indications of depreciation of storage quality of mature fruits, due to treatment.

12. Chemical analysis of the fruit showed increase of total sugars and dry matter in the fruit as a whole, a large part of which contributed to the abnormal development of the stone, resulting in lower proportion in the pulp, and thus contributing to the poorer marketing quality.

13. The translocation of stimulus was discussed and the evidences for the phloem playing the most important role in this furnished.

14. The orderly succession of responses from the time of application of treatment was analysed and correlated with the gradation of growth of the tissues or organs.

15. The general influence of growth regulator treatments in inducing greater vascular development was brought out.

16. The role played by the abnormal development of the stony pericarp in controlling size and by the vascular pattern and cell-elongation effect of growth

regulators in addition, in evolving shape was brought out.

17. The preponderent effect of 2,4-dichlorophenoxy-acetic acid in the mixture applied was explained.

18. The practical applications of the results are listed.

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PLATES

Explanation of abbreviations:

CA	--	cambium
CO	--	cortex
CU	--	cuticle
EP	--	epidermis
FP	--	fleshy pericarp
FS	--	fiber-sheath
HY	--	hypodermal layer
ISP	--	inner stony pericarp
OSP	--	outer stony pericarp
PH	--	phloem
PI	--	pith
PP	--	palisade parenchyma
SP	--	stony pericarp
SPP	--	spongy parenchyma
ST	--	stoma
X	--	xylem
XP	--	xylem parenchyma
CL		Collenchyma
VT		Vascular tissues.

Plate 1

Untreated sour cherry tree

Photographed July 16, 1948

PLATE I

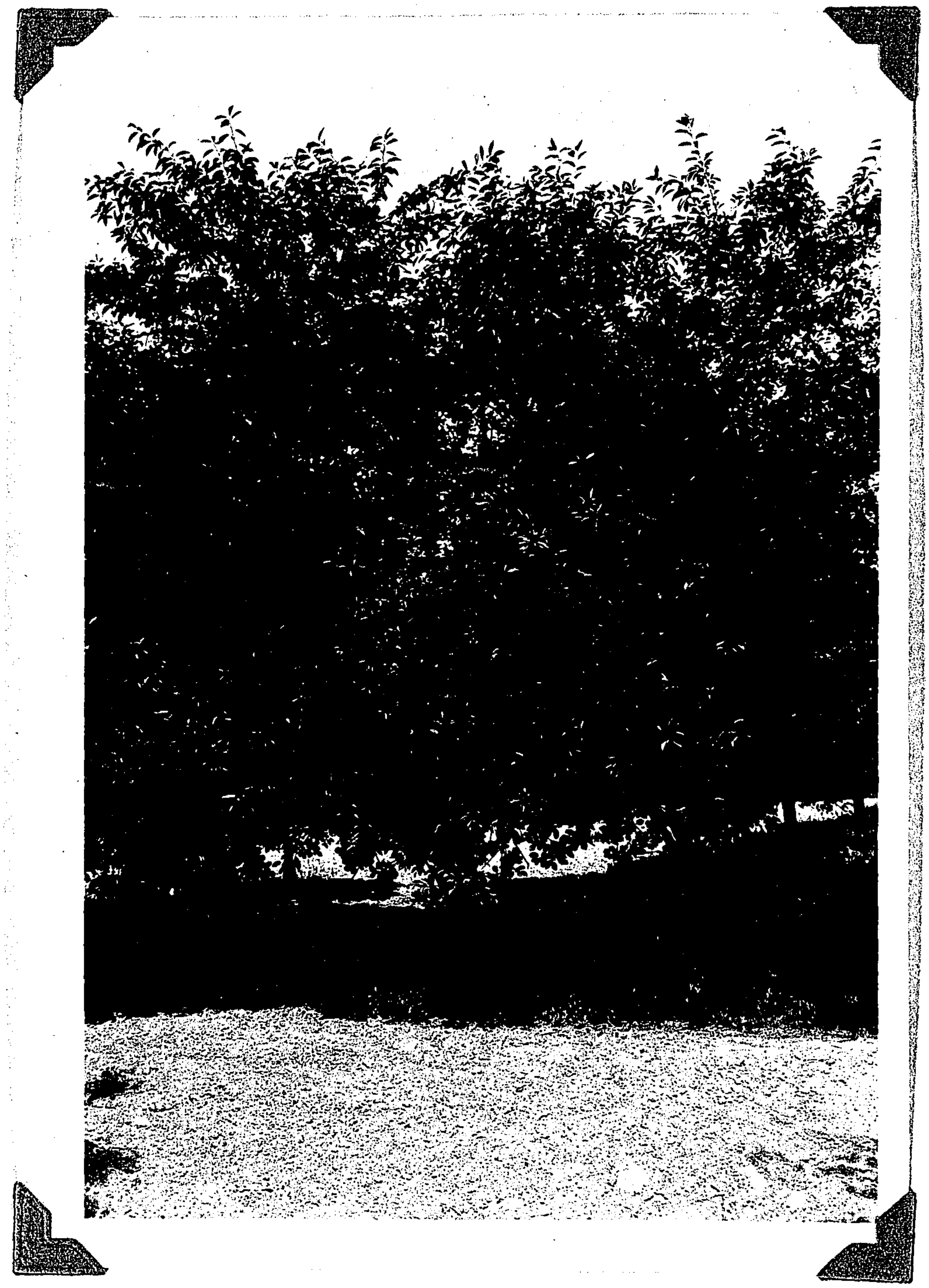


Plate 2

Treated sour cherry tree

Relative bareness of limbs due to killing of leaf-buds, by treatment.

Photographed July 16, 1948

PLATE 3

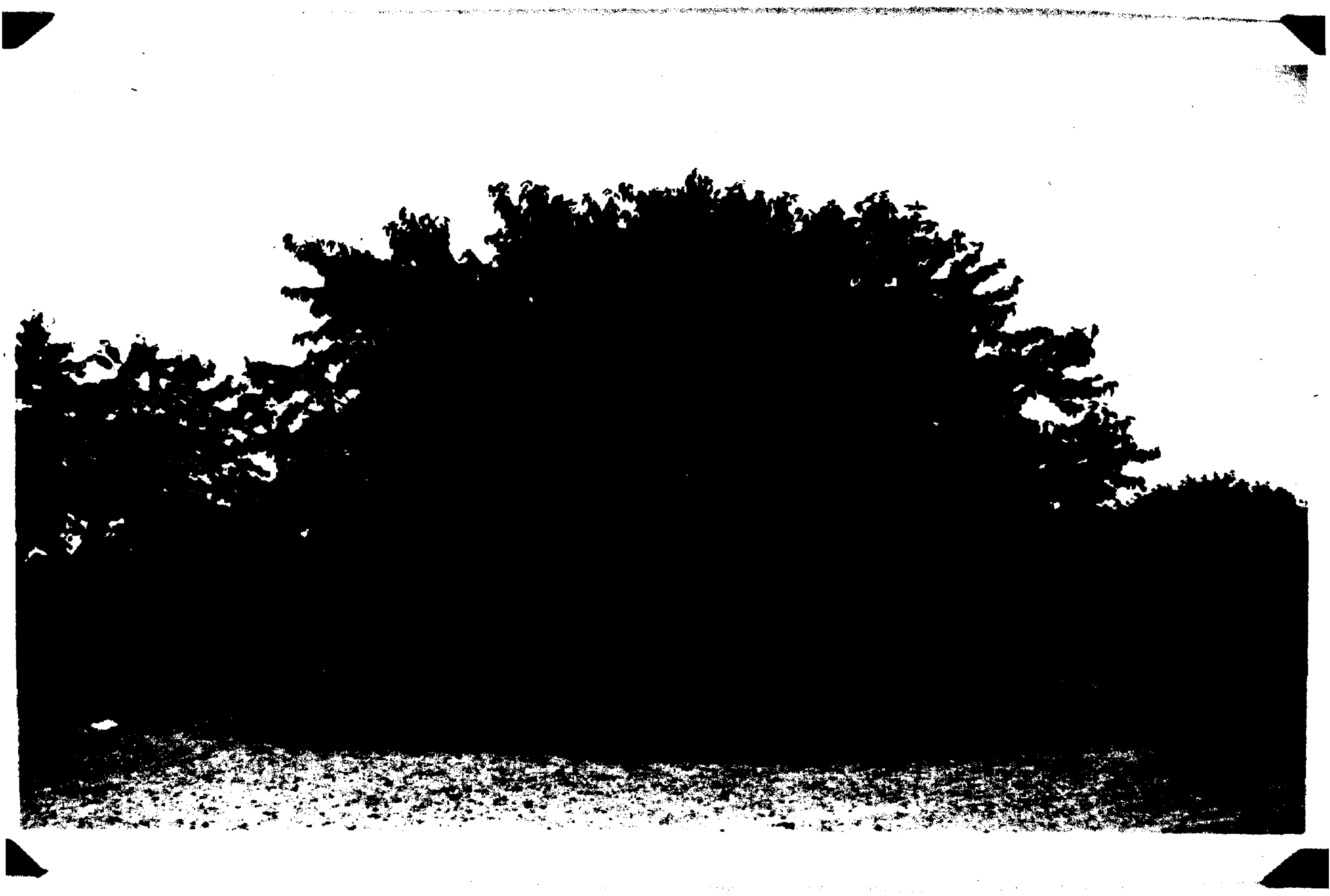


PLATE 2

Plate 3

Untreated sweet cherry tree

Photographed July 16, 1948

PLATE 3

Plate 4

Treated sweet cherry tree

Framework almost bare due to
severe killing of leaf-buds

Photographed July 16, 1948

PLATE 4

Plate 5

Leaves of sweet cherry after emergence

Left - normal emergence from untreated tree
Center and right - bending and twisting of
leaves at emergence

Photographed May 4, 1948

PLATE 5

Plate 6

Sour cherry twig from treated tree
with aborted flowers not abscissing,
and narrow, elongate, mottled leaves

Photographed July 16, 1948

PLATE 6

Plate 7

Modification of size and shape of
leaves due to treatment

Top - sweet cherry
Bottom - sour cherry

Photographed July 16, 1948

PLATE 7

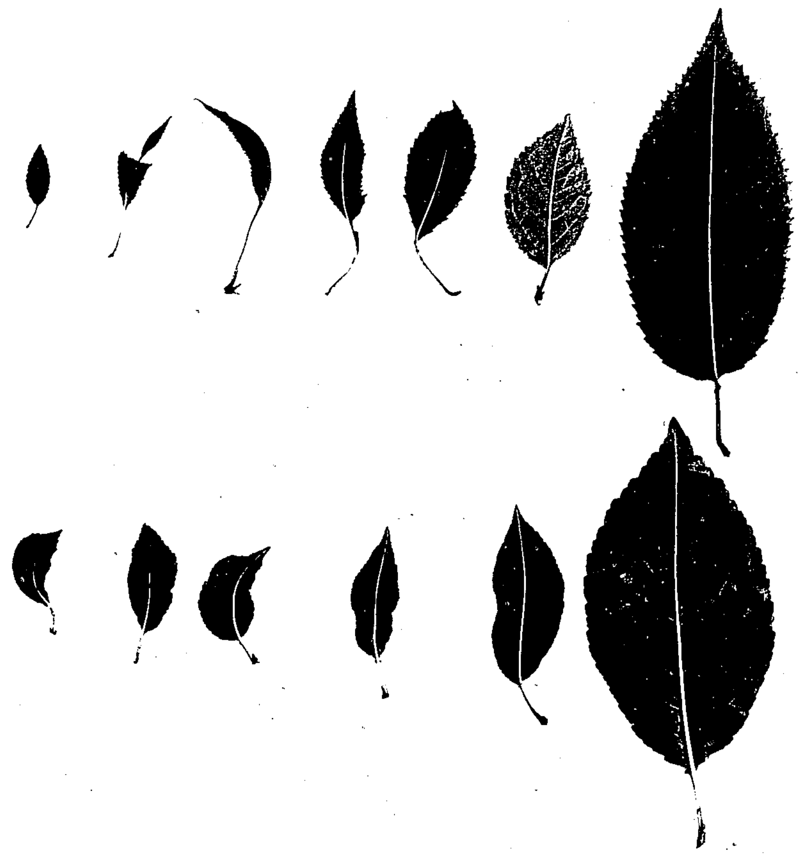


Plate 8

Flower buds of sour cherry on
April 24, 1948

Left - two twigs - treated - flower
buds retarded in opeining.
Leaves of fall not abscissed.
Right - two twigs - untreated

PLATE 8

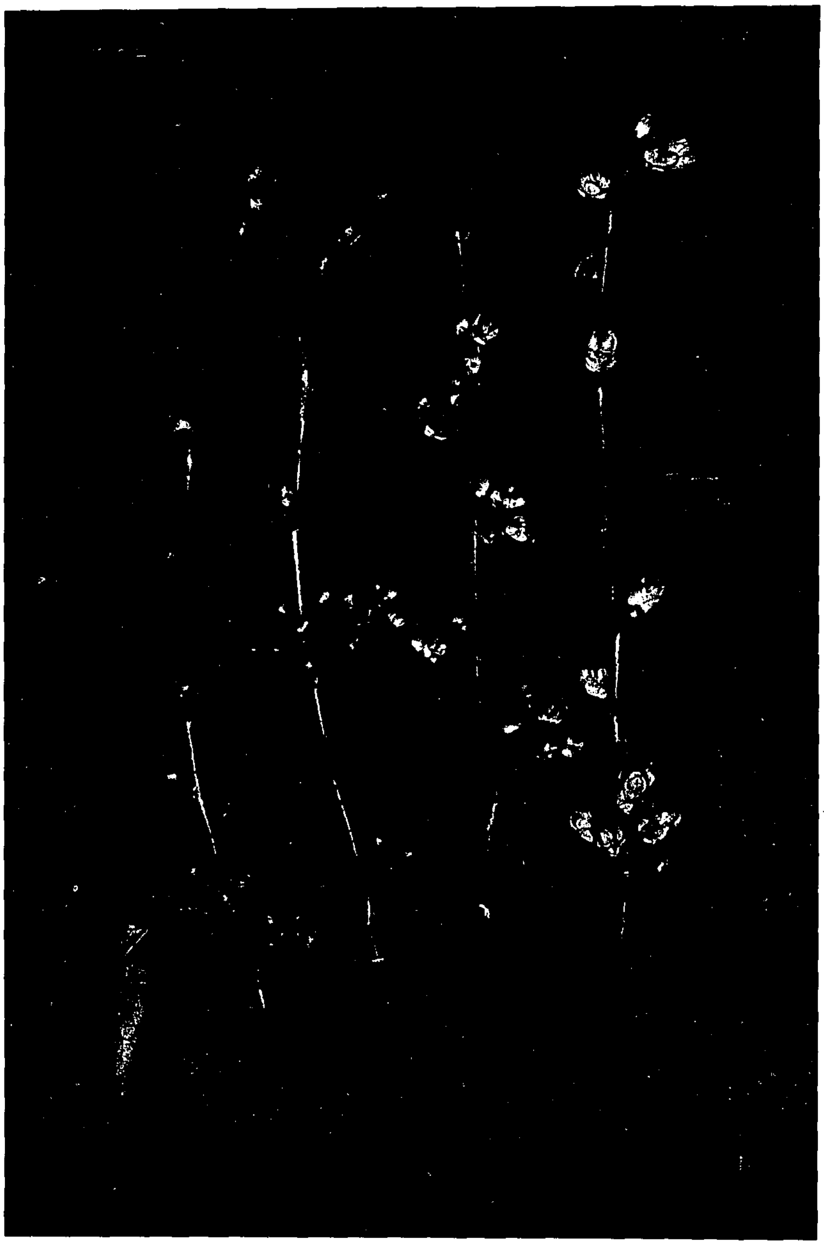


Plate 9

Leaves and open flowers of sour cherry

From left to right:-
1. Leaf from treated tree
2. Flower and single petal from treated tree
   (The incurving of the petals is shown)
3. Flower with petals removed to show the
   incurving of calyx lobes and stamens -
   from treated tree
4. Flower and single petal from untreated tree
5. Flower with petals removed to show the
   normal position of calyx lobes and the
   normal posture of the stamens
6. Leaf from untreated tree

Plate 10

Sweet cherry flowers

Top. Left - single petal from treated tree
Right - single petal from untreated tree

Bottom from left to right-

1. Open flower from treated tree with incurving of petals clearly shown
2. Flower with petals removed with incurving of stamens, greater thickness of filaments, larger sized anthers, pointed at apex, and thicker pedicel, shown
3. Open flower from untreated tree
4. Flower with petals removed

PLATE 10

Plate 11

Mature fruits of sour cherry

Top - untreated
Bottom - treated

PLATE II

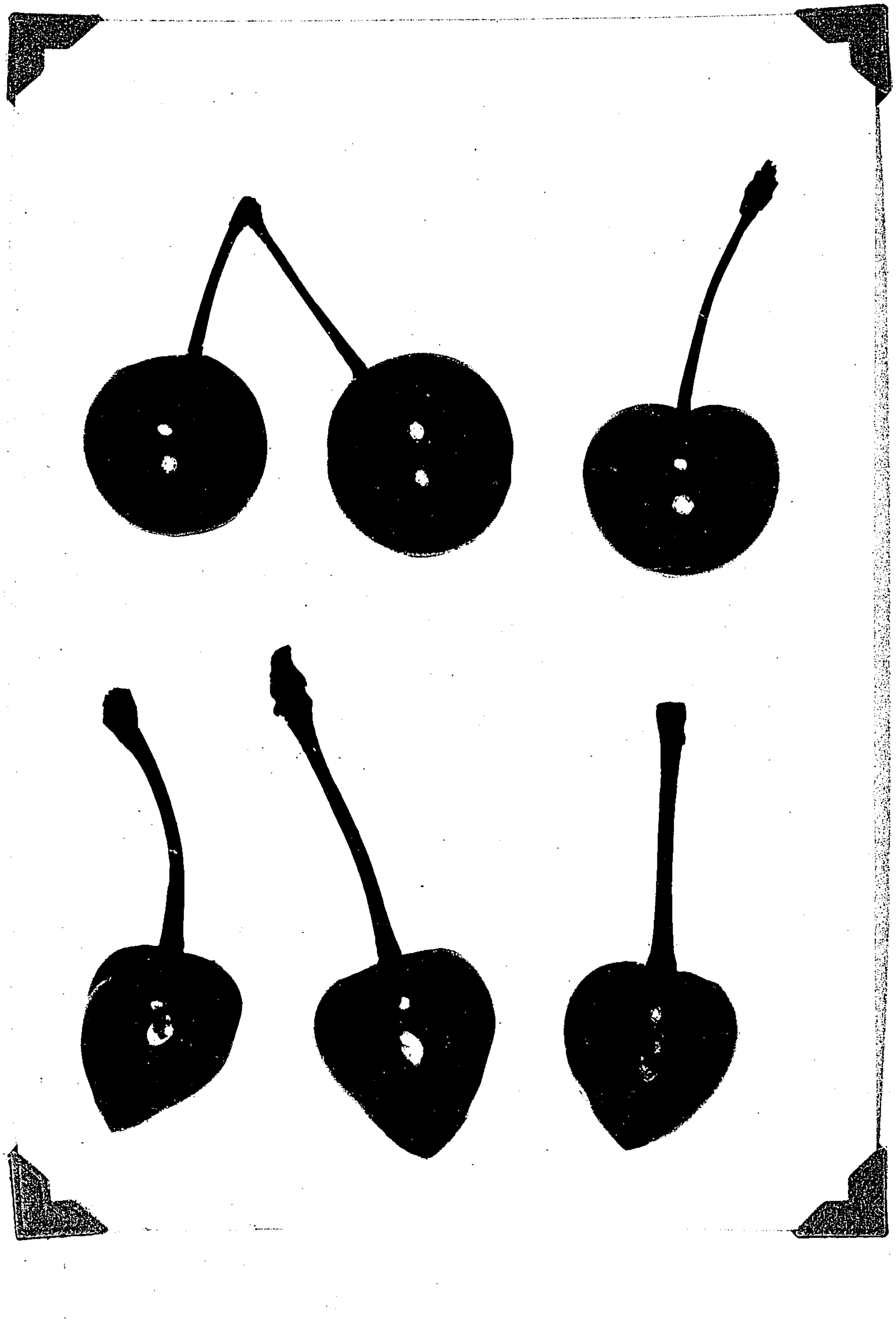


Plate 12

Mature fruits of sweet cherry

Top - untreated
Botton - treated

PLATE 12

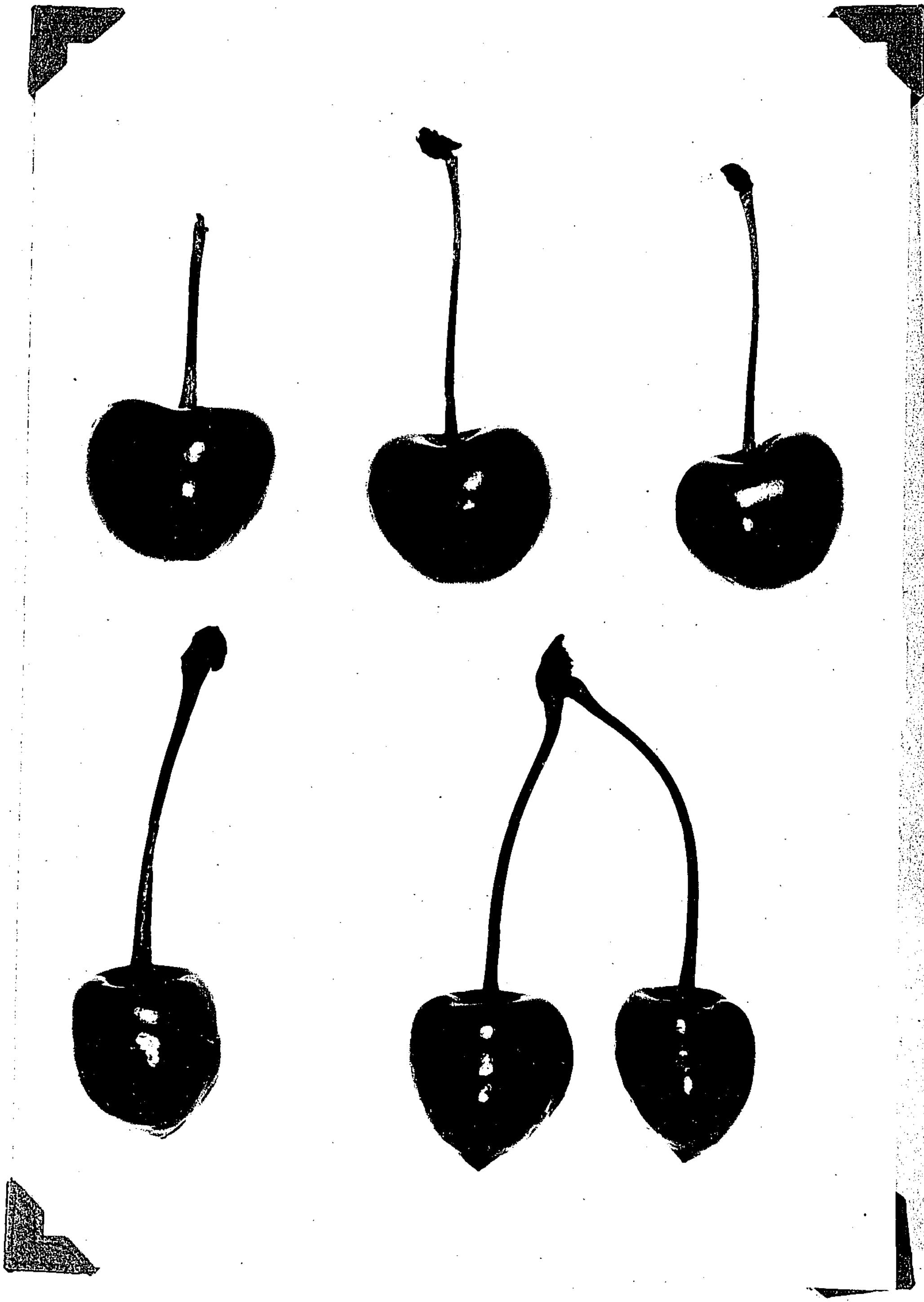


Plate 13

Stones of mature cherry fruits

Top - treated
  left - sweet cherry
  right - sour cherry

Bottom - untreated
  left - sweet cherry
  right - sour cherry

PLATE 13

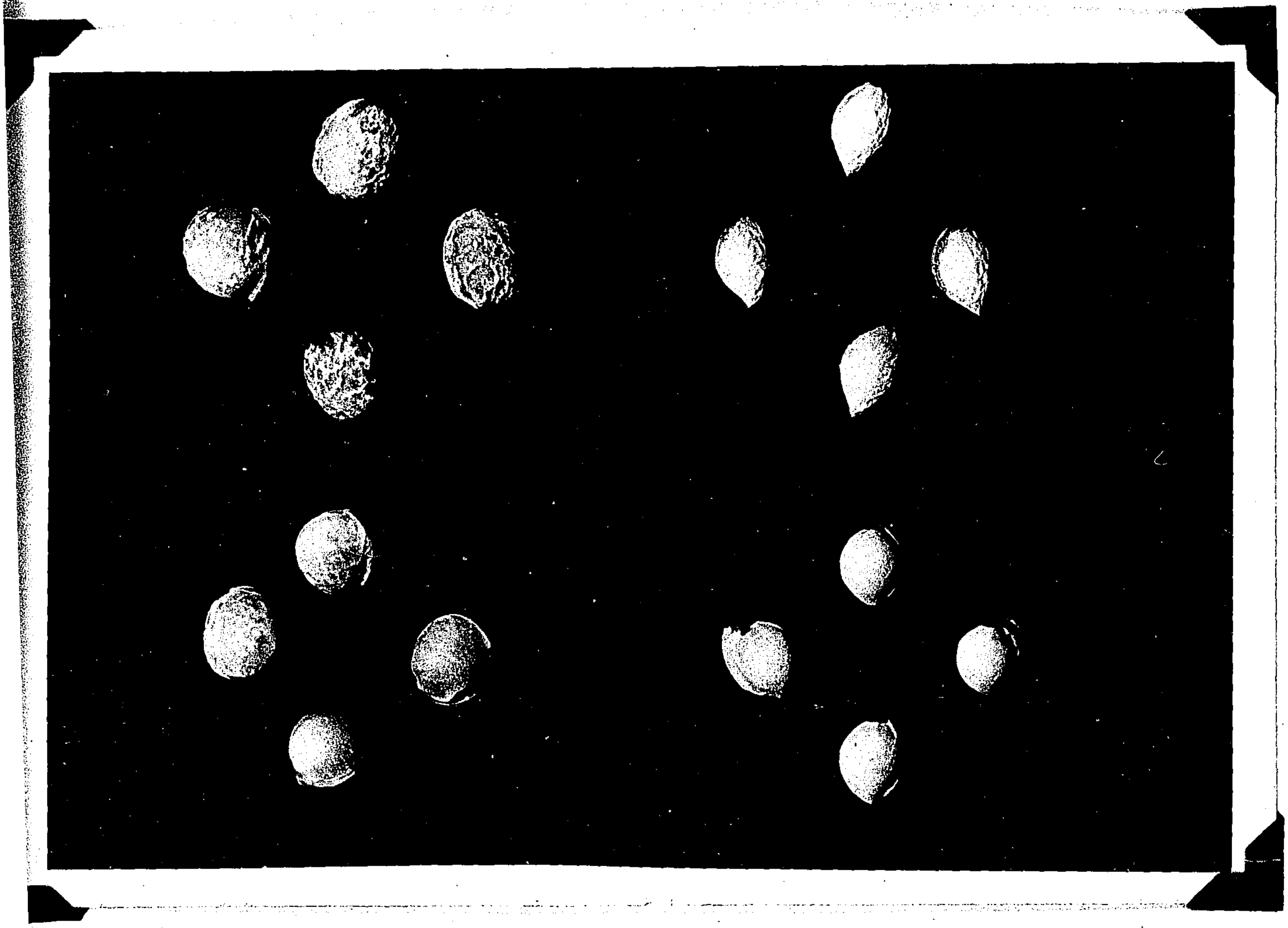


Plate 14

Projectoscope drawings

Fig. 1 - cross-section of sour cherry fruit in early part of Stage II showing tissues of pericarp (tissues in the locule not shown)
A - treated
B - untreated

Fig. 2 - portion of a section through fleshy pericarp of mature fruit showing the nature and position of vascular system
A - treated
B - untreated

PLATE 14.

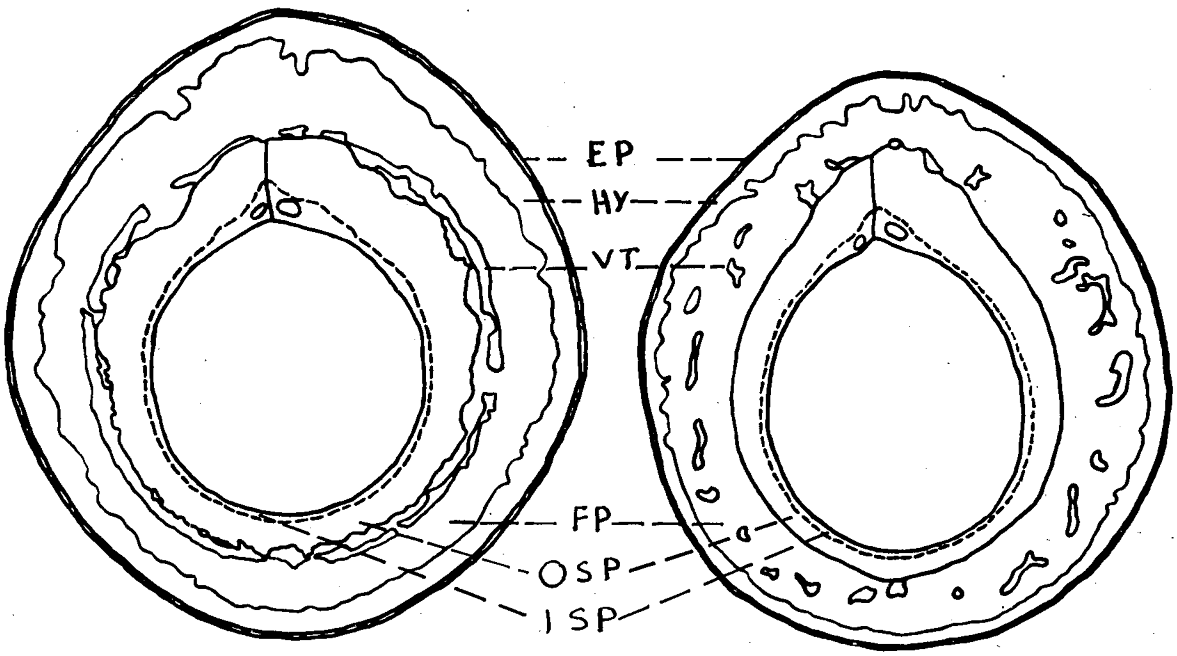


FIG. 1

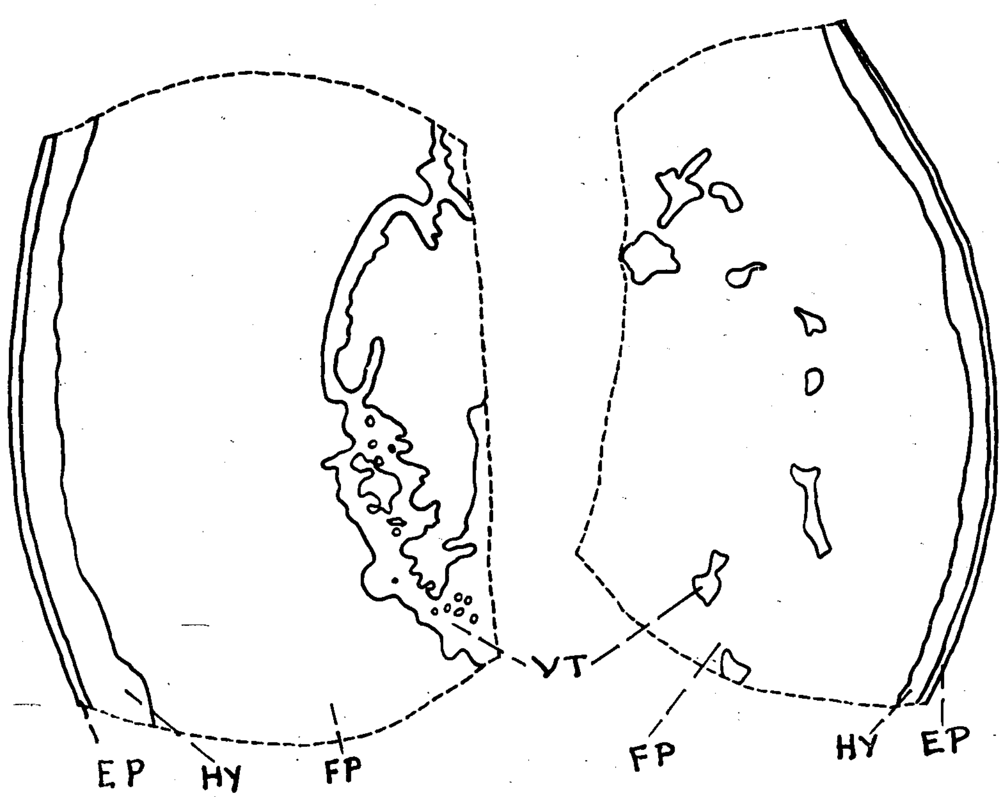


FIG 2

Plate 15

Camera lucida drawings

Cellular details of cross-sections of tissues of the pericarp of sour cherry at commencement of Stage II (mid-stage of ripening)

Left - treated
Right - untreated

PLATE 15

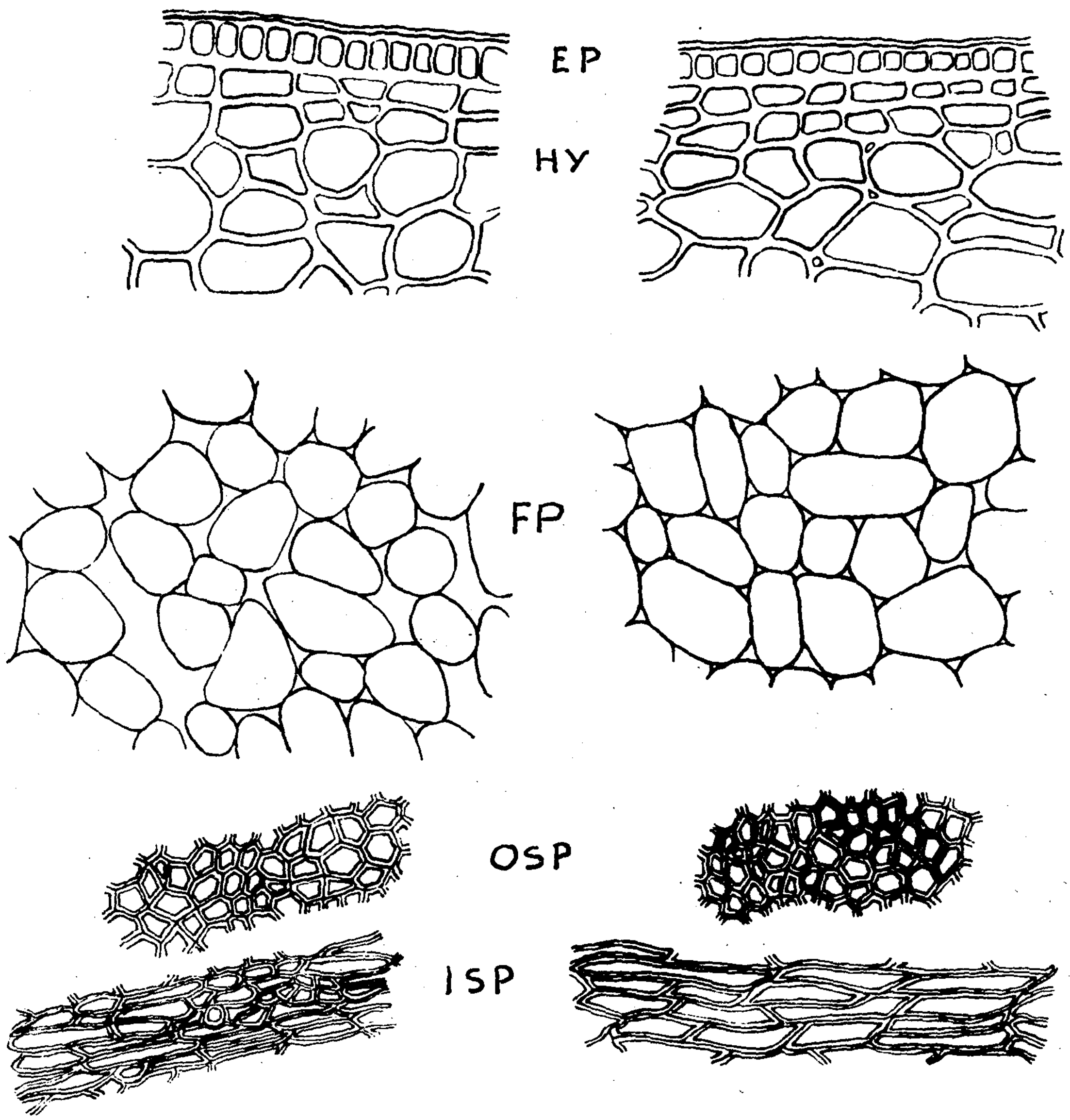


PLATE 15

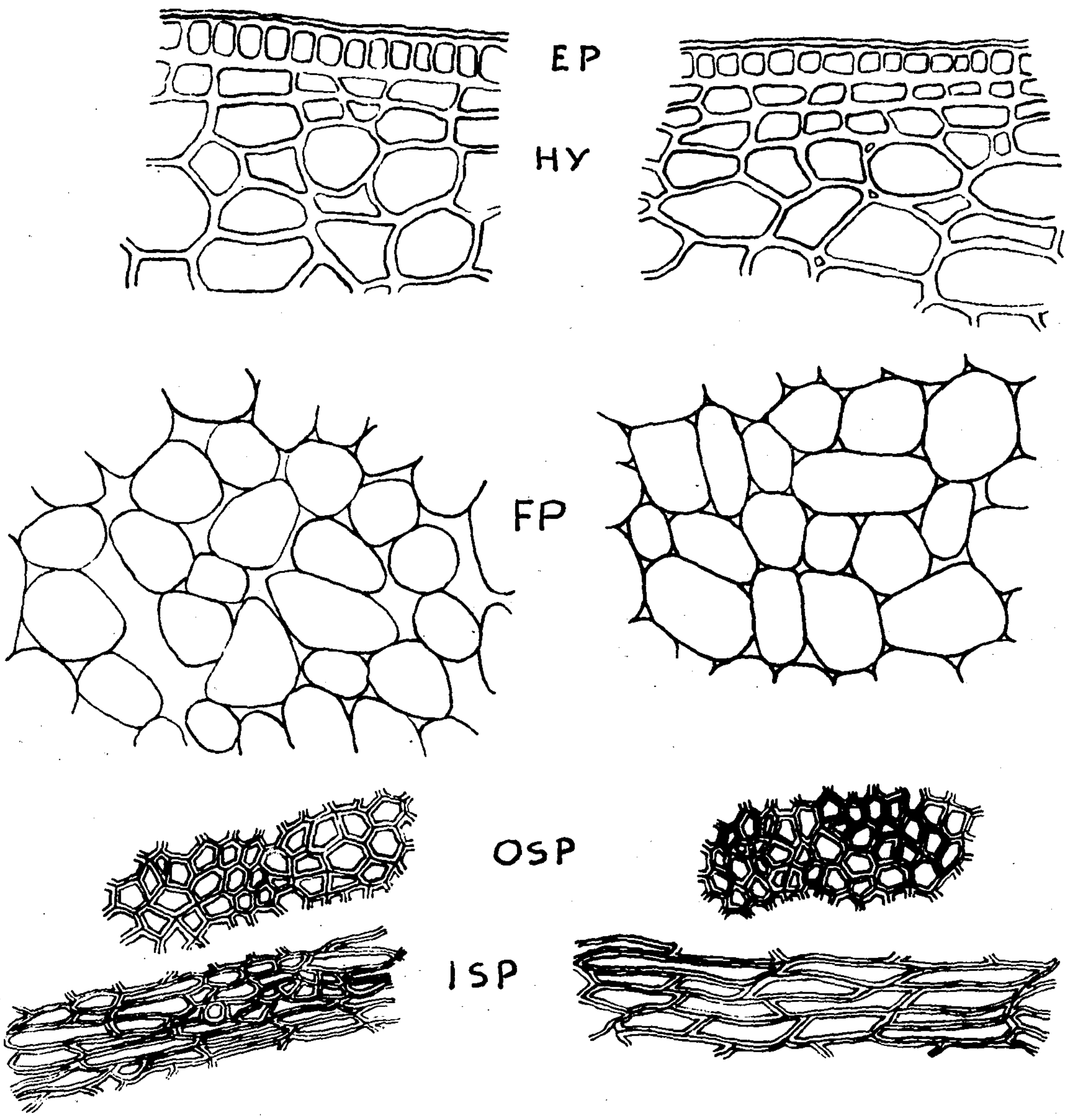


Plate 16

Camera lucida drawings

Cellular details of cross-section of tissues of fleshy pericarp of mature sour cherry fruit

Fig. 1 - untreated
Fig. 2 - treated
a - epidermis and hypodermis
b - cells next to hypodermal layer
c - cells in the middle of the fleshy pericarp
d - cells in inner region of fleshy pericarp

PLATE 16

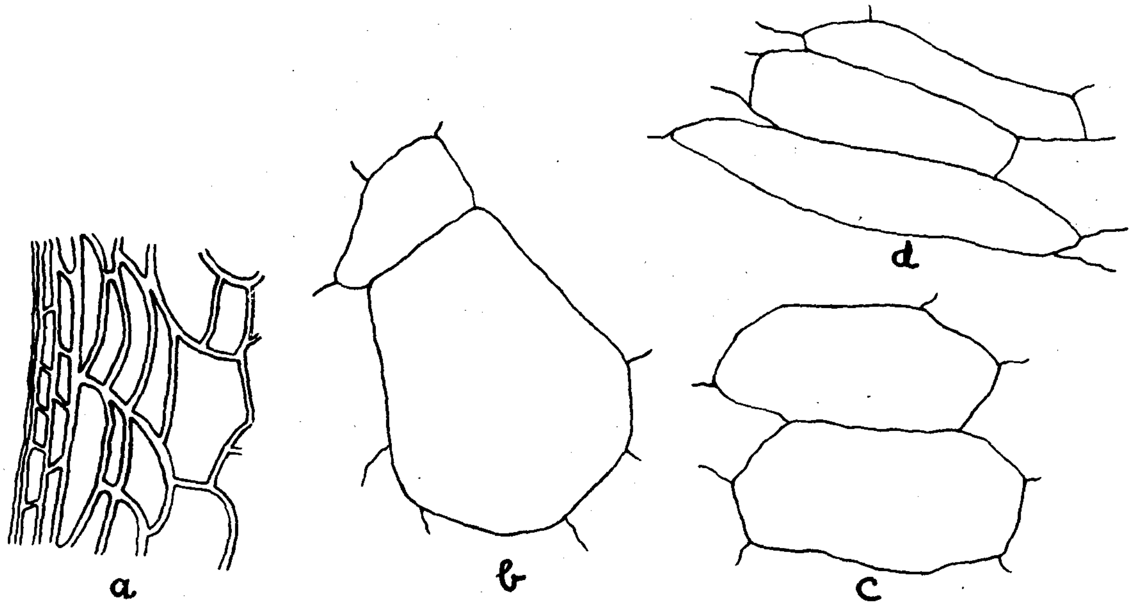


FIG 1

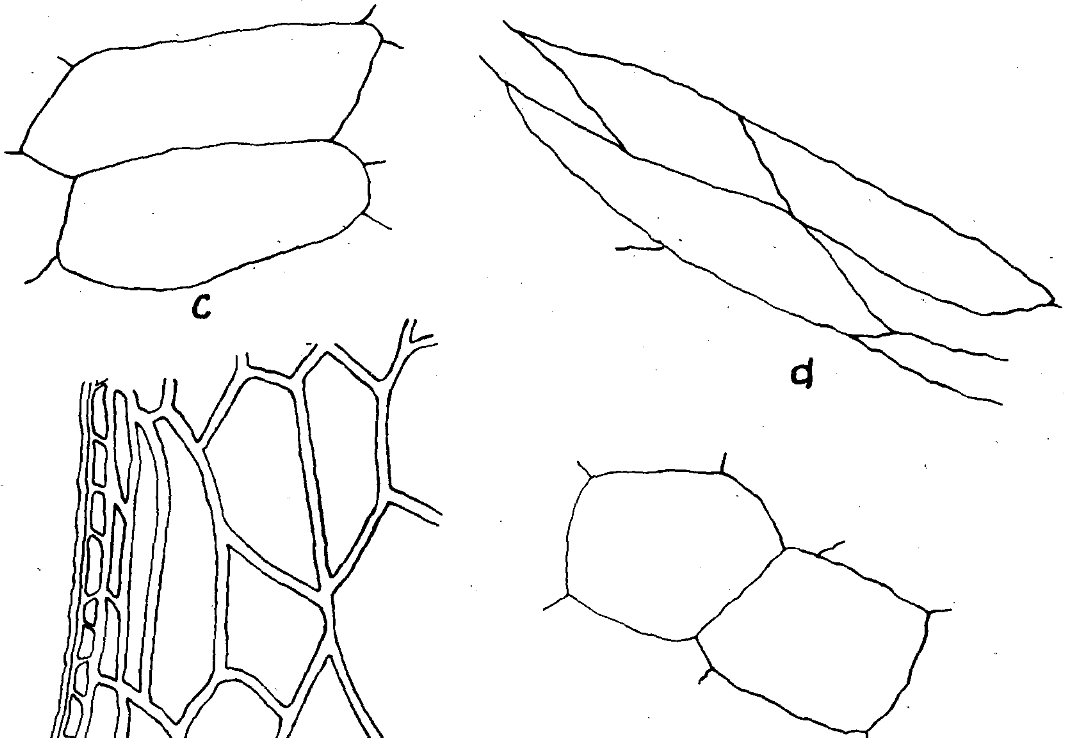


FIG 2

Plate 17

Camera lucida drawings

Cross-section of xylem vessels in stem

A - untreated
B - treated

Projectoscope drawings

Cross-section of stem showing tissue regions

C - untreated
D - treated

Cross-section of pedicel showing tissue regions

E - untreated
F - treated

PLATE 17

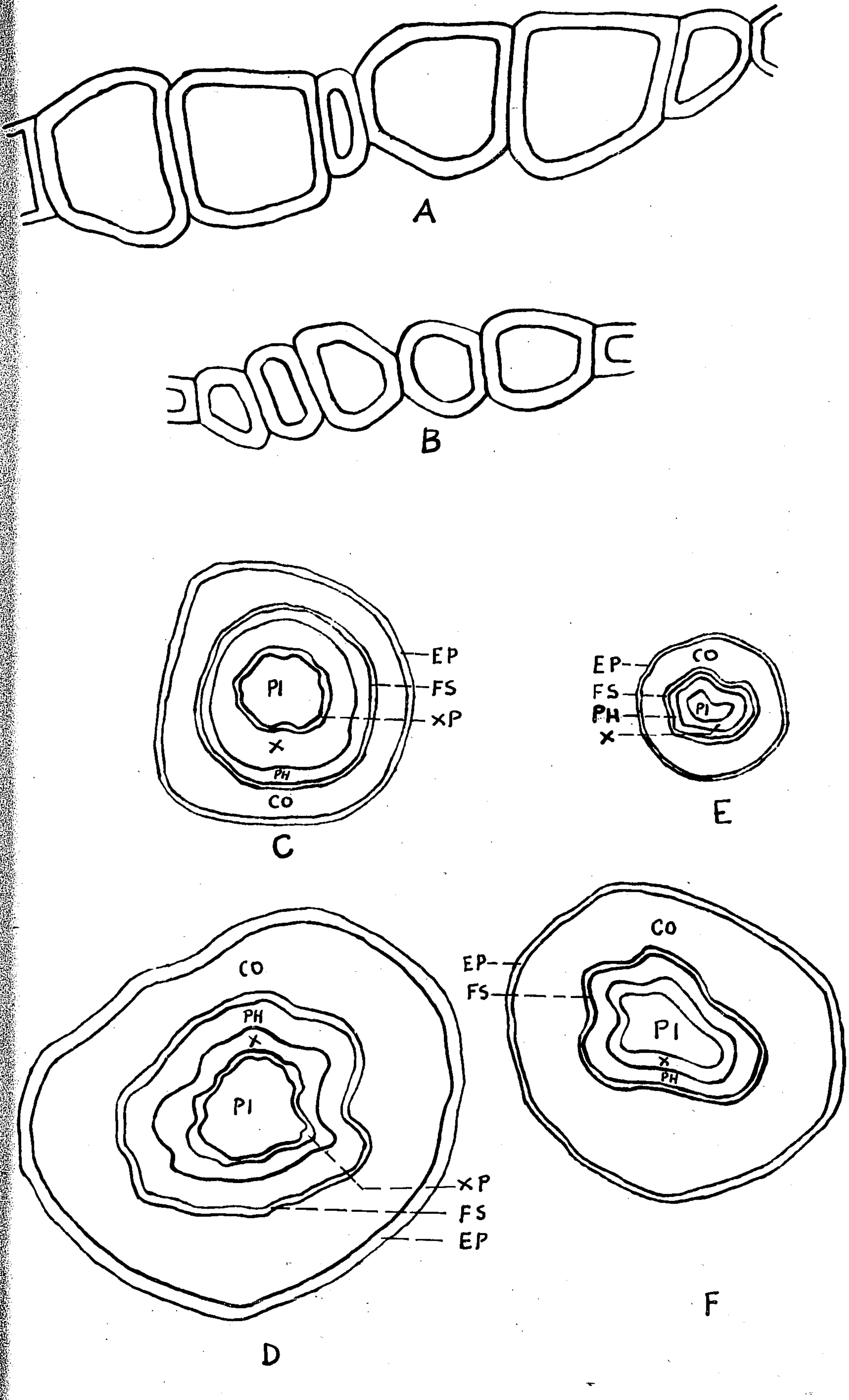

A
B
EP
FS
XP
PI
X
PH
CO
C
EP
CO
FS
PH
PI
X
E
CO
PH
X
PI
XP
FS
EP
D
CO
EP
FS
PI
X
PH
F

Plate 18

Camera lucida drawings

Cross-section of comparable portions of the vascular system in pedicel of sour cherry open flowers

Left - treated
Right - untreated

PLATE 18

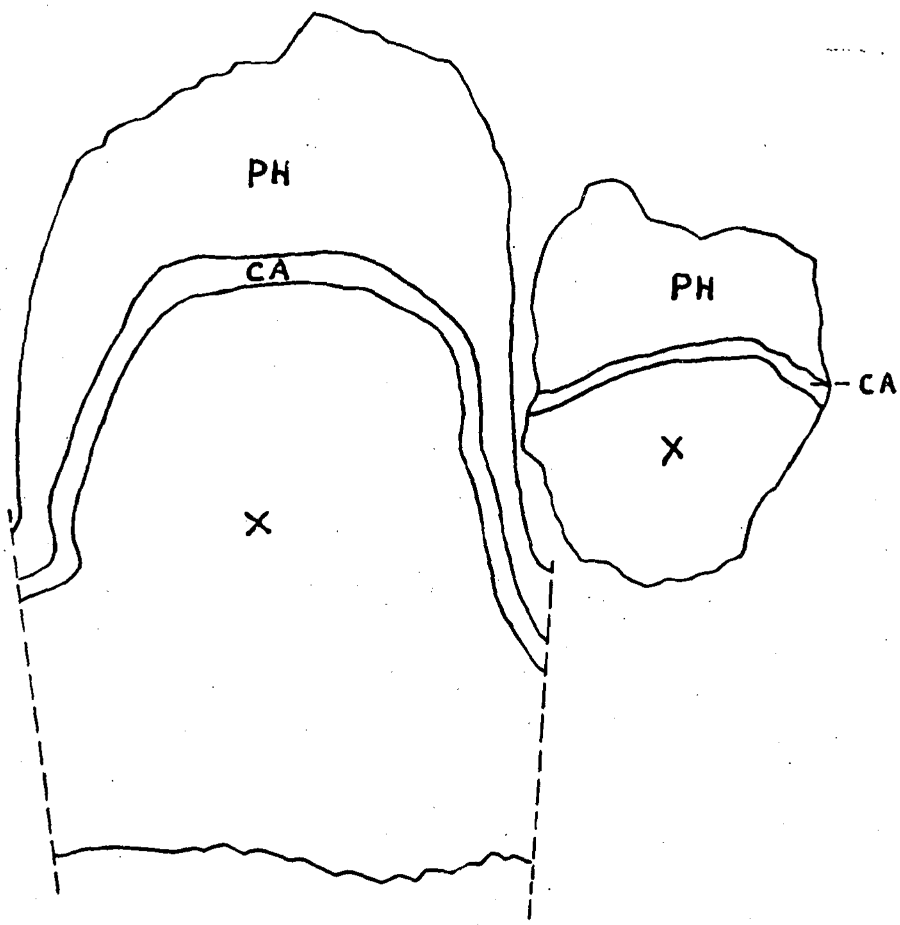


Plate 19

Camera lucida drawings

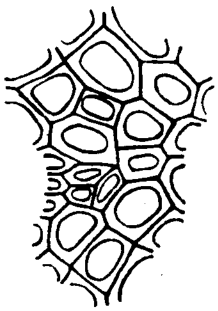
Cross-sections of fiber cells of the pedicels of mature fruits

A — treated
B — untreated

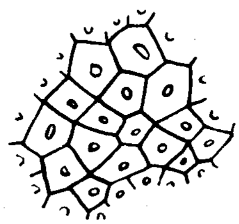
Cross-sections of comparable portions of vascular system in sour cherry pedicels 21 days after the flower opened

C — untreated
D — treated

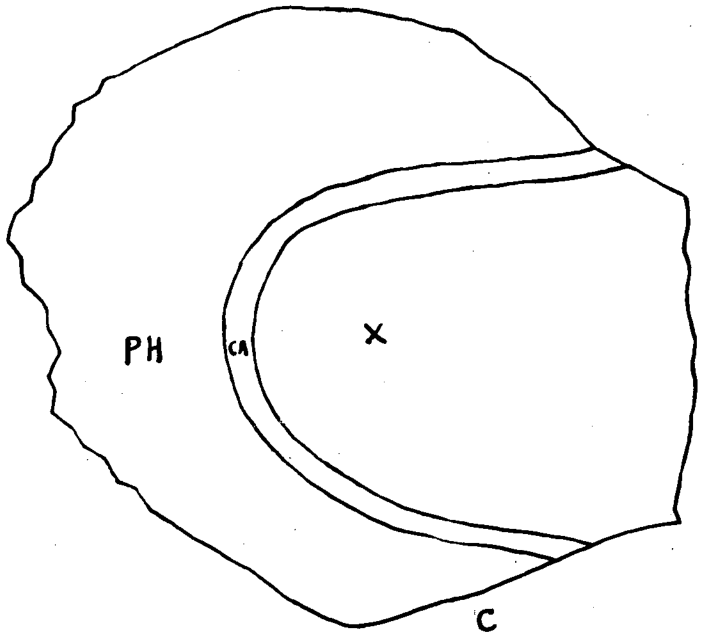
PLATE 19



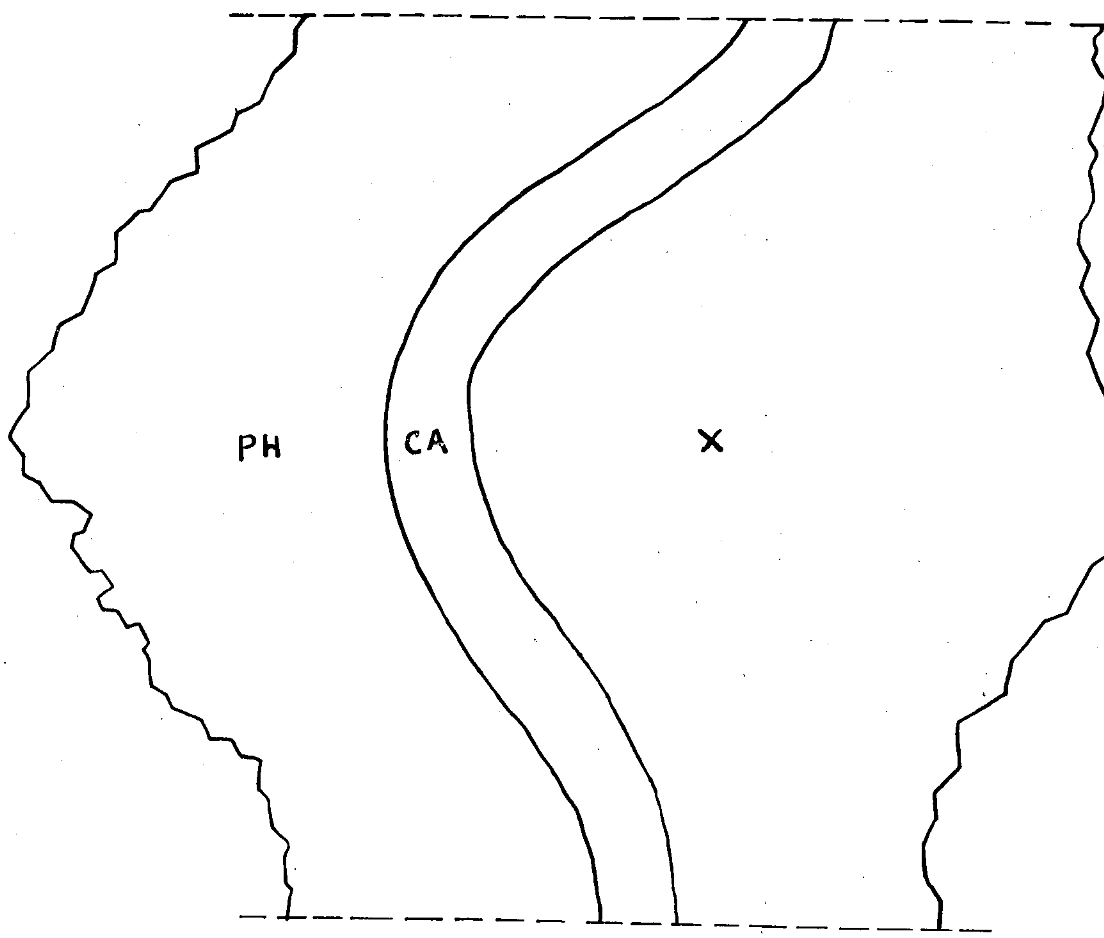
A



B



C



D

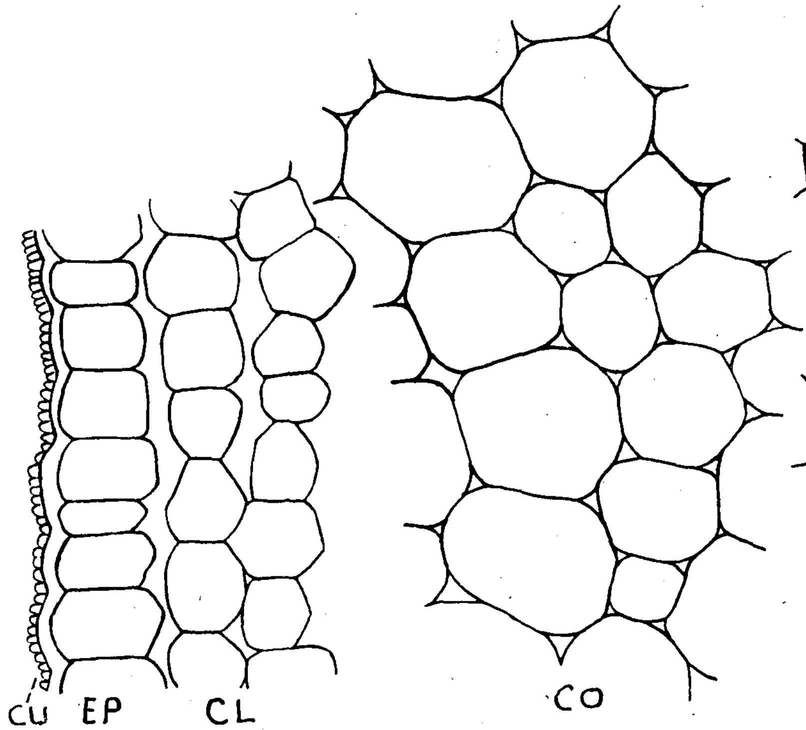
Plate 20

Camera lucida drawings

Cellular details of the cross-sections of tissues of pedicel of sour cherry open flowers

Top - treated
Bottom - untreated

PLATE 20



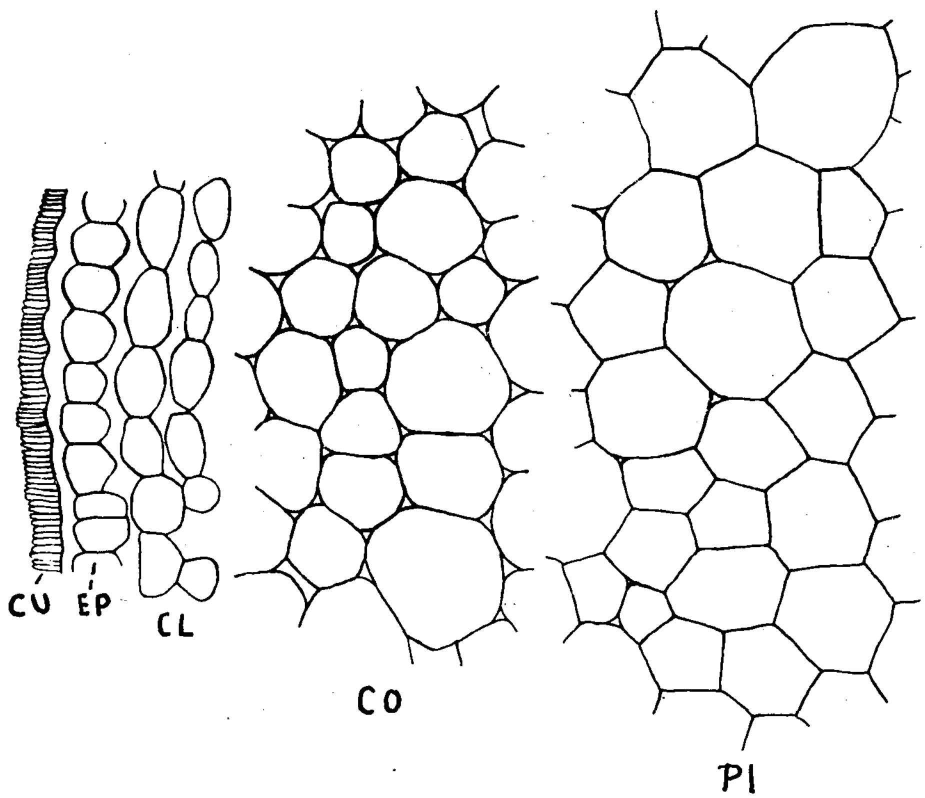


Plate 21

Projectoscope drawings

Fig. 1. Outline of vascular system in the sour cherry petals

Left - untreated
Right - treated

Camera lucida drawings

Fig. 2. Surface view of the epidermal cells of petals

Left - untreated
Right - treated

PLATE 21

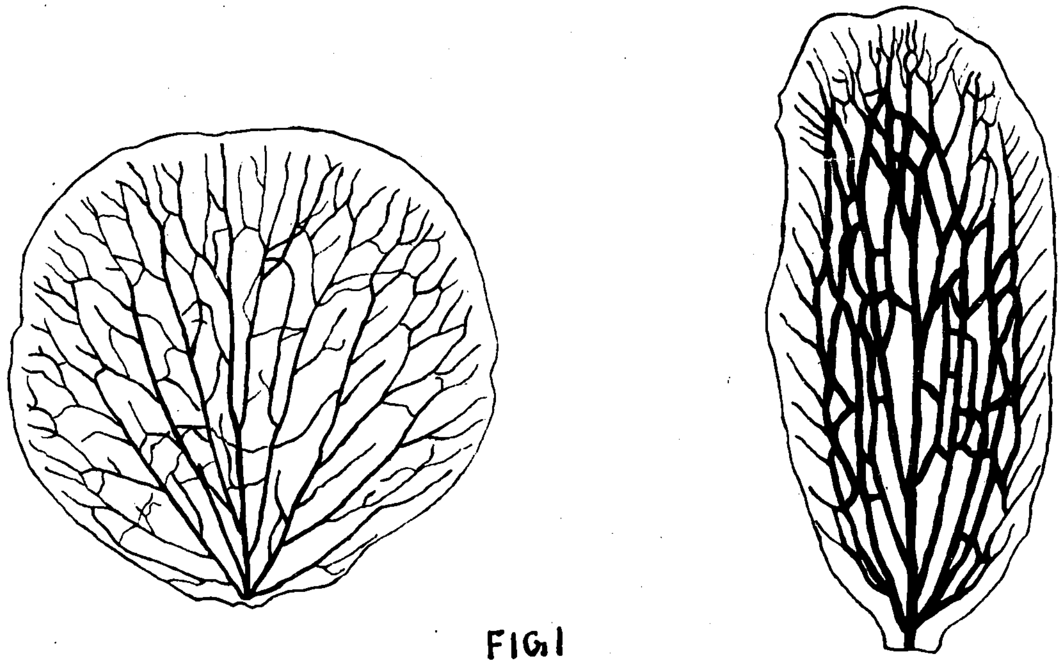


FIG 1

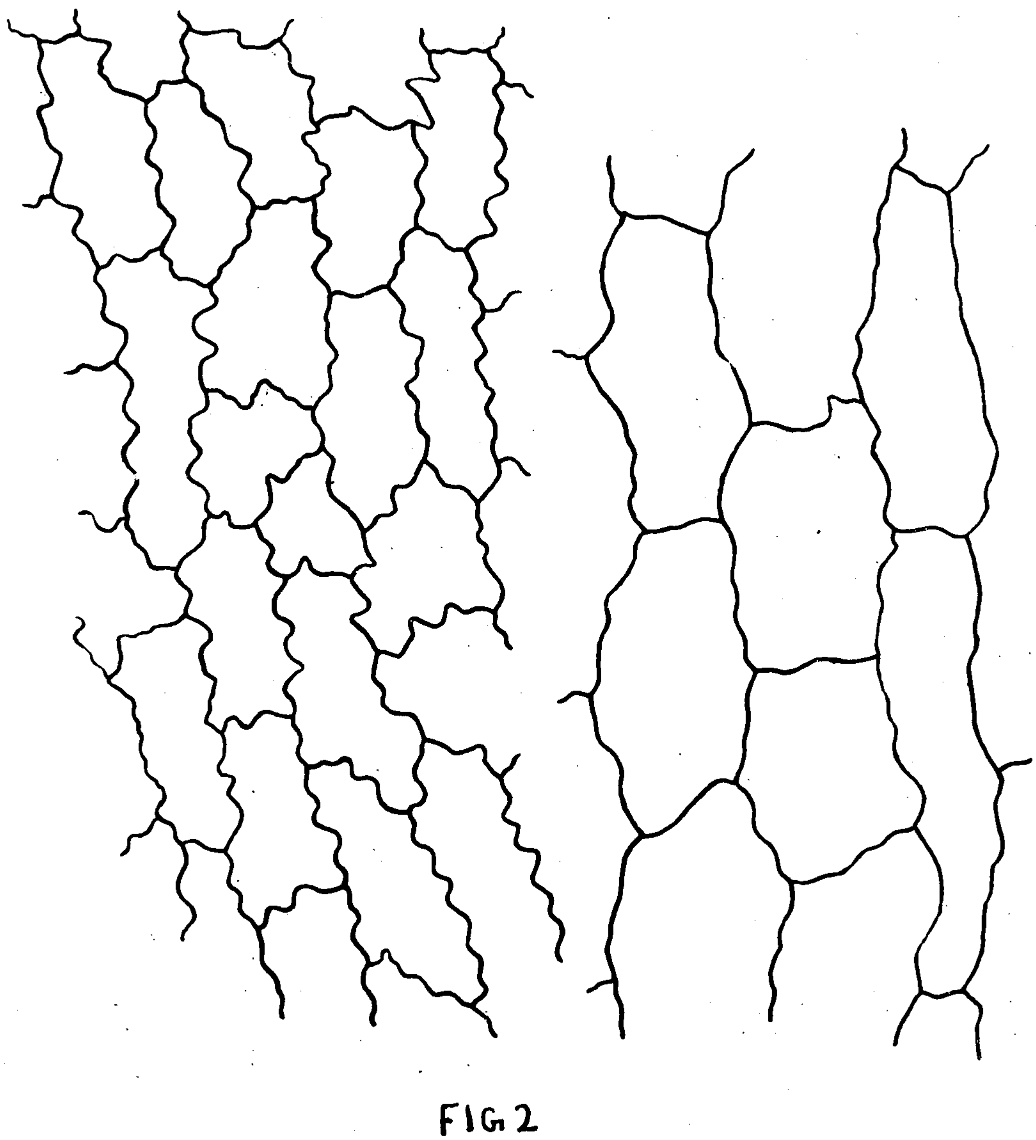


FIG 2

Plate 22

Camera lucida drawings

Surface view of the epidermal cells of sour cherry leaves

A - treated
B - untreated

Segments of cross-sections of the sour cherry leaves

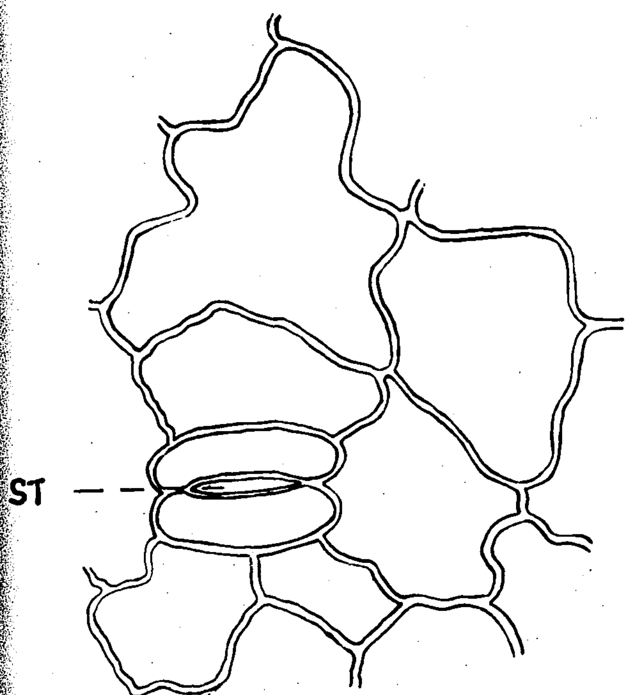
C - treated
D - untreated

Projectoscope drawings

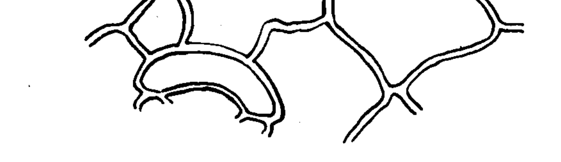
Cross-section of the mid-rib of the sour cherry leaves

E - treated
F - untreated

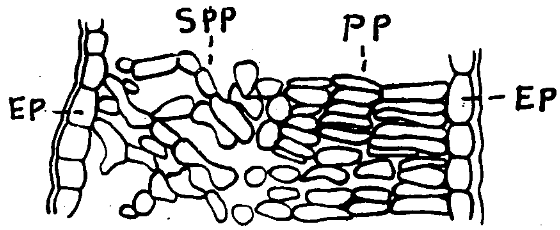
PLATE 22



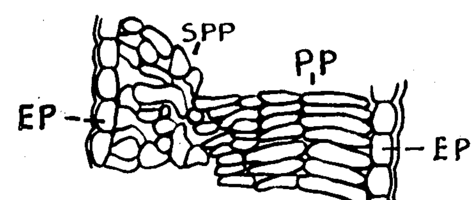
A



B



C



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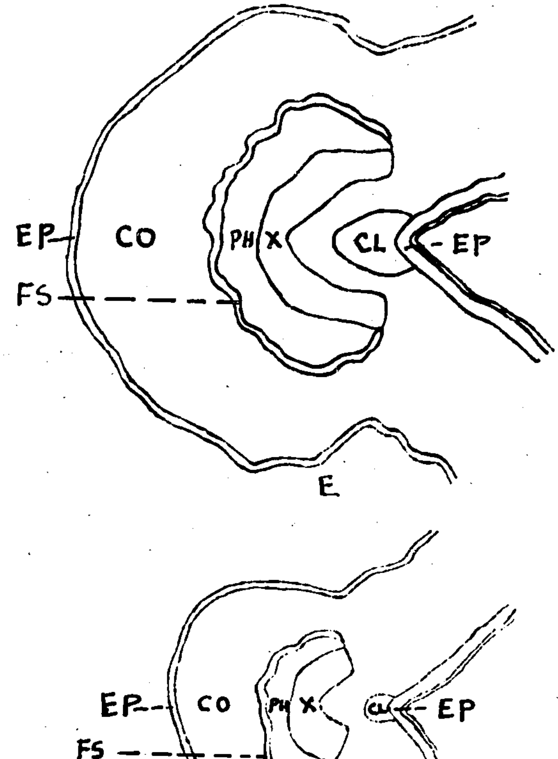
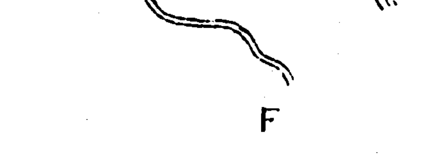


E



F