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The Effects of Plant Growth Regulating Substances on Flower Bud Development and Fruit Set

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ABSTRACT

Flowers of greenhouse-grown tomatoes, var. Master Marglobe, were sprayed with synthetic plant growth-regulating substances (α-naphthaleneacetic acid 20 p.p.m., β-naphthoxyacetic acid 50 p.p.m., and p-chlorophenoxyacetic acid 10 p.p.m.) eight days prior to anthesis, at anthesis, or four days later. The purpose was to determine the effects of these chemicals on fruit-setting and subsequent fruit development when applied at different stages of flower development.

With all chemicals, pre-anthesis treatment resulted in a reduced set of fruit, the majority of which were small, seedless, and misshapen. Treatment at anthesis resulted in an increased set of fruit which were seedless or only partly seeded. The average size of fruits was augmented by β-naphthoxyacetic and p-chlorophenoxyacetic acids, while α-naphthaleneacetic acid reduced fruit size. Sprays applied four days after anthesis, fertilization probably having occurred in most instances, resulted in the greatest yields of fruit, the majority of which was normally seeded.

The application of hormone sprays, α-naphthaleneacetic acid 10 p.p.m., β-naphthoxyacetic acid 25 p.p.m., and p-chlorophenoxyacetic acid, 10 p.p.m., to grape flowers, var. Concord, prior to anthesis or at anthesis markedly reduced set of berries. With treatment at anthesis a large number of small, green, and seedless berries persisted till time of harvest. Post-fertilization sprays did not influence materially the yield of fruit. p-Chlorophenoxyacetic acid tended to increase berry size while α-naphthaleneacetic acid tended to reduce it.

Tomato flower development, as measured by date of opening, growth of ovary, and time of shedding corolla and anthers, was retarded by application of p-chlorophenoxyacetic acid 10 p.p.m., eight days or four days before anthesis. Histological studies revealed that with treatment eight days prior to anthesis approximately fifty per cent of the pollen grains collapsed, while treatment four days later resulted in abnormal growth of the pollen grain wall and of pollen tubes while still in the anther sac. Spraying flower buds at either of the pre-anthesis stages resulted in the cessation of growth in a large number of the ovules, while those which continued to develop did so at a retarded rate. In flowers sprayed at anthesis only a few ovules developed at a retarded rate into seeds. Neither the number nor the rate of development of seeds was reduced materially by spraying flowers four days after anthesis with p-chlorophenoxyacetic acid 10 p.p.m. But α-naphthaleneacetic acid 40 p.p.m., or p-chlorophenoxyacetic acid 20 p.p.m. applied at this same stage caused the majority of the fruits to be seedless or partly so.

The rate of metabolism of flower buds, as indicated by catalase activity, was depressed by the application of α-naphthaleneacetic (20 p.p.m.) and p-chlorophenoxyacetic (10 p.p.m.) acids eight days prior to anthesis. But β-naph-

thoxyacetic acid 50 p.p.m. did not reduce catalase activity when applied at this stage. All three chemicals stimulated activity when applied to fully open flowers.

A discussion is presented of the possible mechanisms of retardation in flower development. Probable causes for the injurious effects of growth-regulating substances on pollen, ovules, and embryos and their relation to the mechanism of retardation are considered.

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The Effects of Plant Growth-Regulating Substances on Flower Bud Development and Fruit Set

D. D. Hemphill

INTRODUCTION

Within the past few years, growth-regulating substances have assumed an important role in the culture of plants. Some of these chemicals are now being used to increase the yields of a number of horticultural crops. Although some plants respond favorably to the treatment with these substances, other crops show no beneficial effect or give reduced yields. What are the factors which determine whether yields will be increased, decreased, or not affected? It would seem to be necessary to learn the influence of these chemicals upon the various plant processes when they are applied at different concentrations and at different stages of growth and development, if they are to be used intelligently and without harmful effects. Many problems, therefore, have arisen in the use of growth-regulating substances in agricultural practice.

All students of plant development recognize that the effect of a growth-regulating substance, whether beneficial or harmful, depends upon the concentration employed. One that stimulates the growth of one plant structure may inhibit another. The concentration of indoleacetic acid, which increases growth of *Avena coleoptile*, for example, will cause inhibition of *Avena* roots (96).* A given strength may have a beneficial or stimulating effect upon one plant process, such as ovary development, and a harmful or inhibiting effect upon another process, such as flower bud differentiation.

The physiological condition and physiological age of the plant are very important in this connection. To produce the desired effect, a plant must be treated at the proper stage of development. The importance of this factor is not as well understood by those who use growth-regulating substances in the culture of plants as is the importance of concentration. Although this factor has been overlooked by many investigators, some have pointed out its significance. In the use of growth-regulating chemicals to increase the set of tomatoes, Roberts and Struckmeyer (62) have emphasized that the flower cluster should not be treated until the majority of the buds have opened.

While studying the value of whole plant spraying of tomatoes to increase fruit set as compared to flower cluster spraying, Murneek et al. (57) (59) (60) at the Missouri Agricultural Experiment Station observed that sometimes the set of fruit was decreased by whole plant spraying due to an inhibiting effect of the growth-regulating substance upon the young flower buds. It was found that whole plant spraying would give increases in yield comparable to that of flower cluster spraying, and often higher, if the tip of the plant containing

*Numerals identify authors and reports listed in Literature Cited, beginning on page 52.

young flower buds was not sprayed. Inhibition was also observed in flower cluster spraying when they were treated while only one or two flowers were open. The curtailment of flower development was particularly noticeable when the more potent growth-regulating substances such as p-chlorophenoxyacetic and 2,4-dichlorophenoxyacetic acids were substituted for the less active indolebutyric and β-naphthoxyacetic acids in the sprays.

In the Missouri investigations it was also found that a reduced yield may result if green bush beans are sprayed before the first flowers open. Furthermore, Murneek and Hibbard (58) have observed that α-naphthaleneacetic acid may reduce or stimulate fruit set in peaches depending on the time when the application is made in relation to flower development.

The present study deals with the various responses that may be obtained when a growth-regulating substance is applied at different physiological stages of growth and development of the flower and young fruit. The specific objectives of this study were to determine at what stages the treatment will be inhibitory, and to study the internal changes of a chemical and histological nature that might be associated with this inhibition.

REVIEW OF LITERATURE

Although literature pertaining to the effect of growth-regulating substances on fruit development is quite voluminous, that relating directly to flower development is very limited. For the most part, the experimental work has dealt with the effect of various substances on induction and development of parthenocarpic fruit. Usually, these chemicals have been applied to the emasculated flower in the form of sprays or pastes at approximately the time that normal anthesis would have occurred.

Such methods of using growth-regulating substances are of fundamental importance, but they cannot be adopted to agricultural practice because of the excessive amount of labor involved. These chemicals have to be applied as sprays or dusts to the whole plant or at least to the whole flower cluster, as in the case of tomatoes.

In connection with the economical use of plant hormones there is need for information, therefore, on the effects of these chemicals upon leaf bud development, flower bud differentiation, and flower development, as well as their influence on fruit set and subsequent growth.

Effects of Growth-Regulating Substances, Natural and Synthetic, on Vegetative Bud Development

For the most part, there is agreement among the investigators of plant development that an auxin or auxin-like substance (or substances) is produced in the terminal bud and/or leaves which controls the growth of axillary buds (44) (72) (84). It has been shown by Laibach (44), Thimann and Skoog (84), and Skoog and Thimann (67) that the lateral buds of decapitated seed-

lings of various *Leguminosae* can be inhibited by the application of auxin, or of hetero-auxin, to the upper cut surface of the main stem.

As to the manner in which auxin acts to inhibit lateral bud and shoot growth there is considerable difference of opinion (44) (73) (75) (84) (87) (95). Moreover, the presence of specific inhibitors of growth have been reported (14) (20) (46) (73) (74) (75) (80) (92), and Snow (73) (75) considers that these inhibitors play a definite role in the correlative inhibition of axillary buds. These substances appear to inactivate auxin or decrease its effectiveness in stimulating growth.

Parthenocarpic Fruit Development

Types of Parthenocarpy. Two types of parthenocarpy may occur in nature. In species where fruit set and develop without the stimulus of pollination we have a type of parthenocarpy called vegetative or autonomic. In species that require pollination or other stimulus for fruit development, this type of parthenocarpy is referred to as stimulative or aitionomic. Goodspeed, as reported by Gardner et al. (19), observed that the Thompson Seedless grape is of the stimulative type. Even though the fruits are seedless because of embryo sac degeneration, the flowers must be pollinated to insure fruit set. Among the fruits reported as vegetatively parthenocarpic may be mentioned the banana (Var. Gros Michel), a number of varieties of the orange and other citrus fruits, and many varieties of figs.

Parthenocarpy Induced by Pollen Extracts. Yasuda (104) probably was the first to produce artificially parthenocarpic fruits of normal or nearly normal size. Egg plant ovaries injected with *Petunia* pollen extract grew to nearly normal size, as did cucumber ovaries injected with cucumber pollen extracts.

Fitting (16) was able to obtain slight growth in the ovary of several species of orchids when the stigma was treated with dead or foreign pollen or drops of their extracts.

After numerous experiments, Gustafson (24), in 1937, supplied evidence to show that there is present in pollen a substance (or substances) which initiates and sometimes causes a continuation of the growth of ovaries into fruits. Chloroform extracts of pollen were used to stimulate the growth of the ovary in a number of plants.

Without doubt, the stimulating effect of pollen extract is due, at least in part, to its auxin content. Laibach (43) and Laibach and Maschmann (45) found auxin to be plentiful in the pollen of several species of orchids and *Hibiscus;* Thimann (82) reported that *Sequoia* pollen is relatively rich in hormones; Mitchell and Whitehead (54) reported that an ether extract of pollen caused significant internode elongation in beans; and Dollfus (13) has reported that pollen contains a growth substance that will induce rooting.

Parthenocarpy Induced by Growth-Regulating Substances. After Gustafson's report (23) that fruit development could be induced by certain growth-

regulating chemicals, a large number of chemicals which were known to have or might have growth-promoting properties were tested (18) (25) (35) (66) (100) (102).

Fruits induced by growth substances and those induced by pollination were compared chemically (35) (38) and histologically (2) (17). Chemically no consistent differences were found and histologically both follow the same pattern of development.

The Cause of Natural Parthenocarpy. Gustafson (26) proposed the hypothesis that the reason some plants produce fruits parthenocarpically is that the ovaries of these plants contain, in the flower bud stage, enough auxin to commence growth without fertilization, while in the ovaries of other plants this occurs only after pollination and fertilization have augmented the auxin supply already present in the ovules.

To test this hypothesis, a comparison was made of the auxin concentration in the ovaries, at flower bud stage, of plants that produce fruits parthenocarpically and of plants that require fertilization. It was found that the auxin content in the ovaries of flower buds from varieties of oranges, lemons, and grapes, which produce fruits parthenocarpically, is higher than in the ovaries from corresponding varieties that do not produce fruits parthenocarpically.

Plant reproductive organs and developing seeds are, in general, a rich source of auxin (27) (29) (41) (45) (42) (54) (100). Gustafson (26) suggests that this auxin is essential to the continued development of the fruit and is actually depleted in this process. Studying changes in growth hormone content of seeded and seedless fruit, he noted that following pollination and fertilization, the auxin content increased appreciably in seeded fruit and at the same time decreased in seedless fruit. Judkins (40) demonstrated a general increase in auxin content of tomato fruit following fertilization.

Van Overbeek et al. (91) came to the conclusion that fruit development under conditions of natural pollination is not initiated by the auxin brought into it by the pollen or pollen tubes, but growth of the ovary results from the activation of the auxin precursor present in the pistil by an agent in the pollen. According to van Overbeek (88), Muir (56) has presented evidence supporting this conclusion. He found that very little auxin diffused out of ovaries and styles of unpollinated flowers, but that this amount increased with the time after pollination.

In an attempt to produce haploid plants, van Overbeek et al. (91) injected a 0.1 per cent solution or emulsion of the ammonium salt of naphthaleneacetic acid into the young ovaries of 4n *Melandrium* and *Datura Stramonium*. *Melandrium* developed parthenocarpic fruits with well-enlarged placentae and ovules of increased size with seed coats and nucellus, but no embryo. *Datura* produced parthenocarpic fruits with greatly enlarged ovules which developed seed coats and often contained a pseudoembryo consisting of several

hundred cells. The pseudoembryo originates by proliferation from the inner layer of the integument (endothelium) surrounding the embryo sac.

Ireland (37) reported that by treating the pistil of an unpollinated cotton flower with a lanolin paste containing colchicine and indoleacetic acid fertile seed were produced.

Effects of Growth-Regulating Substances on Flower Bud Differentiation and Development

There have been a number of attempts made to increase flower bud differentiation by growth-regulating substances, but for the most part these efforts have resulted in a decreased differentiation. Magness et al. (47) tried to influence the initiation of flower primordia in the buds of biennial bearing apple trees during the "on" year. Such trees, when bearing fruit, differentiate few flower buds, which results in a very light crop the following year. They applied lanolin pastes of α-naphthaleneacetic acid and α-naphthalene acetamide to spurs bearing one apple. This application did not increase flower bud initiation. All treated groups formed fewer flower buds than the untreated checks.

Stier and du Buy (78) reported that treatment of tomato seeds with auxin-talc dust mixtures and the subsequent treatment of the plants with solutions of either indolebutyric or α-naphthylacetic acid at the time of field transplanting resulted in a marked acceleration of the time of anthesis of flowers.

Sayre (64) found that hormones in water used at transplanting of vegetables depressed yield, particularly early yields.

The most conclusive evidence that growth-regulating substances have a direct positive effect upon the initiation of flower primordia has been presented by Clark and Kerns (9), Cooper (11), and van Overbeek (89) (91). Working with pineapple, an abnormal parthenocarpic fruit, these investigators found that fifty millimeters of naphthalene acetic acid (5 p.p.m.) or 2, 4-dichlorophenoxy-acetic acid (5 p.p.m.) applied to the center (apical meristem) of the plant is sufficient to cause 100 per cent response. Histological sections showed flower primordia present approximately two weeks after treatment. Clark and Kerns reported that high concentrations of naphthaleneacetic acid applied one month prior to normal differentiation of the inflorescence caused a retardation of four to eight months in the date of flowering.

The delay of the opening of flower buds in the spring has been reported by Winklepleck (99). He states naphthaleneacetic acid (125 mg. / liter) applied to peach trees when the buds began to break retarded their opening by eleven days.

Mitchell and Cullinan (51a) were not able to delay the opening of peach and pear flowers by use of naphthaleneacetic acid, naphthyl acetamide, indoleacetic acid and indolebutyric acid applied after the buds had begun to swell in the spring, but vegetative buds were inhibited by naphthaleneacetic acid.

Hitchcock and Zimmerman (34) and Hitchcock (33) have reported that naphthaleneacetic acid and its potassium salt sprayed on the tree at a concen-

tration of 0.02 per cent (200 p.p.m.) during late July or early August resulted in a delay of one to two weeks in the opening of flower buds and longer for leaf buds of peach, apple, plum, and cherry trees. Other workers (32) (50), however, have not been able to reproduce these results.

Weaver (94) reported that 2,4-D sprayed on red kidney beans and soybeans when the first trifoliate leaves were expanding delayed the onset of fruiting and decreased the amount of pod formation. Stromme and Hamner (81) found that 2,4-D at concentrations of 10 p.p.m. and 100 p.p.m., sprayed on bean plants approximately three weeks before anthesis resulted in a delay of maturity of plants. When a concentration of 1 p.p.m. was used there was no retardation. Flowering was delayed two weeks by 100 p.p.m. This concentration also markedly retarded maturity of the pods and gave a slight decrease in number of pods and yield of seed.

Bryant et al. (6) found that apples harvested in 1946, showed a rather large number of abnormalities that appear to have resulted from application of 2,4-D made to bindweed in July, 1945. Some of the fruits showed only rudimentary or no seed development, and a number of "double" fruit resulted.

Similar observations were made by Marsh and Taylor (48). Oil spray, containing residual 2,4-D, applied on August 29, delayed blossoming the following spring. Few fruit set; these were small and ripened prematurely.

Effects of Growth-Regulating Substances on Ovary Development in Unemasculated Flowers

Pre-pollination Application. In general, the application of most growth-regulating substances just prior (one to two days) to pollination has resulted in the production of seedless or partially seeded fruits. The statement is common in literature that the ovaries are stimulated to begin development and fertilization is prevented. Does the initiation of growth of the ovary prevent fertilization?

Mentioned also in the literature is the reduction of yield when growth-substances are applied prior to pollination contrasted to an increased yield when applied after pollination and fertilization.

Britton (4) found that the time of treatment in relation to pollination was an important variable in his experiments with maize. Application of the growth substance (0.1 per cent naphthaleneacetic acid) twenty-four hours or more before pollination resulted in parthenocarpic development of the treated ovaries. Treatment one, four, six, and twelve hours before pollination reduced the set of seed but did not result in sterility of all ovules.

Clark and Kerns (10) have shown that naphthaleneacetic acid applied to the pineapple two weeks before normal anthesis delayed ripening one week or longer. Plants treated at anthesis gave similar results.

Varrelman (93) reported that apple fruits of the Spencer navel, a stimulative parthenocarpic type, and normal types failed to develop after treatment

of unemasculated and unpollinated flowers with various concentrations of indolebutyric and naphthaleneacetic acids, the flowers being left unprotected for pollination.

According to Roberts and Struckmeyer (62), the application of an aqueous spray of certain growth-regulating substances to the flower buds of tomatoes five or more days before normal anthesis resulted in a poor set (16.7 per cent) of fruit. These investigators also found that spraying before pollination has occurred gave smaller fruits than unsprayed controls.

Application at Time of Pollination. Application of growth-regulating substances at time of pollination, or shortly thereafter, results in parthenocarpic development of a number of fruits, but the amount of seedlessness is not as great as when application is made one or more days before time of normal pollination.

Yields of several plants have been increased by application of those materials at this time, whereas the yields of other plants have not been affected, or there has been a crop reduction. What is the criterion for determining whether the yield of a plant will be increased or decreased by the application of growth-regulating substances at the pollination stage?

It is fully established now that growth-regulating substances affect the set and size of tomatoes grown under greenhouse conditions during the winter months (36) (62) (80). These materials, applied to the flower cluster or to the whole plant, give an increased yield due to increases in number of fruit set and size of fruit.

Burrell and Whitaker (8) found that the set of hand pollinated muskmelons could be increased markedly by applying a small quantity of paste of 1.0 per cent indoleacetic acid in lanolin to one lobe of the stigma after flowers had been hand pollinated.

The method of application is of considerable importance. Whitaker and Pryor (98) found that the most effective procedure was to detach the corolla where it joins the ovary and to apply the material to the torn surface.

A marked reduction in set of fruit of the apple due to aqueous sprays of naphthaleneacetic acid and naphthyl acetamide was reported by Burkholder and McCown (7). These materials were applied at concentrations of .001 and .005 per cent during full bloom. The results of Burkholder and McCown have been confirmed by several other workers (65) (76) (77).

The yield of pods of green bush beans has been increased by the application of growth-regulating substances as aqueous sprays or as dusts (15) (59) (101). These substances, applied after the flowers begin to open, appear to reduce flower bud, flower, and pod drop and to stimulate pod development. However, the average number of seeds per pod is less in treated pods than in untreated pods.

The yields of peas, lima beans and dry shelled beans have not been in-

creased by hormone treatment. These substances seem to depress seed formation (30) (97) (101).

A decrease in seed length was observed by Wong (103) following the treatment of pollinated watermelon flowers with growth-regulating substances.

Post-fertilization Application. The set and development of fruits are affected by the application of growth-regulating substances after fertilization has occurred, but in general, the effect is less pronounced.

Britton (4) found that the longer after fertilization that treatment was delayed the more nearly normal the maize caryopsis would be. Treatment prior to or at the approximate time of fertilization resulted in misshapen ears and kernels.

Greene (21) reported that naphthaleneacetic acid in wax emulsion sprayed on small apple fruits nearly a month after full bloom retarded the growth of these fruits and prevented their normal development.

A fifty per cent reduction in set of dates was observed by Nixon and Gardner (61) when an aqueous spray of naphthaleneacetic acid (.01 per cent) was applied ten days after pollination and followed by a .02 per cent spray of the same material six days later.

Smith (68), endeavoring to reduce dropping of young pecan nuts, found that naphthaleneacetic acid increased the amount of drops. The pecan clusters were dipped at approximately weekly intervals in solutions of .001 and .005 per cent naphthaleneacetic acid after the stigmas of all nuts were dry.

Effects of Growth-Regulating Substances on Pollen

Little is known concerning the effect of weak concentrations of these substances on the development of the male gametophyte; however, very strong concentrations are known to prevent pollen-shedding. Grigsby (22) found that pollen production by old plants of ragweed could be stopped, without causing the immediate death of the plants, by spraying with an aqueous solution of 2,4-dichlorophenoxyacetic acid (500 p.p.m.). Pollen formation could be prevented by spraying ragweed in the stage of growth just preceding flower stalk elongation with 250 p.p.m. 2,4-D. Plants sprayed in this stage appeared to be arrested in development and usually persisted until frost. Few seeds were produced by such plants.

According to Tukey et al. (86), pollen grains of bindweed were plasmolyzed and disorganized and flower development was arrested after the application of 2,4-D at the concentration of 1000 p.p.m.

As to the effect of these substances upon mature pollen grains, Addicott (1) and Smith (70) (71) presented data which indicated that pollen germinated on sugar agar medium was favored by the addition of growth-promoting substances. Concentrations of indole-3-acetic, indole-butyric and naphthaleneacetic acids weaker than 20 p.p.m. were favorably stimulative, but all stronger concentrations were toxic, as indicated by decreased germination, bursting and distortion of tubes.

Chemical Changes in the Plant Due to Treatment with Growth-Regulating Substances

There appears to be a direct or an indirect influence of certain growth-regulating substances upon the carbohydrate metabolism of the plant. Mitchell et al. (52) observed that naphthaleneacetic acid decreased the amount of starch and dextrins in bean leaves. Sugars increased at first then decreased later.

Borthwick et al. (3), using microchemical tests, observed that indole-3-acetic acid applied to the cut stems of tomato plants caused starch and nitrates to disappear from the treated stems. The concentration of protein in the treated stem increased in those areas when cells became abundant.

According to Brown (5), bean seedlings sprayed with 2,4-D at a concentration of 1000 p.p.m. in ethylene glycol (Carbowax 1500) .5 per cent, absorbed 34 per cent less water in a five day period after spraying than controls. Rate of respiration increased nineteen to eighty per cent beginning twenty-four hours after treatment, and solid matter in the tops decreased.

Mitchell and Marth (53) reported that the ripening period of detached fruits of banana, apple, and pear was shortened due to more rapid starch hydrolysis by treatment with 2,4-D as sprays or dips at concentrations of 100 to 1600 p.p.m.

The dry weight of morning-glory plants treated with 2,4-D 1000 p.p.m., was found by Mitchell and Brown (51) to decrease while the weight of untreated plants increased. Carbohydrates (sugar, starch, dextrins) were depleted in all treated plants in the course of three weeks, though sugar was first increased in the treated plants, then depleted rapidly.

However, the above cases may not be applicable to the present investigation, for concentrations used in these experiments were many times stronger than those used in fruit-setting sprays.

THE PROBLEM FOR INVESTIGATION

As stated previously this study deals with the response of flower buds and flowers to a specific growth-regulating substance, when applied at different stages of their growth and development. The problem posed is to determine the effect that a certain concentration of a growth-regulating substance will have on flower development and fruit set when applied at different physiological stages of development and to study some of the internal changes that may be associated with these responses.

The investigation has been divided into a number of phases. In the first one, a study has been conducted on the effects of growth-regulating substances on the yield of mature fruit when applied at different stages of development. For this study crops, whose yields can be increased by the application of these materials, have been used as well as those which are not known to be favored

by the use of these substances. Both the number of fruit set and the size of individual fruit have been taken into consideration.

In the second phase, a histological comparison has been made of the buds, flowers, and young fruits of plants after their treatment at various stages of development. This study has been directed toward finding possible changes from the normal pattern of growth of any structure or tissue due to treatment with a growth-regulating substance. If fruit buds of different physiological ages respond differently, this response may be associated with changes in certain tissues. Particular attention has been paid to the development of the pollen grains, the ovules, and the embryos in plants treated at different stages of development in contrast to these structures in otherwise comparable untreated plants.

The third phase of the investigation consists of studies designed to detect chemical changes in flower buds and young fruits due to treatment with growth-regulating substances.

Catalase determinations should give an indication of the effects of growth-regulating substances upon enzyme activity in these tissues and of the rate of metabolism in flower buds and young fruits.

Microchemical tests have been used to some extent to ascertain possible changes in starch, reducing sugars, proteins, and fats due to treatment. If growth-regulating substances cause alterations in the quantity of any one of these constituents in flower buds and young fruits, such tests should reveal this.

TERMINOLOGY

To avoid possible confusion, the definitions of certain terms as used herein are presented.

1. Auxins—Naturally occurring growth-promoting or growth-regulating substances in plants.
2. Flower Development—The period from differentiation of floral primordia until fertilization.
3. Fruit Development—That phase of development from fertilization until maturity of the fruit.
4. Growth-Regulating Substances, or Growth-Promoting Chemicals, or Hormones—Synthetic chemicals, such as α-Naphthaleneacetic acid, p-chlorophenoxyacetic acid, etc., which cause growth-regulating responses in plants.

ABBREVIATIONS

A list of abbreviations used herein is as follows:

1. ClPA—p-Chlorophenoxyacetic acid
2. CIPP—α-o-Chlorophenoxypropionic acid
3. NA—α-Naphthaleneacetic acid
4. NOA—β-Naphthoxyacetic acid

EFFECTS OF GROWTH-REGULATING SUBSTANCES ON YIELD OF FRUIT

The existing records indicate, in a general way, that the effect of hormones upon yield of fruit is influenced by the stage of development of the plant, particularly the flowers. There seems to be a need for additional evidence on this specific phase of hormone effect. Consequently it was thought desirable to conduct a series of experiments to gain more detailed information on this problem.

Yield studies were made on treated plants grown both in the field and in the greenhouse. The tomato was selected for greenhouse studies, because certain growth-promoting chemicals are often used to increase the set of fruit during the winter months when the amount of light is limited.

The grape was used for field studies. The yield of this plant is not known to be increased by the use of these chemicals, but perchance, it could be improved if application were made at the proper stage of flower development.

Yield Studies with Tomatoes

Tomatoes for all yield studies were grown in the greenhouse in concrete benches. The soil was a steam-sterilized mixture of clay loam, leaf mold, and sand to which complete fertilizer (4-12-4) was applied during the growth period of the plants. They were staked and trained to a single stem. Pollination was facilitated by tapping the clusters twice each week while the flowers were open.

Experiment I—Winter 1946-1947. The growth-regulating substance, p-cholorphenoxyacetic acid (ClPA) was selected for this experiment, because it has been shown by experiments at the Missouri Agricultural Experiment Station and elsewhere that it is one of the most effective of the hormones for fruit-setting. The plants were divided into four groups, fourteen in each, and were treated as follows:

1. ClPA—10 p.p.m.—Bud Stage. Spray applied approximately eight days prior to anthesis.
2. ClPA—10 p.p.m.—Anthesis Stage. Spray applied when flowers were fully open.
3. ClPA—10 p.p.m.—Whole Cluster Treatment. Spray applied when the first three flowers in the cluster were fully open.
4. Control—No treatment.

For treatments 1 and 2, each bud or flower was sprayed individually when it reached the proper stage. A small hand atomizer was used for appplying the growth-regulating substance, a cardboard shield being held so as to limit the distribution of the spray to the bud or flower.

Results from this experiment are presented in Table 1. ClPA, 10 p.p.m., applied to the flower at anthesis gave an increase in number of fruit set and an improvement in average size of fruit. This resulted in an aggregate increase in

Table 1.--Effects of p-Chlorophenoxyacetic Acid on Yield of Tomatoes When Applied at Different Stages of Development

(Conc. 10 p.p.m. Average for 10 plants Winter 1946-47)

Stage of Flower Development When Treated	Number of Fruit Harvested	Increase or Decrease Due to Treatment (Per Cent)	Average Weight per Fruit (Pounds)	Increase or Decrease Due to Treatment (Per Cent)	Total Weight of Fruit Harvested (Pounds)	Increase or Decrease Due to Treatment (Per Cent)
Control No Treatment	120	----	0.258	----	30.98	----
Bud Stage	92	- 23.33	0.241	- 6.58	22.18	- 28.41
Anthesis Stage	158	+ 31.66	0.277	+ 7.36	43.77	+ 42.64
Whole Cluster Flowers and Buds present	144	+ 20.00	0.263	+ 1.93	37.86	+ 22.20

yield of fruit of 42.64 per cent over the controls. When this chemical was applied to the bud, approximately eight days before anthesis, a marked reduction in yield (28.41 per cent) resulted, due both to a decreased number of fruit set and a smaller average size of fruit.

Most of the fruit from the plants treated during the bud stage were seedless, abnormally flattened, ridged, and had very large stem scars. Only a small percentage would have been marketable due to the undesirable shape and general appearance, even though they were of a marketable size. The conspicuous variation in size and form of fruits is illustrated in Figure 1.

The yield from plants treated by spraying the whole flower cluster, at the time when three flowers were fully open, was greater than that of the controls but was less than that of plants with separate flower treatment, when fully open. This indicates that the treatment should be delayed until more flowers in the cluster have opened.

Experiment II—Spring 1947. Although p-chlorophenoxyacetic acid is probably the most effective chemical for fruit-setting in the tomato, other growth-regulating substances are used also for this purpose. It was thought desirable to determine the influence upon yield of certain other growth-promoting chemicals, in addition to p-chlorophenoxyacetic acid, when applied at different stages of development.

The hormones chosen were α-naphthaleneacetic acid and β-naphthoxyacetic acid, which are very effective and are widely used for this purpose.

A group of tomatoes was divided into seven lots of twelve plants each and subjected to the following treatments:

1. p-Chlorophenoxyacetic acid—10 p.p.m.—Bud Stage.
2. p-Chlorophenoxyacetic acid—10 p.p.m.—Anthesis Stage.
3. α-Naphthaleneacetic acid—20 p.p.m.—Bud Stage.
4. α-Naphthaleneacetic acid—20 p.p.m.—Anthesis Stage.
5. β-Naphthoxyacetic acid—50 p.p.m.—Bud Stage.

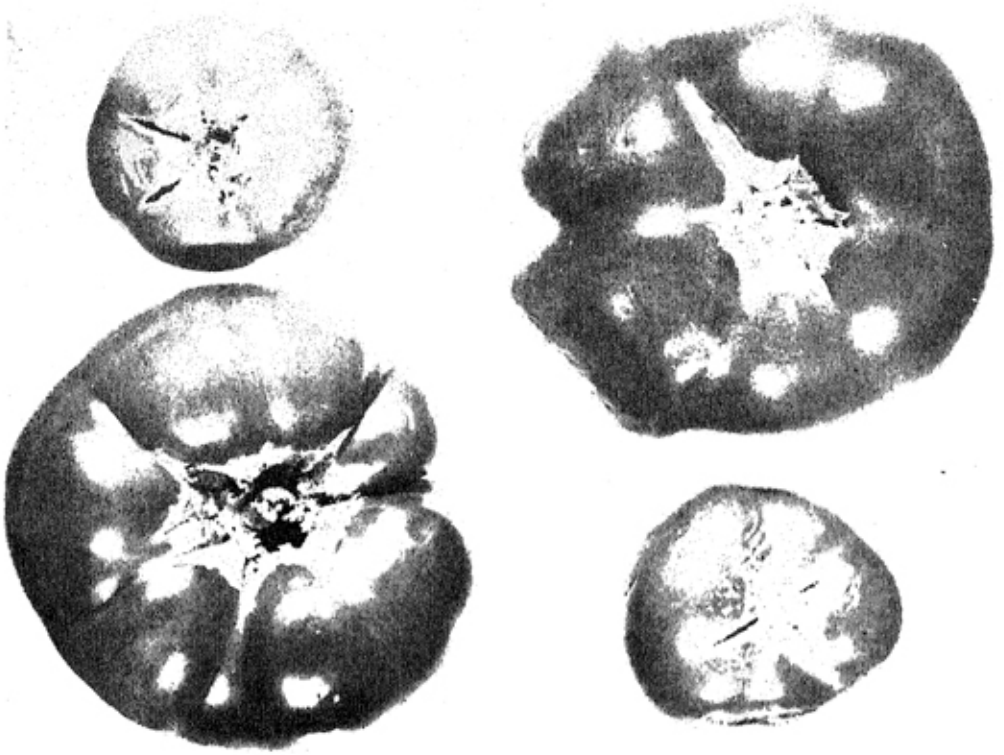

Fig. 1. Mature fruits from tomato flowers sprayed with ClPA, 10 p.p.m., approximately eight days before anthesis. Note variation in size and shape and large stem scars.

6. β-Naphthoxyacetic acid—50 p.p.m.—Anthesis Stage.
7. Control—No treatment.

All growth-regulating substances were applied as aqueous sprays. Each bud or flower was treated individually when it reached the proper stage of development. The buds were sprayed approximately eight days before anthesis. For the "anthesis stage" treatments the flowers were treated as soon as the petals were fully expanded. Probably pollination had taken place in many cases, while fertilization had occurred in but a few, if any, instances in the "anthesis stage."

Data presented in Table 2 show that an appreciable decrease in yield resulted when growth-regulating substances were applied during the bud stage. All three chemicals used in this experiment gave equivalent decreases in total weight of fruit harvested: p-chlorophenoxyacetic, 10 p.p.m., resulted in a 11.24 per cent reduction; α-naphthaleneacetic acid, 20 p.p.m., 13.11 per cent; and β-naphthoxyacetic acid, 50 p.p.m., gave a 10.76 per cent reduction when compared to the controls. This decrease in yield was due both to fewer fruit set and to a smaller average size of fruit.

A considerable increase in yield resulted when p-chlorophenoxyacetic and

Table 2.--Effects of Certain Growth Regulating Substances on Yield of Tomatoes When Applied at Two Stages of Development

(Average for 10 Plants--Variety--Master Marglobe--Spring 1947)

Treatment	Number of Fruit Harvested	Increase or Decrease Due to Treatment (Per Cent)	Average Weight per Fruit (Pounds)	Increase or Decrease Due to Treatment (Per Cent)	Total Weight of Fruit Harvested (Pounds)	Increase or Decrease Due to Treatment (Per Cent)
ClPA--10 p.p.m. Bud Stage	166	- 8.79	0.346	- 2.81	57.48	- 11.24
ClPA--10 p.p.m. Anthesis Stage	206	+ 13.19	0.363	+ 1.97	74.75	+ 15.42
NA--20 p.p.m. Bud Stage	172	- 5.49	0.327	- 8.15	56.27	- 13.11
NA--20 p.p.m. Anthesis Stage	191	+ 4.95	0.337	- 5.34	64.35	- 0.63
NOA--50 p.p.m. Bud Stage	174	- 4.39	0.340	- 4.49	59.20	- 8.59
NOA--50 p.p.m. Anthesis Stage	195	+ 7.14	0.368	+ 3.37	71.73	+ 10.76
Control--No treatment	182	----	0.356	----	64.76	----

β-naphthoxyacetic acids were applied to fully open flowers. This was due largely to a greater number of fruit harvested, although there was also a small increase in fruit size.

α-Naphthaleneacetic acid, 20 p.p.m., did not augment the yield when applied to open flowers. The benefit from a slight increase in number of fruit was offset by a reduction in their average size.

Experiment III—Winter 1947—1948. Investigators have shown (18) that flowers may be stimulated by hormones even several days after anthesis. Therefore, it was thought worth-while to determine their influence upon yield when applied soon after fertilization. The literature indicates that growth-regulating substances tend to prevent fertilization; hence a post-fertilization application might be more beneficial than at earlier stages. The developing seed is a center of production for auxins (26) (101) which stimulate the development of the fruit. Some fruits will make little if any growth when seeds are absent, even though growth-promoting chemicals are applied.

For this experiment flowers four days after they had opened were selected as being in a post-fertilization stage. According to Judkins (39), usually fertilization in the tomato will have occurred in four days or less after pollination.

It was also deemed desirable to determine the influence of a higher as well as the usual concentration of growth-regulating substances when applied at this post-anthesis stage of sexual reproduction.

Ten plants were used in each of the treatments, which were as follows:

1. p-Chlorophenoxyacetic acid—10 p.p.m.—Bud Stage—applied approximately eight days before anthesis.

2. p-Chlorophenoxyacetic acid—10 p.p.m.—Anthesis Stage—applied when flowers were fully open.
3. p-Chlorophenoxyacetic acid—10 p.p.m.—Post-Anthesis Stage—applied four days after flowers were fully open.
4. p-Chlorophenoxyacetic acid—20 p.p.m.—Post-Anthesis Stage—applied four days after flowers were fully open.
5. α-Naphthaleneacetic acid—20 p.p.m.—Bud Stage—applied approximately eight days before anthesis.
6. α-Naphthaleneacetic acid—20 p.p.m.—Anthesis Stage—applied when flowers were fully open.
7. α-Naphthaleneacetic acid—20 p.p.m.—Post-Anthesis Stage — applied four days after flowers were fully open.
8. α-Naphthaleneacetic acid—40 p.p.m.—Post-Anthesis Stage — applied four days after flowers were fully open.
9. Control—No treatment.

All buds and flowers were sprayed individually and only once at the desired stage of development.

At harvesting, fruits were separated into marketable and unmarketable sizes. Those weighing three ounces or more were considered to be marketable.

Fruits of ample size were cut open to determine the effect of the different treatments upon the development of seeds.

This experiment was closed when all fruits had ripened. Toward the end of the harvesting period, when the majority of the fruits had been picked, ovaries of flowers which had not developed into fruit but which had persisted began to enlarge. A considerable number of small, rough, seedless fruits were produced from these persisting flowers. They account for the large majority of the fruit of unmarketable size.

1. *Yield Records.* In Tables 3 and 4 are presented data from this experiment. It will be noted that p-chlorophenoxyacetic acid, 10 p.p.m., applied approximately eight days prior to anthesis caused a marked decrease in yield due to a reduction in number of fruit developed, particularly those of a marketable size. Flowers treated in the bud stage produced, on the average, smaller fruit than when treated in the anthesis or post-anthesis stages. Specifically, α-naphthaleneacetic acid, 20 p.p.m., applied during the bud stage resulted in an appreciable decrease in total yield due to a smaller average size of fruits. A conspicuous reduction in weight of marketable fruit resulted due to their small average size and to the decreased number of fruit which reached a desirable size.

When application was made at anthesis or four days later, a larger yield resulted from plants sprayed with ClPA, 10 p.p.m., than from untreated ones. This improvement in yield was due to the greater number of fruit which reached a marketable size. Post-anthesis appears to be a more favorable time for treat-

Table 3.--Effects of p-Chlorophenoxyacetic and *a* -Naphthaleneacetic Acids Upon Yield of Greenhouse Tomatoes When Applied at Different Concentrations and Stages of Development

(Average for 10 Plants--Variety--Master Marglobe--Winter 1947-48)

Treatment	Total Number of Fruit Harvested	Total Weight of Fruit Harvested (Pounds)	Average Size per Fruit (Pounds)	Number of Fruit of Unmarketable Size*	Weight of Unmarketable Fruit (Pounds)	Number of Fruit of Marketable Size*	Weight of Marketable Fruit (Pounds)
Controls	294	59.25	0.202	142	12.75	152	46.50
ClPA-10 p.p.m. Bud Stage	204	45.25	0.222	87	8.94	117	36.31
ClPA-10 p.p.m. Anthesis	217	55.45	0.256	36	3.07	181	52.38
ClPA-10 p.p.m. Post-Anthesis	240	59.32	0.249	44	4.69	196	54.63
ClPA-20 p.p.m. Post-Anthesis	239	63.92	0.267	49	4.44	190	59.48
NA-20 p.p.m. Bud Stage	265	46.44	0.175	162	13.81	103	32.63
NA-20 p.p.m. Anthesis Stage	253	50.05	0.198	102	9.19	151	40.86
NA-20 p.p.m. Post-Anthesis	286	54.98	0.193	128	10.88	158	44.12
NA-40 p.p.m. Post-Anthesis	261	49.88	0.191	112	9.63	149	40.25

* Fruits 3 ounces and over considered as marketable size.

Table 4.--Effects of p-Chlorophenoxyacetic (ClPA) and *a*-Naphthaleneacetic (NA) Acids Upon Yield of Marketable Greenhouse Tomatoes When Applied at Different Concentrations and Stages of Development

(Average for 10 Plants--Variety--Master Marglobe)

Treatment	Number of Fruit of Marketable Size*	Increase or Decrease Due to Treatment (Per Cent)	Total Weight of Fruit (Pounds)	Increase or Decrease Due to Treatment (Per Cent)
Controls	152	----	46.50	----
ClPA-10 p.p.m. Bud Stage	117	- 23.03	36.31	- 21.91
ClPA-10 p.p.m. Anthesis Stage	181	+ 19.08	52.38	+ 12.65
ClPA-10 p.p.m. Post Anthesis Stage	196	+ 28.95	54.63	+ 17.48
ClPA-20 p.p.m. Post Anthesis Stage	190	+ 25.00	59.48	+ 27.91
NA-20 p.p.m. Bud Stage	103	- 32.24	32.63	- 29.83
NA-20 p.p.m. Anthesis Stage	151	- 0.66	40.86	- 12.13
NA-20 p.p.m. Post Anthesis Stage	158	+ 3.95	44.12	- 5.12
NA-40 p.p.m. Post Anthesis Stage	149	- 1.97	40.25	- 13.51

* Fruits 3 ounces or more considered as marketable size

ment than at anthesis, because the largest number of marketable fruit were produced by plants treated at this time.

The highest yield resulted when p-chlorophenoxyacetic acid, 20 p.p.m., was applied at the post-fertilization stage of sexual reproduction. Fruits from this group of plants were larger than those of any other lot.

α-Naphthaleneacetic acid, 20 p.p.m., depressed the yield when applied at any one of the three stages of development. The greatest reduction (29.83 per cent) resulted when application was made during the bud stage, and the smallest reduction (5.12 per cent) occurred with a post-fertilization application.

A higher concentration (40 p.p.m.) of *α*-naphthaleneacetic acid applied at the post-anthesis stage inhibited the development of a large portion of the

young fruit on the basal cluster. After a period of three or four weeks, they began to enlarge, but a normal size was never reached.

All results seem to indicate that the most beneficial time of treatment is after fertilization has occurred.

With p-chlorophenoxyacetic acid, post-anthesis applications gave higher yields than did treatments at earlier stages of development, and, in the case of α-naphthaleneacetic acid, which depressed yields, the harmful influence was less marked when the application was made at the post-anthesis stage than when made at less advanced stages of flower development.

2. *Seed development.* The effects of p-chlorophenoxyacetic acid and α-naphthaleneacetic acids upon seedlessness are shown in Table 5. ClPA, 10 p.p.m., applied during the bud stage or at anthesis caused practically all fruit to be seedless or have a reduced number of seeds. Spraying four days after anthesis had much less influence, for 61.0 per cent of the fruit from the post-anthesis-treated lot were normally seeded, and only 9.0 per cent were completely seedless. However, the application of a higher concentration (20 p.p.m.) of this chemical at the post-anthesis stage gave 62.0 per cent seedless and only 12.0 per cent normally seeded fruits.

Table 5.--The Influence of Chlorophenoxyacetic Acid (ClPA) and α-Naphthaleneacetic Acid (NA) Upon Seed Development When Applied at Different Concentrations and Stages of Development

(Average for 100 Fruits of Marketable Size--Variety--Master Marglobe)

Treatment	Number of Normal Seeded Fruits	Number of Partly Seeded Fruits	Number of Seedless Fruits
Controls	94	6	0
ClPA-10 p.p.m. Bud Stage	4	36	60
ClPA-10 p.p.m. Anthesis Stage	1	37	62
ClPA-10 p.p.m. Post Anthesis Stage	61	30	9
ClPA-20 p.p.m. Post Anthesis Stage	12	26	62
NA-20 p.p.m. Bud Stage	32	42	26
NA-20 p.p.m. Anthesis Stage	29	63	8
NA-20 p.p.m. Post Anthesis Stage	67	30	3
NA-40 p.p.m. Post Anthesis Stage	41	53	6

α-Naphthaleneacetic acid, 20 p.p.m., applied at the bud stage or anthesis stage caused considerable reduction in the number of fully seeded fruit. The harmful effects are lessened when the application is delayed until after fertilization. When treated at the post-anthesis stage, 67.0 per cent of the fruit were normally seeded, whereas, only 32.0 per cent with bud stage treatment, and only 29.0 per cent when sprayed at anthesis. However, a concentration of 40 p.p.m. applied at the post-anthesis stage inhibited seed development, partially or completely, in approximately 60.0 per cent of the fruits.

3. *Viability of seeds from fruit sprayed with hormones.* A large portion of the seeds from fruits treated with α-naphthaleneacetic acid exhibited brown-colored areas on the seed coats. A number of these seeds were sectioned, and

the embryo and endosperm found normal. The question arose as to the viability of seeds from fruits treated with growth-regulating substances. Fully developed seeds were selected from control fruits, fruits from flowers sprayed with p-chlorophenoxyacetic acid, 20 p.p.m., four days after anthesis; and fruits from flowers sprayed with α-naphthaleneacetic acid, 40 p.p.m., at the same post-anthesis stage.

Results are presented in Table 6. The germination of fully matured seeds from fruits which had been sprayed with p-chlorophenoxyacetic acid, 20 p.p.m., and α-naphthaleneacetic acid, 40 p.p.m., soon after fertilization was not different from that of seed from control plants. Although the majority of the seeds may abort or be inhibited, those few which complete their development are viable.

Table 6.--Effects of p-Chlorophenoxyacetic Acid and α-Naphthaleneacetic Acid Upon Germination of Seed from Fruit Treated After Fertilization

(500 Seeds Tested in Each Group)

Treatment	Number of Seedlings	Percentage Germination	Increase or Decrease Due to Treatment (Per Cent)
Control	474	94.8	----
ClPA--20 p.p.m.--Post Anthesis Stage	478	95.6	- 0.84
NA--40 p.p.m.--Post Anthesis Stage	467	93.4	- 1.48

Yield of Grapes as Influenced by Growth-Promoting Chemicals

These experiments were conducted in a vineyard of American type grapes, Concord variety, with plants trained to the four-cane Kniffin system. Clean cultivation was practiced and a spray program for the control of black rot was carried out. The plants were very vigorous.

Effects of Weekly Application of Hormones Beginning at the Full Bloom Stage. Grapes are not known to respond favorably to hormones; therefore, it was thought desirable to determine the effects of two of the more active growth-promoting chemicals. The stimulating properties of these hormones had been known for a relatively short time, and there were no reports of their use on grapes.

Four groups of eight plants each were used. One half of each vine was treated, the opposite half being used as a control. The treatments were as follows:

1. α-o-Chlorophenoxypropionic acid—5 p.p.m.
2. α-o-Chlorophenoxypropionic acid—10 p.p.m.
3. p-Chlorophenoxyacetic acid—2½ p.p.m.
4. p-Chlorophenoxyacetic acid—5 p.p.m.

Four sprays, at weekly intervals, were applied, beginning at full bloom.

Table 7.--Effects of α-o-Chlorophenoxypropionic and p-Chlorophenoxyacetic Acids Upon Fruit Set and Development of Grapes When Applied at Full Bloom

(Average for 8 Plants--Variety--Concord)

Treatment	Total Weight of Fruit Harvested (Pounds)	Increase or Decrease Due to Treatment (Per Cent)	Date of Harvest
Control to ClPP--5 p.p.m.	50.24	----	August 14
ClPP--5 p.p.m.	37.53	- 25.29	August 14
Control to ClPP--10 p.p.m.	49.08	----	August 14
ClPP--10 p.p.m.	29.65	-47.15	August 14
Control to ClPA--2 1/2 p.p.m.	52.54	----	August 14
ClPA--2 1/2 p.p.m.	45.36	- 13.67	August 14
Control to ClPA--5 p.p.m.	48.19	----	August 14
ClPA--5 p.p.m.	43.28	- 10.18	September 9

All foliage as well as fruit clusters were thoroughly covered by the spray.

As shown in Table 7, the yield was reduced in all cases where growth-regulating substances were used. ClPP gave the greatest decreases: 47.15 per cent for 10 p.p.m. and 25.29 per cent with a concentration of 5 p.p.m. At harvest, the sprayed clusters were "loose" due to a reduced number of fully developed berries per cluster. A number of small berries, one-eighth to one-fourth inch in diameter, were persisting. They were green and seedless. Fertilization had been prevented by the first spray or the embryo had been killed by a later spray, and the growth-promoting chemical did not provide enough stimulus for the berry to develop without the presence of viable seeds.

The effects of p-chlorophenoxyacetic acid were similar to those of α-o-chlorophenoxypropionic acid. A reduction in yield, though not as great, resulted with both concentrations. This was due to a smaller number of mature berries per cluster. As in the case of ClPP, partly developed seedless fruit were still persisting at harvest. But a conspicuous delay in maturity resulted from applications of ClPA, 5 p.p.m. Other groups, including the controls, were harvested August 14, whereas the fruit having received this treatment were not ready for harvest until September 9.

Effects of Growth-regulating Substances on Berry Size and Rate of Development. The small size of the berry is an undesirable characteristic of several varieties of grapes, especially so in the case of the seedless ones. This experiment was planned to determine the effects of growth-promoting chemicals upon berry size and rate of development. Three of the most important or most widely used compounds were chosen for this test, namely, p-chlorophenoxyacetic acid, α-naphthaleneacetic acid, and β-naphthoxyacetic acid. They were applied at the following concentrations:

1. p-Chlorophenoxyacetic acid—2½ p.p.m.
2. α-Naphthaleneacetic acid—10 p.p.m.
3. β-Naphthoxyacetic acid—30 p.p.m.

One half of each of eight plants was sprayed four times at weekly intervals, the first being applied after fruit set when the berries were approximately 3 mm. in diameter. The foliage as well as the fruit bunches were sprayed in the case of the treated portion of the vine.

The records, given in Table 8, indicate that p-chlorophenoxyacetic acid, 2½ p.p.m., hastened maturity and augmented the size of the berries, while α-naphthaleneacetic acid, 10 p.p.m., delayed maturity and reduced berry size.

Table 8.--Effects of Post-Fertilization Sprays of p-Chlorophenoxyacetic, α-Naphthaleneacetic, and β-Naphthoxyacetic Acids Upon Yield, Berry Size, and Date of Maturity of Grapes

(Average for 8 Plants--Variety--Concord--1946)

Treatment	Total Weight of Fruit Harvested (Pounds)	Increase or Decrease Due to Treatment (Per Cent)	Average Weight per Fruit (Grams)	Increase or Decrease Due to Treatment (Per Cent)	Average Number of Fully Ripe Berries per 100 Berries when Harvested	Increase or Decrease Due to Treatment (Per Cent)
Control to ClPA	49.01	----	3.18	----	53.88	----
ClPA--2 1/2 p.p.m.	50.81	- 3.67	3.30	- 3.63	65.17	+ 20.95
Control to NA	47.61	----	3.26	----	77.51	----
NA--10 p.p.m.	43.77	- 8.07	3.01	- 7.67	35.21	- 42.30
Control to NOA	45.99	----	3.06	----	58.94	----
NOA--30 p.p.m.	43.93	- 4.47	3.07	- 0.33	55.30	- 6.18

The slight improvement in yield in the case of ClPA, and the decreased yield in the case of NA, can be accounted for by the effect of the respective chemicals on berry size. β-Naphthoxyacetic acid, 30 p.p.m., appeared to have no appreciable influence on berry size and only a slight delaying effect on maturity.

According to the records, these hormones have no practical value in augmenting the yield of grapes by improving on berry size; however, the difference in effect of p-chlorophenoxyacetic acid, 2½ p.p.m., and α-naphthaleneacetic acid, 10 p.p.m., on maturity is significant.

A Comparison of the Effects of Hormones When Applied at Three Stages of Development. In previous experiments four sprays at weekly intervals were used. If plants respond differently when treated at different stages of development, one treatment may tend to counteract the effects of another. Therefore, it would be desirable to determine the effects of only one application at different stages of development of the flower and fruit.

The plants were divided into nine groups of six plants each, and were treated as follows:

Treatments during Flower Bud Stage

1. p-Chlorophenoxyacetic acid—10 p.p.m.
2. α-Naphthaleneacetic acid—10 p.p.m.
3. β-Naphthoxyacetic acid—25 p.p.m.

Treatments when in Full Bloom

1. p-Chlorophenoxyacetic acid—10 p.p.m.
2. α-Naphthaleneacetic acid—10 p.p.m.
3. β-Naphthoxyacetic acid—25 p.p.m.

Post-Fertilization Treatments

1. p-Chlorophenoxyacetic acid—10 p.p.m.
2. α-Naphthaleneacetic acid—10 p.p.m.
3. β-Naphthoxyacetic acid—25 p.p.m.

These sprays were applied six days after full bloom when berries were approximately 3 mm. in diameter.

As in previous experiments, one half of each vine was sprayed while the other part was used as a control. Both foliage and fruit clusters were thoroughly covered with the spray. To prevent damage to berries by insects and

Table 9.--Yield of Grapes as Affected by Growth-Regulating Substances Applied at Three Stages of Development

(Average for 6 Plants--Variety--Concord)

Treatment	Total Weight of Fruit Harvested (Pounds)	Increase or Decrease Due to Treatment (Per Cent)
Bud Stage		
Control to ClPA	49.50	----
ClPA--10 p.p.m.	38.61	- 22.00
Control to NA	55.50	----
NA--10 p.p.m.	50.50	- 9.00
Control to NOA	45.42	----
NOA--25 p.p.m.	40.76	- 10.26
Full Bloom Stage		
Control to ClPA	52.12	----
ClPA--10 p.p.m.	44.25	- 15.12
Control to NA	49.24	----
NA--10 p.p.m.	33.26	- 32.44
Control to NOA	46.20	----
NOA--25 p.p.m.	25.98	- 43.76
Post-Fertilization Stage		
Control to ClPA	46.84	----
ClPA 10 p.p.m.	42.98	- 6.53
Control to NA	51.20	----
NA--10 p.p.m.	48.76	- 4.84
Control to NOA	39.50	----
NOA--25 p.p.m.	42.26	+ 6.99

birds, all fruit clusters were enclosed in paper bags when the first berries began to ripen.

Data presented in Table 9 indicate that these three growth-regulating substances at the concentrations used in this experiment are detrimental to flower development. Significant reductions in yields resulted when they were applied approximately three weeks prior to full bloom. The lower yields of treated groups were due to the small number of berries per bunch.

Application at the full bloom stage appears to be as harmful, or more so, than treatment at an earlier stage. As was observed in a previous experiment, treatment at this stage resulted in fruit bunches which were loose and which contained a large number of partly developed berries, one eighth to one fourth inch in diameter, which were seedless and which did not ripen properly.

When applied to young fruit, p-chlorophenoxyacetic and α-naphthaleneacetic acids, at concentrations of 10 p.p.m., resulted in slightly lower yields than their respective control groups, whereas β-naphthoxyacetic acid, 25 p.p.m., improved the yield slightly over that of its control. However, none of the differences in yield that resulted from the post-fertilization applications were of sufficient magnitude to be highly significant.

With treatment after fertilization all fruit clusters were well filled. No small seedless fruits were persisting on any sprayed cluster.

One application did not influence maturity to the extent that four applications did in previous studies. There was no appreciable difference in degree of ripeness between controls and treated lots, except for the partly developed berries of groups sprayed during the full bloom stage.

HISTOLOGICAL STUDIES

A number of investigations (2) (17) have been made on the development of parthenocarpic fruit from unfertilized ovaries stimulated by growth-regulating substances; however, little is known of the effects of hormones on flower development and the development of fruits from fertilized ovaries. The objectives of this study were to determine the changes that may occur in the flower and in the young fruit as a result of hormone application.

Materials and Methods

The tomato was chosen for these studies because fruit-setting hormones are used at present to a greater extent on this plant than on any other crop. By commercial growers, applications are made both in the greenhouse and in the field.

The plants for histological observation were grown in the greenhouse in a manner previously described. Treatment, by spraying with p-chlorophenoxyacetic acid, 10 p.p.m., was as follows:

1. Bud Stage—approximately eight days before anthesis.
2. Bud Stage—approximately four days before anthesis.
3. Anthesis Stage—when flowers were fully open.

4. Post-Anthesis Stage—four days after flowers were fully open.

A small hand atomizer was used to treat the buds and flowers, which were sprayed individually and only once.

Collections of buds and flowers for histological examinations were made from the second and third clusters at 24, 48, 96, 192, and 288 hours after treatment.

To carry out this experiment the following plan was used:

Treatments Prior to Anthesis

Time of Collection after Treatments had begun	Control	Sprayed when Sepals began to separate. (Approx. eight days before anthesis	Sprayed four days after Sepals had begun to separate. (Approx. four days before anthesis)
Hours	Plant No.	Plant No.	Plant No.
0	1	--	--
24	2	3	--
48	4	5	--
96	6	7	--
120	8	--	9
144	10	--	11
192	12	14	13
288	22	24	23
384	32	--	33

Treatments at Anthesis and Four Days Later

Time of Collection after Treatments had begun	Controls	Sprayed when Flowers were fully open	Sprayed Four Days after Flowers were fully open
Hours	Plant No.	Plant No.	Plant No.
0	15	--	--
24	16	17	--
48	18	19	--
96	20	21	--
120	25	--	26
144	27	--	28
192	29	30	31
288	34	35	36
384	37	--	38

Material for sectioning was placed in FAA (formaldehyde, acetic acid, ethyl alcohol) for killing and fixing. Dioxane was used as a dehydrating agent and paraffin solvent employing the method described by Sass (63). Microtome sections, ten microns in thickness, were prepared and stained with Delafield's haemotoxylin.

Results

Macroscopic Comparisons. After all samples had been collected, killed, and fixed, but before starting the dehydrating process, treated groups were paired with their respective control lots in order to make general comparisons and photography possible. Observations presented in Table 10 indicate that buds treated eight days or four days before anthesis were somewhat inhibited and that the retardation of development was greater with treatment at the earlier stage. At the time 100 per cent of the unsprayed flowers were fully open, 60 per cent of those treated eight days before anthesis and 90 per cent of those sprayed four days before anthesis had reached a comparable stage.

Table 10.--Effects of p-Chlorophenoxyacetic Acid on Development of Tomato Flowers

(Variety--Master Marglobe)

Time of Collection Days before or after normal time of Anthesis	Control No Treatment	Treated Eight Days Before Anthesis	Treated Four Days Before Anthesis
-8	Sepals have begun to separate	Sepals have begun to separate	Sepals have begun to separate
-4	Buds larger than those of treated group and more nearly open	Development less rapid than that of controls. Buds not as nearly open	----------
0	100% of Flowers Open	60% of Flowers Open	90% of Flowers Open
+4	Corolla and Anthers shed from 80% of flowers	Corolla and Anthers shed from 30% of flowers	Corolla and Anthers shed from 60% of flowers

This decrease in rate of development was evident, also, when time of shedding of corolla and anthers and size of ovaries were compared. These differences are illustrated in Figure 2.

It will be noted by the records of Table 11, that flowers sprayed with p-chlorophenoxyacetic acid, 10 p.p.m., at anthesis or four days after anthesis, are stimulated as measured by rate of development of the young fruit.

Noteworthy is the difference in response to the application of the same concentration of this hormone. Four to eight days prior to anthesis, the flower is not subject to stimulation while at later stages, anthesis and four days beyond, its rate of development is accelerated.

Microscopic Observations. Each treated section was studied to detect any change from the normal pattern of development as represented by the controls. All parts of the flower or fruit were studied with particular emphasis on pollen, pistils, ovules, and seed.

1. *Pollen development.* In buds which were treated approximately eight days before anthesis the first noticeable change was the effect of the growth-regulating substance on pollen development. This was evident 24 hours after treatment. Pollen grains were shrunken and some had failed to stain.

About 48 hours after treatment a large number of the pollen grains were collapsed or the protoplasm was clumped in the center of the cell. Pollen grains which were not shrunken or collapsed appeared to develop more slowly than those of the untreated buds. Wing tips were visible on the majority of the pollen grains from untreated buds, whereas less than 10 per cent of the treated pollen grains had developed this structure. A comparison of the pollen grains of treated and untreated buds is illustrated in Figure 3.

At 96 hours after treatment the percentage of shrunken and collapsed pollen grains was not materially greater than that observed at 48 hours. The amount of defective pollen varied from approximately 25 per cent for some flowers to 100 per cent for others. As measured by wing-tip development, that portion of the pollen which appeared normal was less advanced than that of

Fig. 2. Effects of ClPA, 10 p.p.m., on flower and fruit development of the tomato: (22) controls; (23) sprayed approximately four days before anthesis; and (24) sprayed approximately eight days before anthesis. Note difference in size of ovaries and in number of flowers which have shed the anthers.

Table 11.--Effects of p-Chlorophenoxyacetic Acid Upon Growth and Development of Young Tomato Fruits

(Variety--Master Marglobe)

Time of Collection Days After Anthesis	Diameter of Fruit in Millimeters		
	Controls--No Treatment	Treated at Anthesis	Treated Four Days after Anthesis
4	4.6	6.1	----
6	6.9	----	8.7
8	10.1	14.8	13.4
12	16.1	22.4	21.2
16	21.8	----	26.2

control flowers. Figure 4 shows the pollen of a flower 96 hours after treatment and pollen of an unsprayed flower.

The percentage of defective pollen had not increased at 192 hours after that observed at 48 or 96 hours. A conspicuous difference observed in flowers collected at this stage was the amount of germinating pollen present on stigmas of sprayed and control flowers. A small amount of pollen, a little of which had germinated, was present on the stigmas of only 10 per cent of treated flowers, while 80 per cent of the untreated flowers had germinating pollen present.

Even 288 hours (12 days) after treatment some pollen sacs of sprayed flowers had not dehisced; while untreated flowers had set fruit, and the corolla and anthers had been shed from 80 per cent of the flowers.

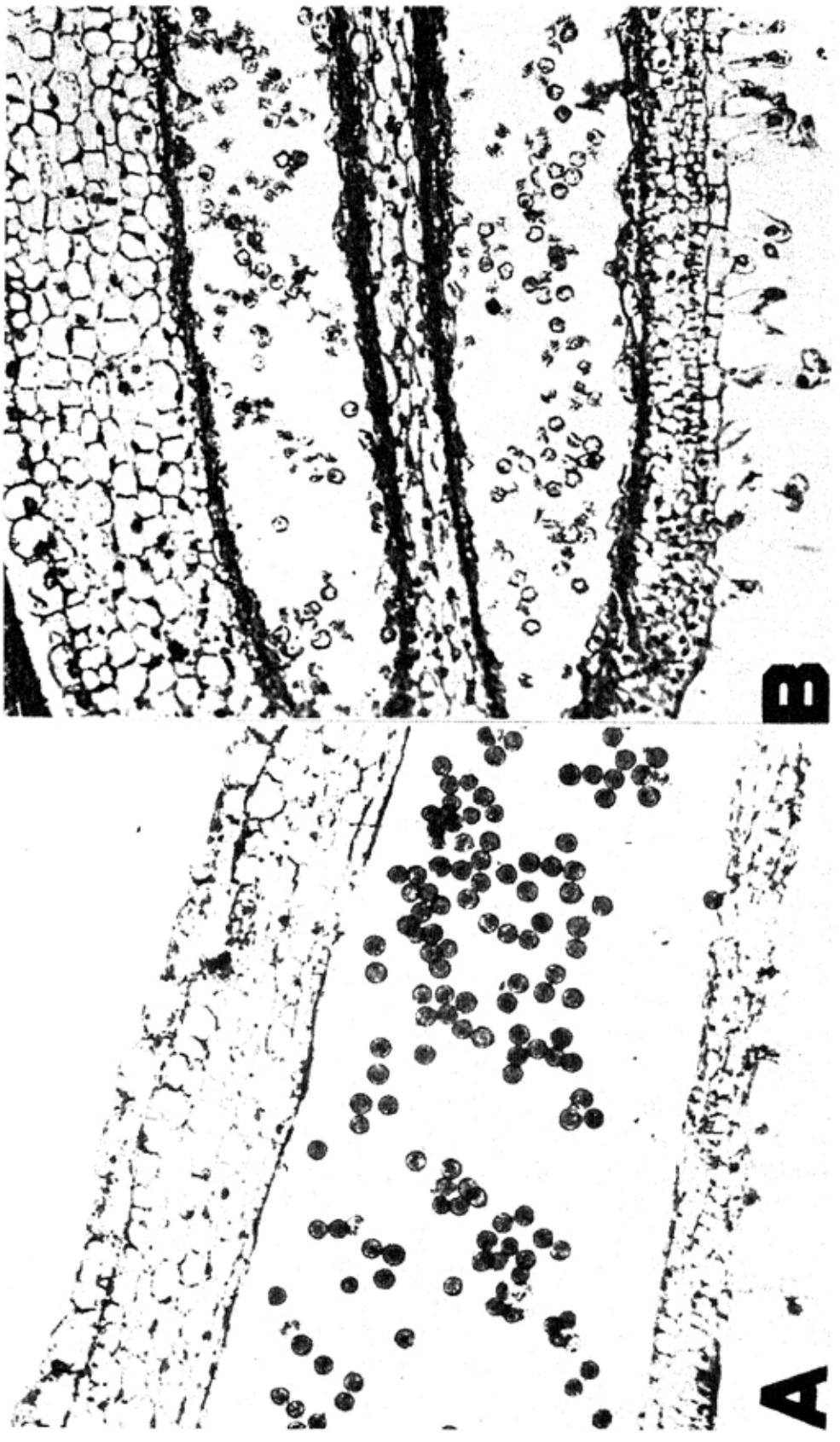

Fig. 3. Effects of ClPA, 10 p.p.m., on tomato pollen development: (A) control; (B) sprayed approximately eight days before anthesis. Flowers collected 48 hours after treatment. Note difference in amounts of collapsed and unstained pollen in treated and untreated sections.

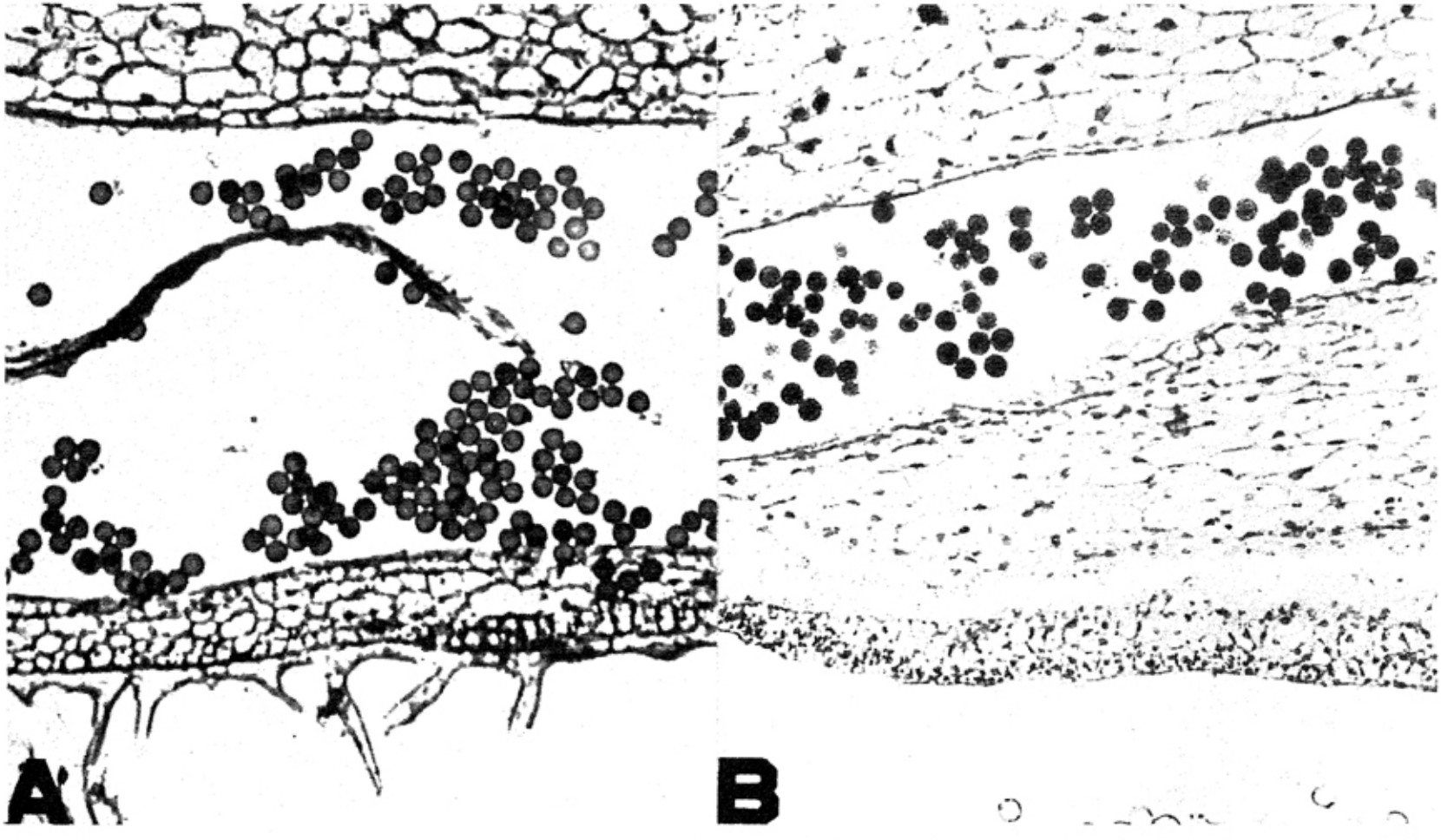

Fig. 4. Influence of ClPA, 10 p.p.m., on pollen development: (A) control; (B) sprayed approximately eight days before anthesis. Collected 96 hours after treatment. Note difference in amounts of shrunken and unstained pollen and in the number of wing tips visible.

The effects of ClPA, 10 p.p.m., upon pollen development, when applied approximately four days before anthesis, appear to be very different from its effects when applied four days earlier. Treatment at the later stage of development seemed to stimulate the growth of the pollen grain wall. The enlargement of this membrane produced a large hyaline area around the protoplasm. This condition was evident in pollen of some flowers 24 hours after spraying. Photographs of pollen of untreated flowers, from those treated eight days before anthesis, and from flowers sprayed four days later are presented in Figure 5. Material for these sections was collected 96 hours after spraying the later-treated group.

In some instances, as illustrated in Figure 6, germination appears to have been initiated, even though the pollen was still in the anther sac. Moreover. some pollen grains exhibited not one but two or three tubes, with one originating at each wing tip.

To determine the amount of defective or abnormal pollen, counts were made in sections prepared from unsprayed flowers, and flowers treated at each of the two pre-pollination stages. Pollen grains which were shrunken, collapsed, plasmolyzed, or exhibited enlarged cell walls or premature germination were considered as defective. The results are presented in Table 12. The amount of defective pollen of untreated flowers was approximately 2 per cent, while that of sprayed flowers was much greater: 45 per cent for those treated at eight days before anthesis, and 37 per cent for those sprayed four days later.

Table 12.--Effects of p-Chlorophenoxyacetic Acid Upon Pollen Development When Applied Prior to Anthesis

(Average for 10 Flowers)

Time of Collection Days before or after Normal Date of Anthesis	Defective Pollen*		
	Controls--No Treatment (Per Cent)	Treated Eight Days Before Anthesis (Per Cent)	Treated Four Days Before Anthesis (Per Cent)
-4	1.8	46.1	----
0	2.5	44.9	35.7
+4	2.1	43.2	38.1

* Collapsed, shrunken, enlarged cell wall, or premature germination

2. *Ovule development.* Another obvious result of hormone application was its effects on ovule development. Measurements of ovule lengths were made from sections prepared from material collected at stages from eight days before anthesis until sixteen days after. The results are presented in Table 13 and Figure 7.

Buds treated eight days before anthesis contained ovules which had developed little, if any, after treatment and ovules which continued to develop, but at a slower rate than that of untreated ovules.

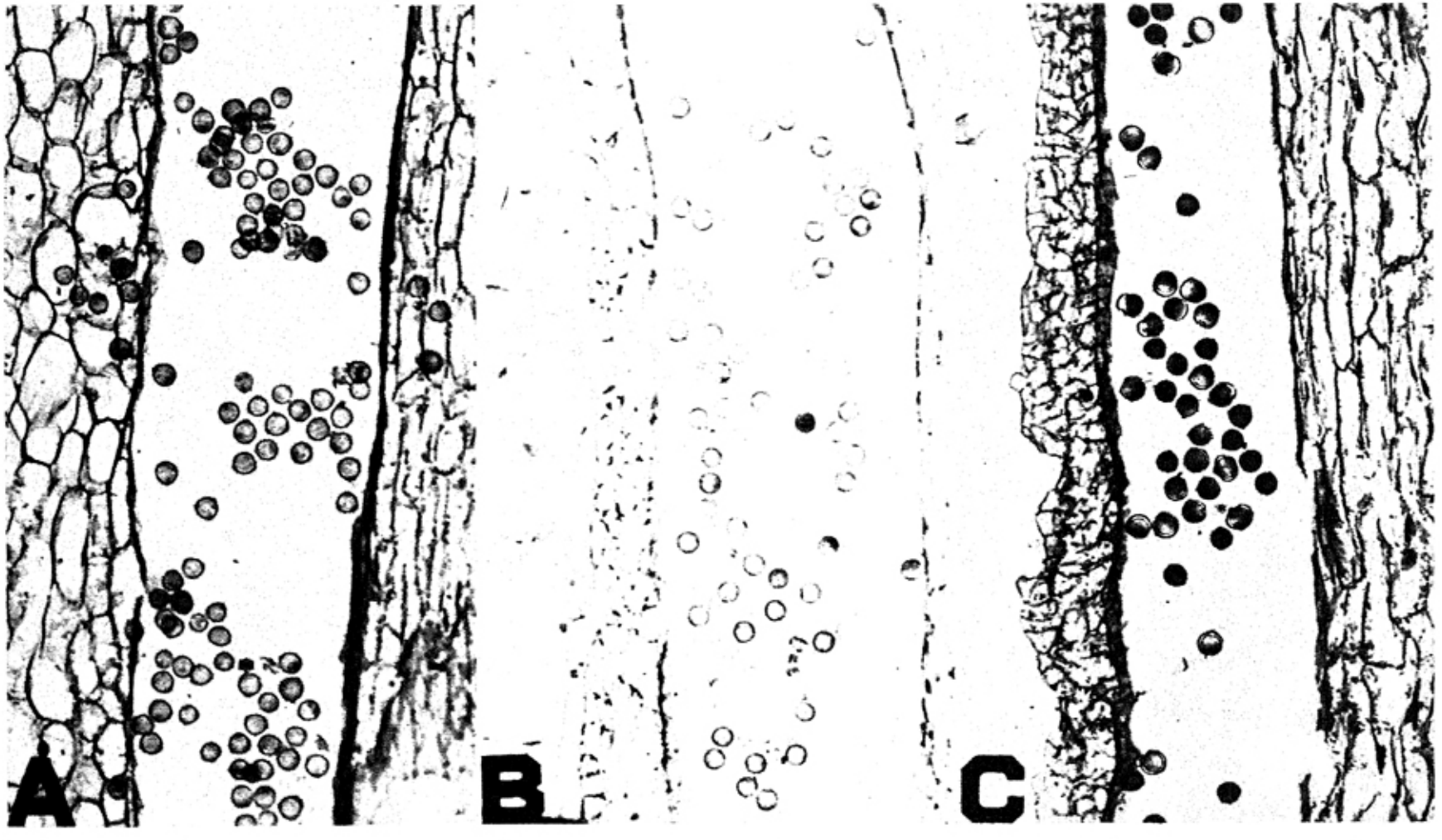

Fig. 5. A comparison of the effects of ClPA, 10 p.p.m., on tomato pollen development: (A) control; (B) sprayed approximately eight days before anthesis; and (C) sprayed approximately four days before anthesis. Sections from flowers of comparable ages. Note collapsed pollen grains and absence of wing tips in (B). Note hyaline area due to enlarged pollen grain wall in (C).

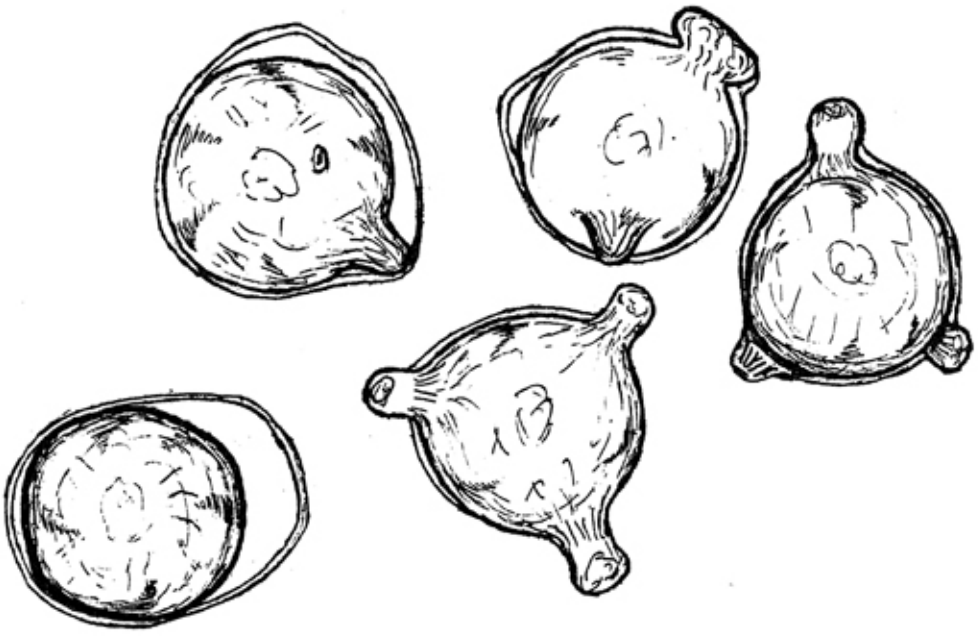

Fig. 6. Pollen grains from tomato flowers sprayed with ClPA, 10 p.p.m., four days before anthesis. Note enlarged wall and pollen tube growth.

Table 13.--Effects of p-Chlorophenoxyacetic Acid Upon Ovule Development When Applied Approximately Eight Days or Four Days Before Anthesis

Days Before or After Anthesis	Length of Ovules in Millimeters		
	Controls--No Treatment	Treated Eight Days Before Anthesis	Treated Four Days Before Anthesis
-8	.216	----	----
-7	.222	.220	----
-6	.230	.220	----
-4	.253	.228	----
-3	.264	----	.256
-2	.270	----	.255
0	.293	.230	.255
+4	.400	.245	.303
+8	.635	----	.510

An application of ClPA, 10 p.p.m., four days later gave similar results. Some ovules ceased to enlarge while others continued to grow, but at a retarded rate.

In buds sprayed at either of the above stages, the embryo sac disintegrated in those ovules which were completely inhibited. This is illustrated in Figure 8.

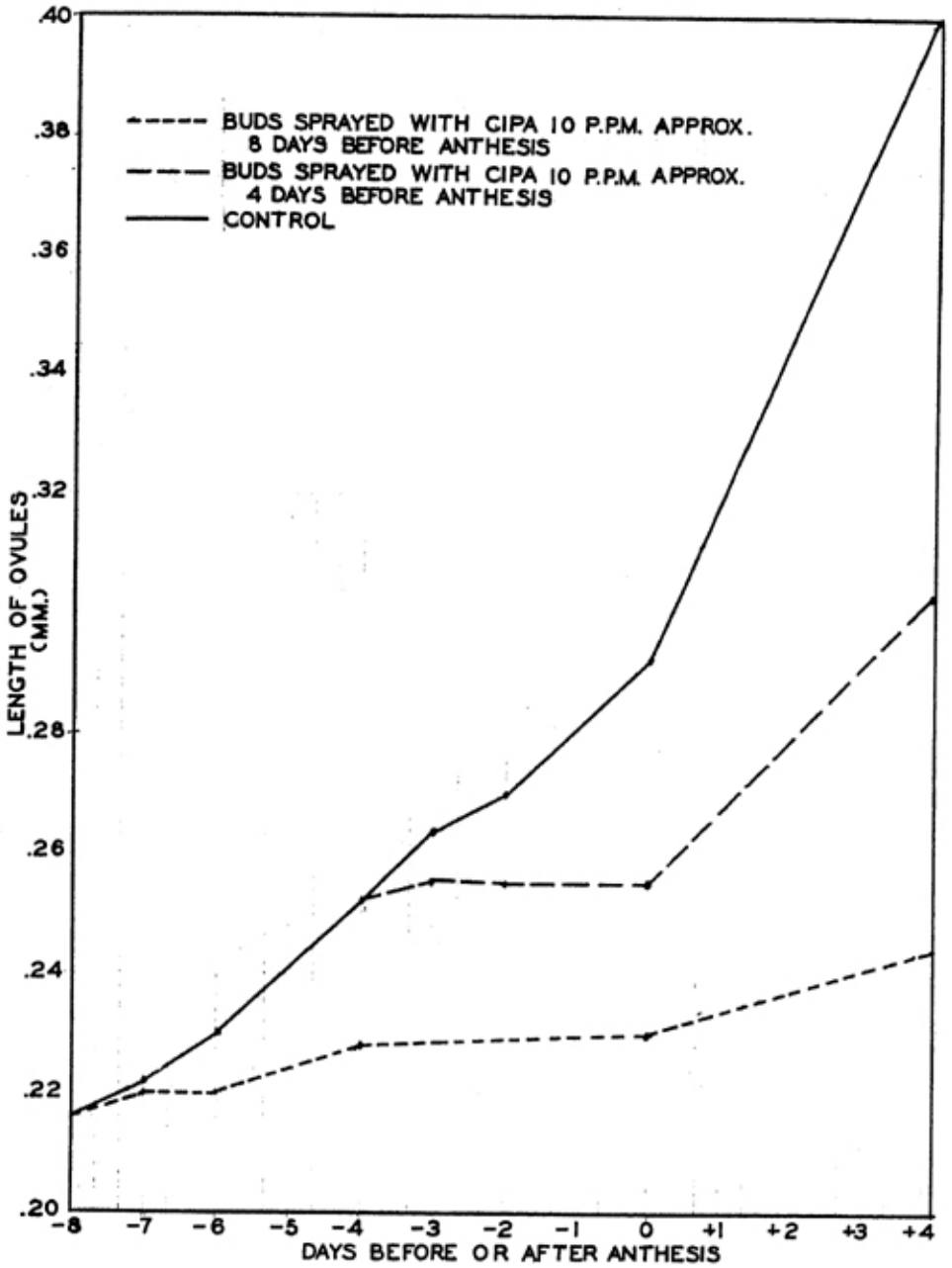

Fig. 7. Effects of a growth-regulating substance on ovule development in the tomato flower.

3. *Seed development.* As indicated by Figure 9, CIPA, 10 p.p.m., applied at anthesis materially inhibited seed development. A large portion of the ovules ceased to grow soon after treatment, and later the embryo sac disintegrated. In addition, those ovules which developed into seeds were somewhat inhibited. Measurement records presented in Table 14 and Figure 10 show that

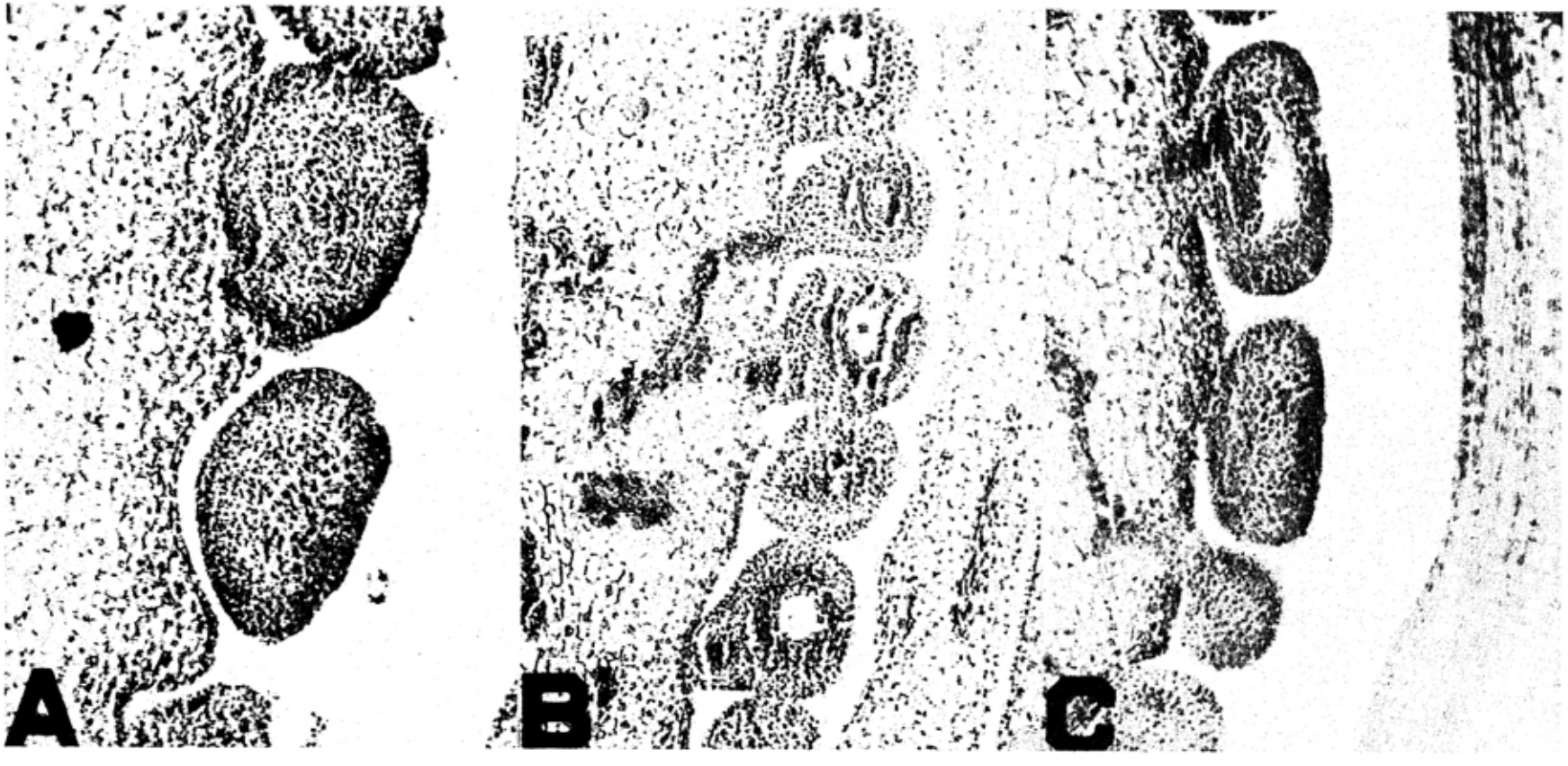

Fig. 8. Development of tomato ovules as influenced by ClPA, 10 p.p.m.: (A) control, no treatment; (B) flowers sprayed approximately eight days before anthesis; (C) flowers sprayed approximately four days before anthesis. All sections of comparable magnification. Note difference in size of ovules between sprayed and unsprayed flowers. Embryo sacs appear to have disintegrated in some ovules in treated sections.

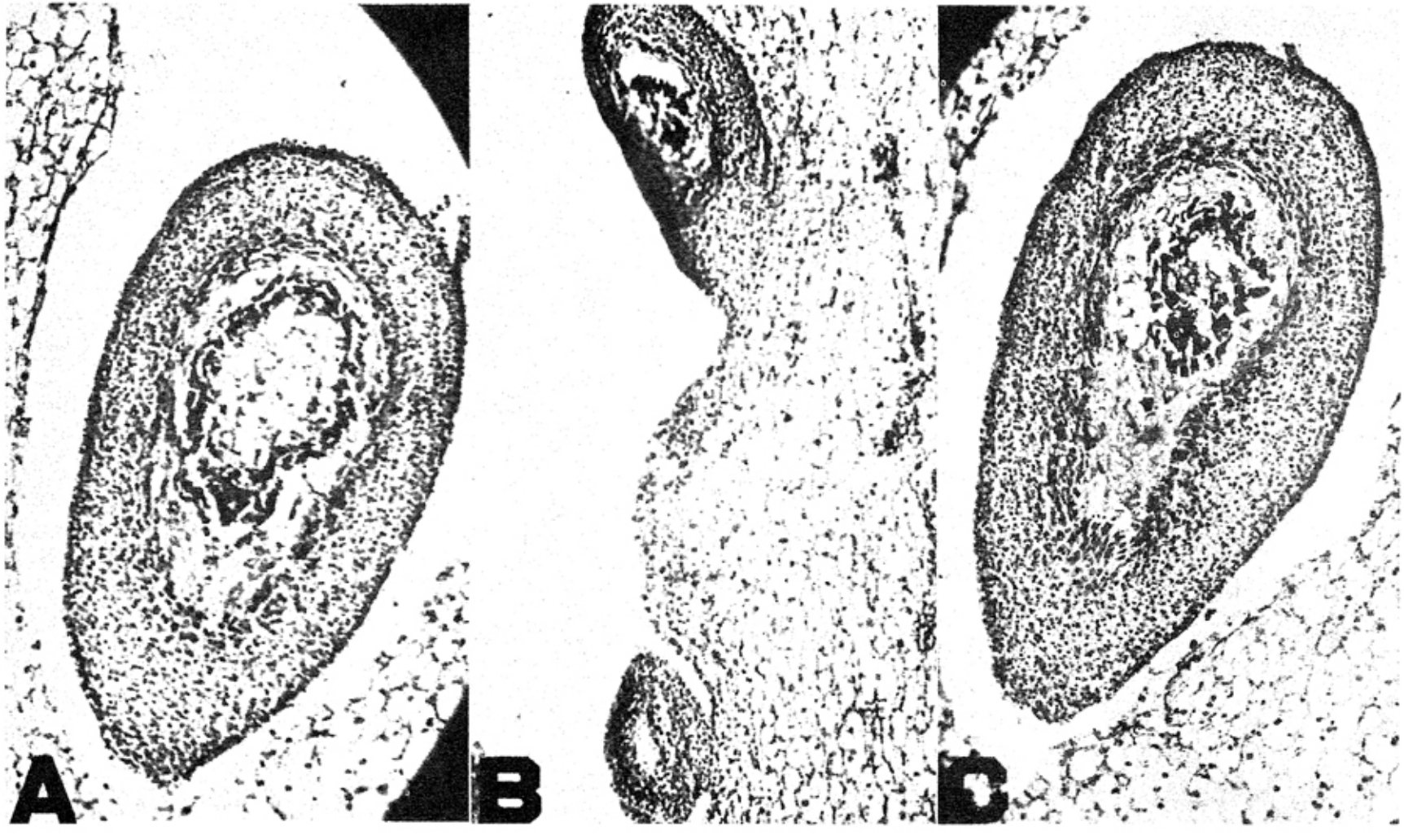

Fig. 9. Effects of ClPA, 10 p.p.m., on seed development in the tomato: (A) control; (B) flowers sprayed when fully open; (C) flowers sprayed four days after fully open. All sections of comparable magnification. Note failure of seed to develop when flowers were sprayed at anthesis.

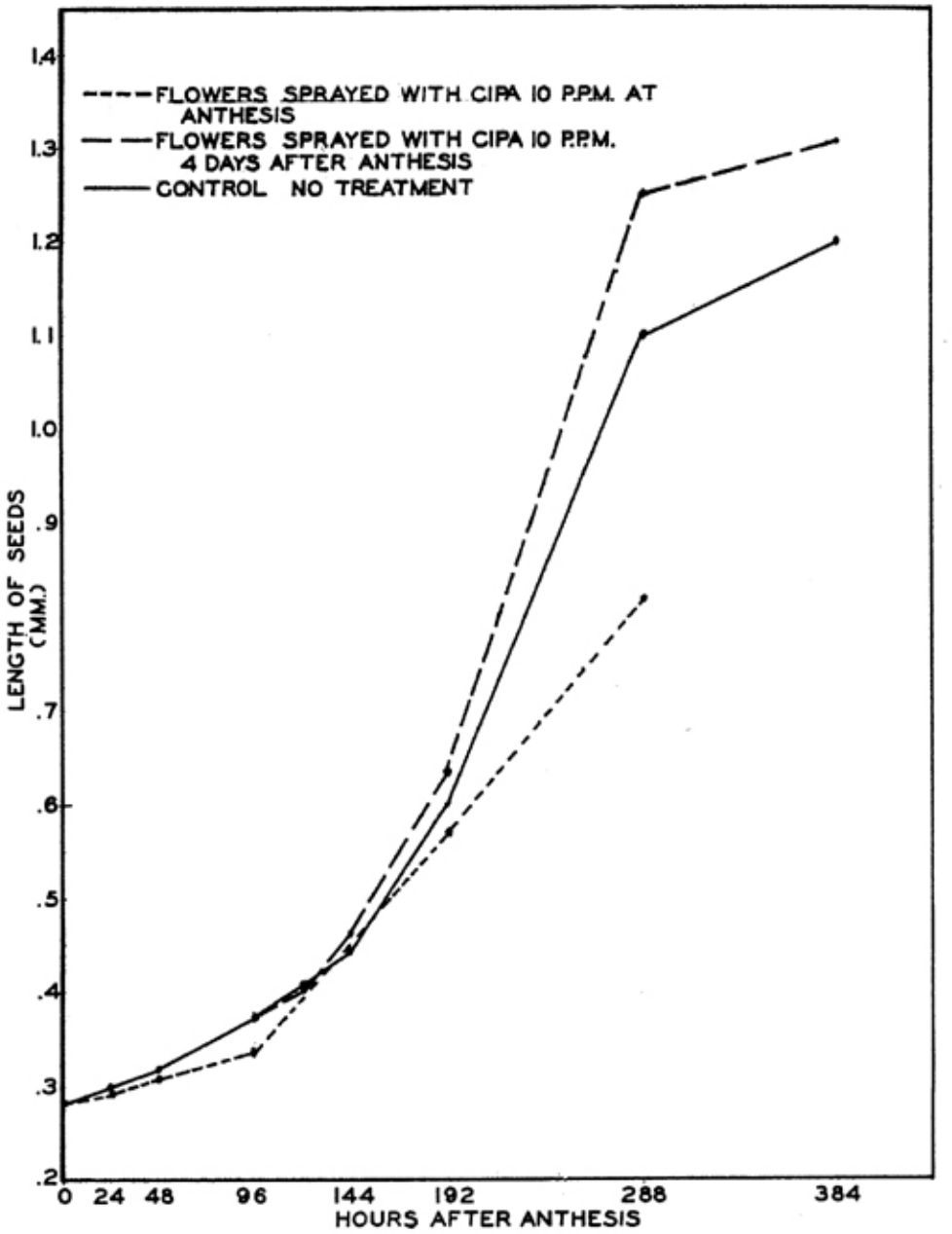

Fig. 10. Influence of p-chlorophenoxyacetic acid on seed development in the tomato.

Table 14.--Effects of p-Chlorophenoxyacetic Acid Upon Seed Development When Applied at Anthesis or Four Days After Anthesis

Time After Anthesis (Hours)	Length of Seeds in Millimeters		
	Controls	Treated at Anthesis	Treated Four Days After Anthesis
0	.283	----	----
24	.300	.297	----
48	.320	.312	----
96	.375	.335	----
120	.410	----	.405
144	.445	----	.460
192	.600	.570	.635
288	1.095	.820	1.250
384	1.205	----	1.305

the rate of development of seeds in fruit of untreated plants was greater than that of treated plants.

However, this was not the case with treatment four days after anthesis. Less than one-third of the seeds aborted, and there appeared to be a slight acceleration in elongation of those seeds which continued to develop.

These results are in agreement with those presented in Table 5.

EFFECTS OF P-CHLOROPHENOXYACETIC ACID (CLPA) ON VIABILITY OF POLLEN AND OVULES IN THE TOMATO

When histological studies revealed that p-chlorophenoxyacetic acid, 10 p.p.m., may cause harmful effects on pollen and ovule development, it was thought desirable to investigate further this phase of hormone activity. The immediate objectives of this study were to determine whether seedlessness of fruit is due to the effects of p-chlorophenoxyacetic acid on the pollen or on ovules.

Material and Methods

Plants, grown in four-gallon jars, were divided into five groups of ten each and treated as follows:

1. Untreated Ovules and Treated Pollen—Untreated flowers emasculated and hand pollinated with pollen from flowers sprayed with ClPA, 10 p.p.m., approximately eight days before anthesis.
2. Treated Ovules and Treated Pollen—Flowers sprayed with ClPA, 10 p.p.m., eight days before anthesis and pollinated with pollen from similarly treated flowers.
3. Treated Ovules and Untreated Pollen—Flowers sprayed with ClPA, 10

p.p.m., eight days before anthesis, emasculated, and pollinated with pollen from untreated flowers.

4. Treated Ovules and Untreated Pollen—Flowers sprayed with ClPA, 30 p.p.m., eight days before anthesis, emasculated, and hand pollinated with pollen from untreated flowers.
5. Control—Untreated flowers emasculated and hand pollinated with pollen from untreated flowers.

Results

Records presented in Table 15 indicate that seedlessness in tomatoes may result from both the influence of ClPA on viability of the pollen and viability of the ovules.

Table 15.--Effects of p-Chlorophenoxyacetic Acid on Viability of Pollen and Ovules in the Tomato

(Fruit Yield for 10 Plants)

Description of Treatment	Number of Fruit Harvested	Number of Fruit Seedless	Number of Fruit Partly Seedless	Number of Fruit Fully Seeded
1. Untreated flowers emasculated and hand pollinated with pollen from untreated flowers	282	12	38	232
2. Untreated flowers emasculated and pollinated with pollen from flowers sprayed with ClPA, 10 p.p.m., eight days approximately before anthesis	206	48	124	24
3. Flowers sprayed with ClPA, 10 p.p.m., eight days before anthesis. Hand pollinated with pollen from similarly treated flowers	164	66	92	6
4. Flowers sprayed with ClPA, 10 p.p.m., eight days before anthesis. Emasculated and pollinated with pollen from untreated flowers	182	50	124	8
5. Flowers sprayed with ClPA, 30 p.p.m., eight days before anthesis. Emasculated and pollinated with untreated pollen	20	20	0	0

Untreated flowers emasculated and pollinated with pollen from sprayed flowers set a small number of fruit, most of which were seedless or only partly seeded, while untreated flowers emasculated and pollinated with pollen from unsprayed flowers set a large number of normally seeded fruit. Moreover, in the case of flowers sprayed eight days before anthesis, a poor fruit set resulted, and most of the fruit were seedless or contained only a few seeds, even though these flowers were pollinated with pollen from untreated plants. It appears that fertilization was not possible, in the majority of the ovules, after the bud had been sprayed at an early stage of development.

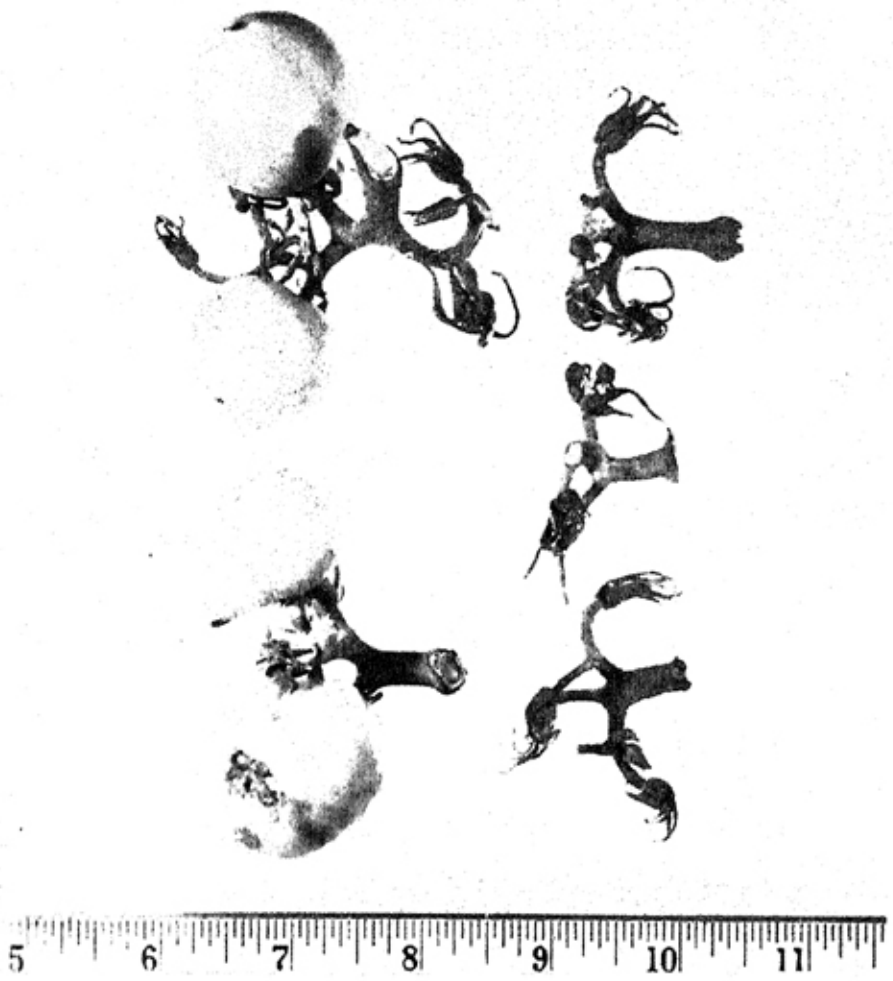

Fig. 11- Inhibiting effects of p-chlorophenoxyacetic acid, 30 p.p.m., on flower development and fruit set. Buds sprayed in late October and early November. Photographed February 19. Flowers which have been inhibited for more than two months have begun now to develop into small seedless fruits.

The inhibition of flower development by ClPA, 30 p.p.m., was very conspicuous. For the most part, the buds never opened. The petals dried and were shed without expanding. The pedicel enlarged but the bud was inhibited for several weeks. The treatments were made during October and November, and only twenty fruits, which were small and seedless had matured by February 19. Several ovaries began to develop during the first part of February. Figure 11 shows the stage of development on February 19, when practically all fruit on plants of other groups had ripened. Plants of this group were larger and more vigorous than those of other lots. Axillary shoots were being produced even on the leaves at the points of attachment of the leaflets. An abundance of assimilated food evidently was present in these plants, but it was not used for fruit development.

INFLUENCE OF GROWTH-REGULATING SUBSTANCES ON CATALASE ACTIVITY

Catalase activity is frequently used as a measurement of the metabolic rate of plants. It was thought desirable to learn whether the activity of this enzyme is influenced by growth-promoting chemicals, when applied at different stages of flower development.

Material and Methods

The tomato was chosen for this investigation for reasons previously stated. The plants were grown in four-gallon jars, and buds and flowers of the second, third and fourth clusters were used for this study. Eight lots of five plants each were treated as follows:

1. ClPA Buds—Sprayed with p-chlorophenoxyacetic acid, 10 p.p.m., when the sepals began to separate—collected four days after treatment.
2. NA Buds—Sprayed with α-naphthaleneacetic acid, 20 p.p.m., when the sepals began to separate—collected four days after treatment.
3. NOA Buds—Sprayed with β-naphthoxyacetic acid, 50 p.p.m., when the sepals began to separate—collected four days after treatment.
4. Control Buds—No treatment—collected four days after the sepals had begun to separate.
5. ClPA Flowers—Sprayed with p-chlorophenoxyacetic acid, 10 p.p.m., when fully open—collected four days after treatment.
6. NA Flowers—Sprayed with α-naphthaleneacetic acid, 20 p.p.m., when fully open—collected four days after treatment.
7. NOA Flowers—Sprayed with β-naphthoxyacetic acid, 50 p.p.m., when fully open—collected four days after treatment.
8. Control Flowers—No treatment—collected four days after fully open.

Immediately after collection, buds and flowers were placed in a refrigerator at a temperature of —20°C. until sufficient material had been collected. One series of treatments and collections was made in February and a second series was made in April from a different group of plants, similarly treated.

The method used for the estimation of catalase activity is essentially that described by Morrow and Sandstrom (55). A five-gram sample was ground in a mortar with quartz sand to which had been added one gram of pure precipitated calcium carbonate. After the addition of 50 ml. distilled water, the mixture was filtered through muslin cloth to remove the sand and calcium carbonate. The filtrate was transferred to a 100 ml. volumetric flask, and distilled water was added to bring it to volume. For each determination, 10 ml. of the extract and 10 ml. 3% H_2O_2 were used. Triplicate determinations were made for each sample. The reactions were carried out in a constant temperature water bath. The number of ml. of O_2 released during a five-minute period was used as a basis for measurements.

Results

Data from this experiment are presented in Table 16. ClPA, 10 p.p.m., applied during the bud stage, depressed catalase activity but increased it when the application was made to fully open flowers. NA, 20 p.p.m., tended to depress activity of this enzyme when applied at either stage of development. NOA, 50 p.p.m., gave a slight increase in activity, which probably was not significant, when applied during the bud stage and markedly stimulated activity when sprayed on fully open flowers. These data seem to substantiate the observation that growth-regulating chemicals, at the concentrations used for fruit setting, may have an inhibiting effect on flower development.

Table 16. Effects of Growth-Regulating Substances Upon Catalase Activity When Applied at Two Stages of Flower Development

(Ml. O_2 released from 3% H_2O_2 in 5 Minutes)

Treatment	Buds		Flowers	
	February	April	February	April
ClPA Buds--Sprayed with ClPA, 10 p.p.m., when sepals began to separate--Collected four days after treatment	26.4	34.0	----	----
NA Buds--Sprayed with NA, 20 p.p.m., when sepals began to separate--Collected four days after treatment	30.3	40.3	----	----
NOA Buds--Sprayed with NOA, 50 p.p.m., when sepals began to separate--Collected four days after treatment	33.2	45.1	----	----
Control Buds--No treatment--Collected four days after sepals had begun to separate	32.0	44.1	----	----
ClPA Flowers--Sprayed with ClPA, 10 p.p.m., when fully open--Collected four days after treatment	----	----	24.2	38.4
NA Flowers--Sprayed with NA, 20 p.p.m., when fully open--Collected four days after treatment	----	----	20.2	29.3
NOA Flowers--Sprayed with NOA, 50 p.p.m., when fully open--Collected four days after treatment	----	----	26.5	42.1
Control Flowers--No treatment--Collected four days after fully open	----	----	22.5	36.2

EFFECTS OF P-CHLOROPHENOXYACETIC ACID AND α-NAPHTHALENEACETIC ACID UPON THE CHEMICAL CONSTITUENTS OF FLOWERS AND FRUITS IN THE TOMATO

Investigations (3) (52) (53) have shown that growth-promoting chemicals may cause changes in some of the major chemical constituents of plants. It was thought desirable to determine if there may be a correlation between inhibition of flower development and changes in the important chemical components. The immediate objective of this study was to determine the influence of ClPA and NA upon the amounts of starch, sugars, proteins and fats present in the flower and young fruit.

Materials and Methods

A group of plants was divided into six lots and treated as follows:

1. ClPA, 10 p.p.m., sprayed on buds when sepals began to separate—collected eight days after treatment.
2. NA, 20 p.p.m., sprayed on buds when sepals began to separate—collected eight days after treatment.
3. Controls—buds collected eight days after sepals had begun to separate.
4. ClPA, 10 p.p.m., sprayed on fully open flowers—collected four days after treatment.
5. NA, 20 p.p.m., sprayed on fully open flowers—collected four days after treatment.
6. Controls—flowers collected four days after fully open.

Immediately after collection sections were prepared using a freezing microtome for this purpose. Microchemical tests were made for starch IKI reagent, Fluckiger's reaction for sugars, Eosin and Millon's reagent for proteins, and Sudan III for fats. Sections were studied with both the microscope and the unaided eye.

Results

A comparison of treated and untreated sections of both flowers and young fruits showed no appreciable differences. By the use of these reactions, no marked change in amounts of starch, sugars, proteins, or fats could be detected, either macroscopically or microscopically, as a result of the application of p-chlorophenoxyacetic acid, 10 p.p.m., or α-naphthaleneacetic acid, 20 p.p.m., to flower buds eight days before anthesis or to fully open flowers. Although other investigators have reported alterations in the amounts of the major chemical constituents as a result of application of growth-regulating substances, the concentrations employed were much greater than those commonly used in fruit-setting sprays.

DISCUSSION

The effects of growth-regulating substances on the physiology of sexual reproduction deserves more study than it has received heretofore. Investigations pertaining to the development of parthenocarpic fruits from emasculated flowers are numerous, but information concerning the influence of synthetic growth-regulating chemicals on flower initiation and later development is very limited. Observations by some investigators, especially Murneek (57) (60), that retarded flower development and reduced fruit set may result from the pre-anthesis application of hormone sprays, have emphasized the fundamental importance of this phase of hormone activity and have shown the need for specific information on this effect of growth-regulating substances.

That the influence of growth-regulating chemicals on sexual reproduction and fruit development depends on the stage of flower development when appli-

cation is made has been fully demonstrated in this investigation. Data presented indicate that hormone sprays applied to flower buds several days before anthesis tend to retard flower opening and reduce fruit set and size, while application at anthesis or a few days after may result in some plants in greater yields due to augmented fruit set and size over that of untreated plants.

To help visualize how growth-regulating substances may influence sexual reproduction or fruiting of plants, some of the important physiological factors involved in this process should be considered. Evidence at the present indicates that initiation of reproduction is of a catalytic nature and that a hormone may be the responsible agent. The synthesis of the flowering hormone, which appears to be produced in the mature leaves, is dependent on both temperature and photoperiod. Upon translocation to certain meristems the hormone affects the apical cells in some manner that causes them to change from the production of vegetative organs to that of floral primordia (Murneek 56a).

The number of floral primordia which develop into functional flowers is dependent upon environmental conditions (supply of soil nutrients, temperatures, and amount of light). Elimination of a large proportion of the developing flowers may take place due to the competition for the available food supply. Also, the development of the floral organs after inception may be promoted by catalysts or hormones produced concomitantly with these structures (Murneek 56a).

A peak in growth hormone concentration in the tassels of the corn plant following microgametophyte differentiation in the male flower buds has been demonstrated by Wittwer (100). He has shown that this rise in hormone content is associated with chromosome conjugation at synapsis and that there is a stimulation of growth of the plant that extends beyond the reproductive to the vegetative parts of the plant.

The presence of large amounts of growth-regulating substances in pollen extracts has been reported by a number of investigators (16) (24) (43) (45) (104), and Wittwer (100) found that in corn, pollen and tassel extracts seem to be specific for stimulating cell, stem, and internode elongation. He suggests that this growth substance may be concerned particularly with flower stalk growth and pollen tube growth.

Fertlization and development of the embryo are the most important features of sexual reproduction of plants. Seeds and fruits are produced without fertilization only in exceptional cases. If fertilization does not occur, the ovary will not enlarge, and the flower will usually absciss in a few days.

Subsequent to fertilization there is a notable increase in metabolism of the ovule, ovary, and accessory tissues (Murneek 56a). This acceleration of metabolism probably is due to the production of hormones as a result of fertilization. Wittwer (100) has reported a rapid accumulation of growth substances in the pistillate inflorescence of corn concomitant with syngamy and a marked increase in dry matter production following this process.

That fruit development is stimulated by the embryos is a well-known fact. Their size, shape, time of maturity and chemical composition are influenced (Janes 38, Murneek 56a, Tukey 85). Most fruits will cease growth or their size will be decreased if the embryo is destroyed. Undoubtedly the auxins produced by the seeds play an important role in this influence of embryos on fruit development. Gustafson (26) has suggested that plants produce parthenocarpic fruits because the ovaries contain enough auxin to cause them to commence growth without fertilization. Also, he has shown that the auxin in ovaries which develop parthenocarpically decreased as growth progresses, indicating that it is actually used up in the process, while auxins in seeded fruits increased appreciably for a period after fertilization. This increase has been demonstrated also in the corn kernel (100) and in the young tomato fruit (40). Moreover, Wittwer (100) found that corn kernel extract was more effective in producing seedless fruit in peppers than .05 per cent indolebutyric acid.

The present investigation has provided some facts which may help us to understand how growth-regulating substances may alter the normal sequence of reproduction. Data from macroscopic, microscopic, and chemical studies indicate that flower development is hindered and fruit set and size are reduced in the tomato by hormone sprays applied approximately eight days before anthesis. Application of the same spray when the flower is fully open or four days later results in increased set and size of fruits. Why the difference in response to the same substance?

Histological studies revealed that the develoment of the pollen grains and ovules are markedly influenced, probably more than other structures, by these chemicals. When sprayed several days before anthesis nearly one half of the pollen grains collapsed and a number of ovules ceased to develop further. Also, catalase activity, which is used as an indication of the rate of metabolism in the plant, was found to be depressed in most instances.

It is a well-known fact that vegetative buds are inhibited by both natural and synthetic growth-regulating chemicals (67) (69) (79) (84) (87) (95). However, there is no agreement as to the mechanism of this inhibition. The presence of specific growth inhibitors has been postulated by some investigators (14) (20) (73) (74) (80).

The present study has been concerned, primarily, with flower bud development subsequent to initiation. As to the effects of growth-regulating substances on flower bud development, the literature, although meager and inconclusive, seems to indicate that a retardation of development may result from their application. Delayed fruiting of beans may result from the application of non-herbicidal concentrations of 2,4-D before the appearance of any flower buds (81) (94), and a delay in the opening of flower buds of fruit trees may result from the application of growth-regulating substances in the spring when swelling of buds begins (99), or in the late summer of the preceding year (33) (34). The application of a spray containing residual 2,4-D to apple trees

August 29, delayed flowering and markedly reduced set of fruit the following spring (48).

Possibly, an explanation of the effects of these chemicals on flower development would lie in their influence on the natural hormone or catalytic system of the plant.

Since pollen has been shown to contain and probably produce relatively large amounts of growth-promoting substances (16) (24) (45) (100) (104), which evidently are essential to the proper functioning of the flower buds, it would logically seem that destruction of some of the pollen grains, as a result of the application of synthetic growth-regulating chemicals, would retard flower development by decreasing the supply of natural growth substance. Likewise, injury to the ovules may play an important part in the retardation of flower development. However, their influence may not be as great as that of the pollen.

Although ovules have been shown to contain auxin (25) (41), the amount is relatively low until after fertilization (13) (100), and their destruction might not have as much effect on auxin supply as would a decrease in amount of developing pollen.

Although evidence has been presented in this study that pollen grains may collapse and ovules may cease to develop as a result of spraying the tomato bud with synthetic growth-regulating chemicals, the question as to the immediate cause of these effects has not been answered. However, it appears that it is not due to a direct toxic effect of the chemical. Van Overbeek et al. (91) found that the unfertilized ovules of *Datura* and *Melandrium* were stimulated by a 0.1 per cent solution or emulsion of the ammonium salt of naphthaleneacetic acid injected into the ovary cavity. The ovules of *Datura* often contained a pseudo-embryo consisting of several hundred cells. When, however, the material was applied externally the ovary developed parthenocarpically, but there was no development of the ovules. Moreover, the concentration used by these investigators was 20 to 100 times as great as those used in this study.

It would seem, more likely, that the collapse of the pollen grains and the cessation of growth in the ovules are due to the abnormal growth response initiated by the application of the growth-regulating substances. The external application of the hormone causes a thickening of the sepals and an increase in the diameter of the pedicel while the inner parts of the flower bud are not stimulated. Perhaps there is a delicate balance between the different tissues of the flower bud in the utilization of essential substances which are, probably, of an enzyme or hormone nature. The excessive or premature stimulation of growth in the external portions of the bud may deprive the inner parts of these essential substances and result in the death of pollen grains and ovules.

A second alternative might be that there is a critical sequence of development in the flower bud that is changed by the application of a growth-regulating substance.

When hormone sprays of concentrations higher than commonly used for fruit-setting were sprayed on fertilized ovaries, there was considerable abortion of seeds. It appears that seed failure was due to the withdrawal of essential catalysts or foods from these structures. As mentioned previously, van Overbeek (91) has shown that ovules were stimulated by the injection of naphthaleneacetic acid into the ovary cavity while external application stimulated only the ovary wall. Therefore, it does not seem to be due to a direct toxic or inactivating action of the hormone spray. In the case of the tomato, the growth of carpellary tissues was greatly accelerated by the hormone application, especially in the case of p-chlorophenoxyacetic acid. This abnormally rapid growth of the carpellary tissues may deprive the developing seeds of essential catalytic substances or food materials.

Evidence of a similar nature has been presented by Britten (4). He attempted to induce the abortion of the embryo in the maize ovary by the application of 0.1 per cent naphthaleneacetic acid at about the time of fertilization. It was hoped that the growth-regulating substance would produce a hyperplastic condition in the maternal tissues and that the collapse of the embryo would result from starvation as a result of the excessive growth in the pericarp and nucellus. Although abortion did not result, he indicated that a condition approaching somatoplastic sterility had been achieved because the embryos were retarded appreciably in their development. Perhaps a complete collapse of the embryo would have occurred if there had been a greater acceleration of growth in the maternal tissues.

The present investigation has dealt primarily with the tomato. The fact that the fruit of our commercial varieties of tomatoes is an abnormal structure should be considered. The size of the fruit, the number of locules, the number of seeds, and the amount of carpellary tissues are different from that of its wild ancestor. It is abnormal not only from a morphological but undoubtedly also from a physiological standpoint. Because of these abnormalities, the response that this plant exhibits to growth-regulating substances may be different from that of other plants.

The growth of the tomato fruit appears to be somewhat independent of its seeds. Plants of our commercial varieties will produce partly or fully parthenocarpic fruits by stimulation from environmental factors and any one of several growth-regulating substances (Gustafson 25, Howlett 36). Growth hormone production may have been taken over by the carpellary tissues or the catalytic substances, essential for fruit growth, may come from the leaves. Man in his selection may have chosen strains which have a tendency to produce parthenocarpic fruit. Seedless fruits, although small and of poor quality, are very common on plants grown in the greenhouse during winter months, and Hawthorn (31) has reported that a cross between the Large Cherry variety and the Bonnie Best produced seeded fruits during the spring and fall, but bore seedless fruits which were of good quality during mid-summer.

It would seem that this tendency for fruit development to be somewhat independent of the seeds is an important criterion in determining whether or not the yield of a crop will be increased by hormone sprays applied to open flowers.

The yield of tomatoes and green bush beans can be increased because the carpellary tissues of the fruit will grow due to the stimulation of the hormone sprays even though no seeds or a reduced number of seeds are present, while the berries of the Concord variety of grapes will not obtain a normal size in the absence of viable seeds.

Due to the limited information available at the present, there is a great need for further study of this problem, for the effects of synthetic growth-regulating chemicals on sexual reproduction of plants are important from both the theoretical and the practical points of view. The present investigation, though limited largely to the tomato, has supplied information which indicates how these chemicals may affect flower and fruit development. This problem cannot be fully elucidated until there is more definite information as to the normal physiological changes of sexual reproduction. Unless the normal sequence is known, it will be difficult to detect changes due to the influence of an external supply of growth-regulating substances.

APPLICATION TO HORTICULTURAL PRACTICE

An evaluation of the experimental data in terms of practical usage is desirable. The growth-inhibiting effect of hormones on young flower bud development has not been emphasized sufficiently, although the retarding effect of these chemicals on vegetative buds is recognized by some and is utilized at the present to delay sprouting of potato tubers and root crops while in storage (12) (69), to retard shoot development on rose plants during common storage (49), and to suppress axillary growth in decapitated tobacco plants (79).

In the case of tomatoes, results of both yield and histological studies indicate that the application of "hormones" to increase set and size of fruit will be successful only if it is made at the proper stage of flower development.

These chemicals must be applied when the buds have reached a stage of development where they will be stimulated, not inhibited, by the treatment.

For flower clusters, spraying should not be commenced until at least three flowers of the first and second clusters have opened and four or more flowers of later clusters. Since it has been shown that flowers are still receptive to stimulation several days after opening, the sprays can be withheld until the petals of the first or first and second flowers to open have begun to wither. Puffy or rough fruit sometimes result from the use of hormone sprays. Perhaps these undesirable fruits result from the spraying of young buds as was shown to occur in these experiments (Figure 1). Pruning the large flower clusters, which is practiced by some growers, would remove small, weak buds that open later and that may give rise to undesirable sized and shaped fruits.

If whole plant spraying is practiced, the spray should be directed to cover only that portion of the plant which contains fruit, flowers and buds near the opening stage. The terminal portion of the plant containing clusters of young buds should be avoided.

When production is measured in terms of seed numbers as in tomato seed production, yields are not likely to be increased by hormone sprays. Histological studies have shown that pollen, ovules, and young embryos are very readily injured or inhibited in development by growth-regulating substances. Relatively low concentrations may stimulate the growth or function of these organs, but concentrations higher than 10 p.p.m. of most growth-regulating chemicals are likely to be injurious.

The fruit thinning effect of hormone sprays on apples, peaches, and cherries is probably due to the action of these substances on the pollen, ovules and embryos.

To obtain the desired result from hormone treatment not only must the proper concentration of a specific chemical be used but the application must be accurately timed. This cannot be overemphasized. A given concentration may retard growth at one stage of development while later it may accelerate it. There may even be a stimulating effect on one part of the plant and an inhibiting effect on another.

SUMMARY

It has been observed that growth-regulating substances at concentrations usually employed in fruit-setting sprays may in some cases decrease yields of horticultural crops. This study has dealt, primarily, with this phase of hormone effect. Experiments were designed to provide specific information concerning the stage of development of flowers and fruits when hormone application would give inhibitory effects and to determine by histological and chemical methods the nature or possible causes of this inhibition.

1. Tomato plants whose flowers were sprayed with ClPA, 10 p.p.m., approximately eight days before anthesis set a smaller number of fruit than the controls, and many of these were small and misshapen. Treatment of individual flowers at anthesis or the treatment of the whole flower clusters after three flowers had opened resulted in a yield of more and larger-sized fruits than that of the controls. Treatment of the individual flowers after fully open gave the largest yield.

2. A comparison of the effects of ClPA, 10 p.p.m., NA, 20 p.p.m., and NOA, 50 p.p.m., showed that all three hormones when applied approximately eight days before anthesis lowered yields due to their effects on number of fruit set and size of fruits. When sprayed on fully open flowers ClPA and NOA gave a considerable increase on yields of marketable fruit, but due to the small size of fruits, plants treated with NA yielded less than the controls.

3. Application of ClPA after fertilization has occurred resulted in a greater increase in yields than application at anthesis. In the case of NA, which tended to lower yields, the depression was less when treatment was made at the post-fertilization stage. The majority of the fruits was fully seeded when either of these hormones were sprayed on the flowers at this late stage of development; however, higher concentrations of these chemicals reduced considerably the number of normally seeded fruits. Although growth-promoting chemicals reduced the number of seeds which developed, fully mature seeds from sprayed fruits appeared to be as viable as those from untreated fruits.

4. The yield of grapes was not augmented by hormone sprays. Application before full bloom or during full bloom resulted in reduced yields due to fewer berries per bunch. At harvesting a number of partly developed seedless berries were persisting on those clusters sprayed during full bloom. Four applications of NA at weekly intervals beginning at full bloom tended to delay maturity. Post-fertilization applications of ClPA gave a slight improvement in berry size, while NA seemed to decrease it.

5. Histological studies of flowers treated approximately eight days before anthesis, approximately four days before anthesis, at anthesis, or four days after fully open, indicated that flower development was retarded by pre-anthesis treatments. Flower opening was delayed and the growth-promoting chemicals exerted, directly or indirectly, a detrimental effect upon pollen and ovule development. Some pollen ceased to develop and collapsed when treatment was made approximately eight days before anthesis, while spraying four days later seemed to cause premature germination. When sprayed at either stage, some ovules ceased to develop and the embryo sac disintegrated while those which continued to grow did so at a retarded rate. Seed formation was prevented, to a large extent, by hormone sprays at anthesis or prior to anthesis. The influence was much less when application was made after fertilization.

6. Seedlessness of tomato fruits was shown to result from the effect of growth-regulating substances on both pollen and ovules. Unsprayed flowers, emasculated and pollinated with pollen from sprayed flowers, set a small number of fruit, most of which were seedless or only partly seeded. Likewise, flowers which were sprayed eight days before anthesis set poorly and the fruit were largely seedless or partly so, even though the flowers were pollinated with untreated pollen.

7. Hormones sprayed on flower buds tended to depress catalase activity; however, when fully open flowers were treated, the activity of this enzyme was increased.

8. By use of microchemical reactions, no marked change in amounts of sugars, starch, proteins, or fats could be detected in flowers or young fruits after spraying with ClPA, 10 p.p.m., or NA, 20 p.p.m.

9. As indicated by data presented in the literature and that obtained in

this investigation, the application of fruit-setting sprays to young flower buds is likely, in most all plants, to retard flower development and reduce yields due to the injurious effects of these chemicals on pollen and ovule development; whereas, application to fully open flowers may increase yields if the plant is somewhat independent of the influence of its seeds and will produce parthenocarpic fruit as a result of the stimulation of growth-regulating substances.

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