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Growth Hormone Production During Sexual Reproduction of Higher Plants

With Special Reference to Synapsis and Syngamy

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ABSTRACT

Two cytogenetically important processes associated with sexual reproduction in higher plants were found to stimulate growth. The first is the synaptic reaction initiated within the immature flower bud during gametophyte maturation. The second occurs in the embryo sac at the approximate time of fertilization. These stimulating phases were demonstrated in the cucumber, strawberry, and sour cherry by the treatments of disbudding, deflowering, and defruiting. The greatest growth, accumulation of total nitrogen, and synthesis of carbohydrates occurred in the defruited plants, the least in the disbudded. Deflowered plants were intermediate in growth and nutrient accumulation. The inception of synapsis and syngamy is typified by marked alterations in movement and elongation of the flower stalk (pedicel). In spinach, maximal vegetative extension of the male plants follows the period of most intensive pollen production.

Catalase determinations made periodically of the developing inflorescences of the corn plant and pear tree portrayed two peaks in enzyme activity. The first followed synapsis in the microspore mother cells, the second nuclear fusion in the embryo sac. Changes in the relative growth hormone concentration of developing reproductive organs of corn were ascertained by alcohol extraction and bean seedling internode assay. Subsequent to synapsis in the immature tassel and syngamy in the young kernel, there was a marked increase in growth substances. Crude extracts of the unripe corn grain were unusually active in the setting and parthenocarpic induction of fruit in the tomato. Evidence supporting the concept of two stimulating phases in sexual reproduction is reviewed in other germane investigations. Such data are given a new interpretation and are correlated with the results herein reported. The probable developmental role played by the hormones produced in the reproductive organs is discussed.

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Growth Hormone Production During Sexual Reproduction of Higher Plants

With Special Reference to Synapsis and Syngamy

S. H. WITTWER[1]

Almost all students of plant development recognize the existence of a close relationship between sexual reproduction and vegetative growth. Interest has been focused recently on the question as to what type of vegetative growth is conducive or detrimental to sexual reproduction, and in what ways and to what extent reproductive development of the plant influences vegetative extension. These questions become particularly applicable when referred to plants which have a continuous or indeterminate type of growth.

The effects of developing flowers and fruit on vegetative increase have been amply demonstrated by Murneek (86) (87), and there is no doubt about the conspicuous physiological control that these organs exert on the metabolism of the plant. To most observers such control over vegetative development seems to be evident only after reproduction has progressed well toward the point of completion, viz., fruit and seed formation, when the influence is exhibited by various degrees of inhibition in the subsequent development of the plant.

If one examines in detail, however, some of the earlier, very significant though less evident, stages of reproduction, quite a different phenomenon seems to be in operation with respect to their effect on plant metabolism. Instead of an inhibitory or retarding influence, as is exhibited during fruit and seed development, one finds more or less specific stimulations of vegetative growth coincident with flowering and fruit setting (Murneek, 88, 92, 93).

The present study deals with these stimulating aspects of reproduction on vegetative growth and extension. Two "crucial" stages of sexual reproduction especially considered in this connection are those of syngamy (fertilization or gametic union) and synapsis (chromosome conjugation in meiosis). Both experimental and observational evidence are presented in this publication for critical evaluation of the concept that the process of fertilization in plants with its two cytologically important phases, union of nuclei (syngamy) and union of chromosomes (synapsis), respectively, results in two usually distinct periods of growth acceleration.

This study, perforce, has led into the realm of plant growth hormones.

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REVIEW OF LITERATURE

Because of the relatively small amount of experimental work performed and recorded in the literature pertaining to the above problem, the subject limits itself by virtue of its newness. Aside from the concepts introduced by Murneek (88) on the effect of gametic union on the growth of the tomato and some later observations by the same investigator (91) (92) on a similar stimulation induced at synapsis, practically no work has been done that is concerned directly with the stimulating stages of sexual reproduction in plants. However, evidence gathered from other, though germane, investigations may be used with a new interpretation in support of the above concepts. In some of the published literature the authors are seemingly unaware of the significance of their data in relation to definite periods in sexual reproduction of plants.

Accumulation of Dry Matter and Total Nutrients as Influenced by Removal of Reproductive Organs

One of the best experimental approaches to the study of the problem has been to subject series of uniform plants to either disbudding, deflowering, or defruiting treatments. After due time the accumulation of dry matter, total absorbed soil nutrients, and vegetative extension are measured. The objectives in such experiments, of course, would be first, to prevent synapsis by early removal of all flower buds on a series of plants and then to note their relative growth performance as compared with plants from which the buds were removed much later, perhaps at full bloom (defloration), i. e. after synapsis and gametophyte maturation had occurred; and, second, to prevent syngamy by removal of flowers (defloration) before fertilization, on one group of plants, and on another comparable group, allow fertilization to occur and remove the young fruits, comparing as above the effect on vegetative extension, accumulation of dry matter and of plant nutrients.

It is obvious that plants will vary in the degree of response to the above treatments, depending on the kinds of plants used, the soil nutrient levels and many other factors. It is also much easier to "prevent" gametic union by flower removal than to eliminate synapsis by bud removal. At the time when synapsis occurs in the flower buds of most plants thus far tested, it is almost impossible to effect a removal of the inflorescence without considerable injury to the young vegetative growing point.

The concept, that gametic union causes a stimulation of growth extending beyond the reproductive parts, was first formulated and demonstrated by Murneek (88). Tomato plants were divided into three groups. The treatments of defloration (removal of flower buds just prior to anthesis), defruiting (removal of young fruit when 0.5 - 1 centimeters in diameter), and normal fruiting were used as experimental procedures. The subsequent rate of growth, increase

in fresh and dry matter, and accumulation of the various plant nutrients or end products of metabolism were determined. The highest values for all substances and all measurements were obtained from the plants in which syngamy was permitted but the fruit were removed soon thereafter. From the data obtained it was concluded that the act of gametic union caused a marked increase in metabolism, and that this increase or stimulation in a measure extended throughout the body of the plant. It was suggested that the mechanism responsible for such growth stimulation was perhaps enzymatic or hormonic in nature.

In later publications Murneek (91) (92) (93) has reaffirmed this contention and in addition presented observational evidence for the existence of another stimulating phase, at the time of synapsis. Recently Murneek and Wittwer (96) (125) have presented similar experimental results obtained from several different species of horticultural plants. When allowed to flower, they accumulated more dry matter, total nitrogen, and carbohydrates than plants from which the buds were removed. Also, it was shown that specimens from which the young fruit were removed, in turn, produced more of the above products of metabolism than plants which were deflorated.

Other investigators have reported similar results to those presented above. Dearborn (22) found that cucumber vines on which fertilization occurred had a higher rate of vegetative extension for a period of ten to fourteen days after pollination than deflorated plants. The defruited individuals accumulated more dry matter than the plants from which the flowers were removed. Dearborn's conclusions for the cucumber were similar to Murneek's for the tomato: *Fertilization of the egg results in a growth stimulation which extends beyond the reproductive organs.* McCollum (77), also working with the cucumber, reached conclusions somewhat different from those of Dearborn. Being more concerned with the inhibiting effects of developing fruit, he postulated that fertilized eggs produced a growth-regulating substance, which retards rather than stimulates vegetative growth.

Studying alternate bearing in the sugar prune, Bowman (12) subjected trees to deflowering and defruiting treatments. In most instances the vegetative growth of the defruited trees (fruit removed sixteen days after bloom) was equal to or greater than the deflorated trees (buds removed four days prior to anthesis) in spite of the extra nutrients required for fruit development in the former group.

Rather divergent results from the above are presented by Bartholdi (10) who found, with the potato, that both flowering and fruiting caused significant reductions in vegetative growth and tuber formation. The evidence presented, however, is none too consistent or convincing. Moreover, the potato is usually a non-flowering and, with few exceptions, a non-fruiting plant.

Periodic Growth Cycles and Movement of Reproductive Organs and Adjacent Parts Associated with Internal Floral Development

As plants become reproductive and begin to form flower buds and fruit, a spurt in growth is a familiar, though not always recognized, accompanying phenomenon. Certain characteristic movements of flower parts, especially the pedicel and peduncle, also seem to be correlated with floral development. Rather careful examination of the literature reveals that there are usually two quite definite periods of growth acceleration. Similarly, bending movements of the pedicel or other allied flower parts very frequently characterize these periods of maximal increase. One naturally becomes curious as to the phases of reproduction these periodic changes in growth are associated with. Can any relationship be established between the crucial stages of synapsis and syngamy in sexual reproduction and these periodic growth phenomena?

Vöchting (120) and Fitting (30) report a general correlation between flower bud development (particularly post-floral) and movement and growth of adjoining tissues.

Reed and Holland (101), working with *Helianthus*, and Zollikofer (130), with *Tussilago*, observed that the greatest growth and drooping of the floral and adjacent parts occurred after flowering or, more specifically, after fertilization. Similar observations were made by Schmitt (103) for *Digitalis*, *Althaea*, and *Linaria*, and an upward bending of pedicels of the apple flowers (fruit) after fertilization was noticed by Murneek (91a). It was concluded that fertilization was essential for floral movement, and that a minimum in enlargement occurs at full bloom followed by renewed growth after fertilization.

Of significance is the work of Miyake (83) who found that the growth of the flower stalk of *Taraxacum* exhibited two maxima, with a minimum between. The first maximum occurred during bud development, reaching a peak one to four days before anthesis. The least amount of growth took place during full bloom. This minimum was followed by a second, somewhat greater, spurt of growth, reaching a second maximum one to two weeks after anthesis. A possible correlation between these growth maxima and definite stages in sexual reproduction in this genus may be questioned, however, in view of the frequent occurrence of parthenogenesis.

Errera (29) presents interesting results for the growth of the fungus *Phycomyces* which are strikingly similar to those of Miyake for the dandelion. As above, growth is characterized by two periods of maximal increase separated by a minimum that occurs during the formation of the sporangia. Due, however, to the inadequate knowledge pertaining to reproduction in the lower fungi (particularly sexual), any conclusions from such data would be premature.

Söding (105) and Uyldert (119) conclude that it is the young inflorescence which controls the growth of the flower stem. The former, working with species of *Cardamine, Cephalaria, Chrysanthemum, Heliopsis,* and *Helenium,* and the latter with *Bellis,* found a decrease in growth of the flower stalks upon removal of the reproductive organs while still in the bud stage. Söding has shown that growth of the disbudded stems could be accelerated again by replacing the young inflorescence on the cut surface in moist contact, while Uyldert demonstrated that a growth regulating substance, obtained from oat coleoptiles, accelerated the elongation of the decapitated stalks quite as well. It has been further suggested by these investigators, that the growth acceleration of the flower stalk may be due to growth promoting hormones produced within the flower bud and young fruit. This suggestion was reaffirmed by Söding (106), Snow (104), and Kostytschew (64), the latter postulating a periodic formation of growth hormones by the developing floral parts.

Katunsky (56) (57) has published some especially pertinent results for *Papaver* and *Crepis,* showing that growth and floral movements correspond with definite phases in the development of the female gametophyte. Two maxima were observed. By analyzing the material histologically and microscopically, he determined that the first growth maximum occurred during the development of the nucellus. The second, a much larger spurt in growth, had its beginning subsequent to the rapid cell divisions connected with synapsis and female germ cell formation. Katunsky also determined that the intervals of most intense growth corresponded with the periods of greatest accumulation of growth promoting hormones. It was established that the center of hormone production was the female gametophyte, or more exactly, the developing ovules.

Recently, Duncan and Curtis (25) (26) (27) have presented interesting results on the growth phases of the orchid fruit in relation to embryo sac development. They found, in fruits of *Cattleyeae* and *Phalaenopsis,* three phases of increase (in diameter) coinciding, respectively, with (a) proliferation of the placentae and initiation of ovule rudiments, (b) ovule development and maturation of the macrogametophyte, and (c) growth of the embryo and seed ripening. In *Cypripedium* and *Paphiopedilum,* only two phases of increase were observed, corresponding with, first, macrogametophyte maturation and, second, seed development. Meiosis and fertilization were suggested as crucial stages in the periodic development of the fruit.

Miscellaneous Effects of Flowering and Fruiting

There are numerous examples showing that flowering and early reproductive development exert a catalytic or hormonic influence on tissues connected with the flower parts and upon the plant as

a whole. Murneek (89), studying intermittent sterility in *Cleome*, gave evidence that developing fruit were directly responsible for initiation and maintenance of sterility in the plant. Gametic union was considered the primary cause of the mechanism. Murneek (90) in another investigation with bearing apple spurs, found that flowering and fruit setting caused a marked increase in all active forms of carbohydrates and nitrogen in the fruit spur following fertilization. Presumably, these available forms of food are directed into the fruit by the young embryos.

According to Sande-Bakhuyzen (102), fertilization in wheat is a critical stage in the life of this plant. At gametic union growth hormones, produced in the inflorescence, no longer leave the flowers. This is responsible for a "catabolic impulse" which spreads through the entire plant, causing a decrease in respiration and moisture content, senescence, and final death of the plant.

Das and Palit (20) have affirmed that in *Mimosa pudica*, the power to transmit excitation along the connecting tissues becomes depressed when the flowers make their appearance. Restoration of conductivity is obtained upon removal of the inflorescence.

Cambial activity seems to be associated with flower development, according to Gill (31) and Snow (104). Gill found that expanding catkins of *Populus* and *Salix*, which were within the bud at the beginning of spring, activated the cambium immediately below. Trees whose floral parts were exposed throughout the winter showed no such effect in the spring. The significance of these results will be mentioned later, in the discussion.

Specific Effects of Developing Embryos

The influence of developing embryos on the growth of the fruit and adjoining tissues has been emphasized by some investigators. The experimental results of Tukey and Lee (118) demonstrate that under conditions of low nutrition the developing seed may draw food from the fruit. Tukey further found (117) that the fruit of early varieties ripened sooner when the embryo was destroyed a short period after fertilization. Such a procedure, it seems to the present writer, would allow for the full benefits of stimulation from gametic union, yet prevent undue food diversion to the seed.

Dorsey (24), in studying the cause of "buttons" in the J. H. Hale peach, concluded that these small fruit developed because of incomplete "double fertilization" in the embryo sac. The stimulus to fruit growth arising either from gametic union, or from triple union, was found sufficient to induce the fruit to set, but not adequate for the production of normal size. Dorsey demonstrated that both embryo and endosperm formation were essential for normal fruit development. These results would suggest a stimulus arising from triple union as well as from gametic union in so far as fruit growth is concerned.

In analyses of chemical differences between artificially produced parthenocarpic fruit and normal seeded fruit in the tomato, Janes (54) came to the conclusion that developing seeds exert a profound influence upon the chemical composition of the fruit.

The phenomenon of nucellar budding or apogamy in *Citrus* seems to be a direct consequence of gametic union and the presence of developing embryos. According to Webber (121) and Strasburger (111), a prerequisite for the production of apogamic (vegetative) embryos, is the growth stimulus from the fertilized egg. Nucellar budding does not occur in the absence of nuclear fusion. The interesting results of Swingle (112), demonstrate that the *Citrus* clones arising from nucellar embryos were more vigorous in growth than other vegetative clones.

Growth Promoting Substances in Reproductive Organs

General occurrence in reproductive tissues.—Plant reproductive organs are, in general, a rich source of auxin. Growth substances were found in peas, beans, lentils, tomatoes, oranges, and lemons by Maschmann and Laibach (76). Kögl, Haagen-Smit, and Erxleben (61) isolated auxin a and auxin b from barley malt, and oil made from *maize* embryos. More recently, Haagen-Smit, Leech, and Bergen (44) (45) have identified heteroauxin in corn meal.

Boysen-Jensen (13), and Went and Thimann (123), in their monographs on plant growth hormones, emphasize the presence of these substances in young vegetative and reproductive tissue. Avery, Burkholder, and Creighton (2), in an analysis of the production and distribution of growth substances in shoots of *Aesculus* and *Malus*, revealed that auxin is produced in greater quantity by reproductive shoots than by vegetative ones, the centers of hormone production being in the terminal buds.

The presence of a growth stimulating hormone in reproductive organs has been demonstrated by Grainger (33), who found it possible to increase the rate of vegetative extension of first-year plants of *Sisymbrium alliaria* by inoculating them with a water extract from an inflorescence of the same species.

Presence of growth promoting substances in specific reproductive organs.—The presence of growth hormones in reproductive organs has been amply demonstrated. Seemingly, certain structures involved in sexual reproduction are more plentifully supplied with these catalysts than others. Moreover, growth stimulating substances seem to be produced periodically in specific reproductive organs during the internal development of the floral parts.

1. *Pollen.*—The microgametophyte (pollen) contains growth stimulating substances. Fitting (30) noticed that falling of the flower and swelling of the gynostemium of some tropical orchids after flowering could be accomplished by the presence of pollen grains. A water extract of the pollen proved almost as effective. Fitting

ascribed this action to a hormone in the pollen. Recently, Duncan and Curtis (27) have reaffirmed this hormonal effect of pollen extracts on the orchid fruit.

Laibach (67) and Laibach and Maschmann (68) found auxin to be plentiful in the pollen of several species of orchids and *Hibiscus*, and Thimann (114) reported that *Sequoia* pollen is relatively rich in growth hormones.

Gustafson (35) and Yasuda (128) noticed that pollen extracts would stimulate the growth of plant ovaries, in some instances causing fruit to develop without seeds. According to Dollfus (23), pollen contains a growth substance that will induce rooting.

Significant bean internode elongation, following the application of an ether extract of corn pollen, is reported by Mitchell and Whitehead (81).

2. *Flower buds.*—Flower buds have been found to be very high in auxin during certain stages in their development. Gustafson (36) and Katunsky (56) found that ovules contain auxin. Whence does the auxin arise, which is present in the pollen and ovules, and does any relationship exist between the formation of growth promoting substances and the developmental stages of the gametophytes within the flower bud?

That young inflorescences may produce growth stimulating chemicals has already been noted in the work of Söding (105) (106) and Uyldert (119). Corn tassels were found by Moulton (84) to be exceedingly high in auxin. According to Kerling (59), the center of growth hormone production in *Zephyranthes* is probably the stamen and pistil of the immature flower.

Of special interest are Söding's experiments. He (107) reported that young flower buds, before full bloom, were exceptionally rich in growth substances. Later, analyzing the problem more carefully, he (108) found that in the flower buds of *Heliopsis laevis*, the auxin content varied considerably with their stages of development. Starting relatively low in the very young bud, the auxin content displayed a rapid increase, reaching a peak some time before full bloom. The concentration of growth substance dropped again rather sharply as anthesis approached.

Katunsky (56) reports similar results for *Papaver* and *Crepis*. Extracts of ovules taken from flower buds in the drooping stage showed, by Went's method, forty to sixty and more times the auxin content of extracts prepared from ovules in the preceding and following stages of floral development. He found a direct correlation between auxin content, growth, and floral movements. These, in turn, were associated with stages of the female gametophyte during which cell divisions proceeded with maximum intensity.

Thimann and Dolk (113), studying the conditions governing the production of plant growth hormones by *Rhizopus*, found auxin synthesis to be correlated with formation of sporangia. Two maxima

in production, with a minimum between, were noticed. The correlation between the growth curves of Errera and these hormone production curves for a similar fungus are indeed striking.

3. *Young embryos.*—Since Haberlandt's (46) postulation of a hormone in the embryo of vascular plants, evidence has accumulated showing that the fertilized egg is a rich source of growth hormones. Indeed, growth substance accumulation in the embryo seems to follow fertilization, reaching a peak within a short time thereafter and falling off rapidly with seed ripening.

Dollfus (23) and Söding (107) observed that the fertilized egg and very young fruit were exceedingly high in auxin whereas ovules not fertilized were very low. A general rise in auxin content of tomato fruit following fertilization was demonstrated by Judkins (55).

Gustafson (38) found that ovules and developing seeds were much higher in auxin content than other parts of the fruit. The same investigator (37), studying changes in growth hormone content of seeded and seedless fruit, noted that following pollination and fertilization, the auxin content increased appreciably in seeded fruit and at the same time decreased in seedless fruit.

Nutman (97), in vernalization studies of cereals, suggested that a growth promoting hormone is produced in the embryo sac of the developing grain.

Of particular significance in this connection is the work of Laibach and Meyer (69) and Meyer (78). Using alcohol extraction and lanolin paste methods, they determined auxin changes during the ontogeny of *Zea mays* and *Helianthus annuus.*

The appearance of growth hormones associated with reproductive development was interesting. In corn, there was a rapid decrease in growth substance upon germination. Seedlings thirty centimeters high had no measurable auxin. With the production of male flowers, there was a sudden appearance of growth substance even before the emergence of the tassels. In the young ears, there was no detectable auxin, but immediately following syngamy a great increase was noted, reaching a maximum ten to twenty days after fertilization, whence it dropped off sharply. It is interesting that two peaks seem to appear, one following the formation of the male inflorescence and the other, somewhat greater, subsequent to fertilization. Similar data were presented for *Helianthus annuus, Cucurbita pepo,* and *Cucumis sativus.*

Recently, Avery, Berger, and Shalucha (8) have confirmed the work of Laibach and Meyer for corn. A study was made of the amount of auxin in corn kernels as they developed from the time of pollination to maturity. At pollination little or no auxin was present. Immediately after, however, it accumulated sharply, and the highest concentration was reached in two to three weeks. From the peak, which was at the milk stage, there was a marked drop in auxin as the grain matured.

Hatcher and Gregory (47) obtained similar results for winter rye. The auxin content increased rapidly from a very low value at the time of pollination to a maximum five to six weeks later, attaining an increase of one hundred fold during the interval. At maturity, the concentration of growth substance had decreased to about twelve per cent of the maximum.

THE PROBLEM FOR INVESTIGATION

That definite processes, associated with sexual reproduction in higher plants, are growth stimulating, is a concept assumed in this investigation. In the study herein reported two of these phenomena, synapsis and syngamy, there may be others, have been subjected to experimental analyses. The problem posed is to determine whether or not chromosome conjugation and nuclear fusion, respectively, are responsible for a marked acceleration in plant growth.

The investigation may be divided into five phases. First, a study of the effect of bud, flower, and fruit removal on general plant growth. Such a method would allow one to "eliminate" the influence of synapsis and syngamy by the timely removal of the reproductive organs. Stimulating effects of these sexual processes should be revealed in the subsequent accumulation of dry matter, total soil nutrients, and vegetative extension of the plants under study. Many well known horticultural species might be adapted for such an investigation.

Second, an analysis of growth of the flower stalk of reproductive plants. Periods of maximal elongation may be correlated with the proposed crucial stages of floral development. A spurt in growth associated with flower bud and fruit initiation is a common phenomenon. Perhaps the best method of demonstrating this increase would be to grow large numbers of a given plant, and periodically harvest representative samples, noting the daily or weekly increments in dry weight, nutrient uptake, and products of metabolism. Such a procedure would, however, require a large space for the growing of plants and considerable labor. Similar results should be attained by simply making periodic measurements of flower stalk elongation and correlating them with the development of the flower buds.

The third phase would consist of a study of catalase activity in developing inflorescences. The relative enzyme concentration might be determined in the reproductive organs and accessory tissues, beginning with the pre-synaptic period in the bud and continuing until well after fertilization had occurred. Dioecious and monoecious plants should be particularly fitted for such an analysis.

The fourth phase should deal with the extraction and estimation of growth hormones, obtained from reproductive organs of plants during their development. It may be assumed that if periods of growth acceleration follow the processes of synapsis and

syngamy in the sexual development of higher plants, and if this acceleration is hormonic in nature, then the substance(s) causing such growth stimulations should be secured by extraction of the proper reproductive organs. In other words, the structures themselves in which these vital phenomena occur should yield the growth stimulating factor(s) by proper isolation procedures. That the flower bud following synapsis should be the source material in the one case, and the young fertilized egg, in the other instance seems only reasonable.

Finally, some practical aspects of the problem may possibly be taken into consideration with special reference to the setting and development of fruit. If hormones are produced in the reproductive organs following the fusion of gametes, they might have as their specific functions the induction of fruit setting, and the stimulation of growth in the ovary.

EXPERIMENTS ON REMOVAL OF REPRODUCTIVE PARTS

Material and Methods

Representatives of three general types of horticultural plants were used in experiments on the removal of reproductive parts. The cucumber was selected as a fast growing herbaceous annual, having little food reserve. Sour cherry twigs were chosen as being representative of woody perennial plants with a large accumulation of stored nutrients, and the strawberry plant as a perennial with moderate food reserves.

The Longfellow variety of cucumber was grown under greenhouse conditions in ten inch pots. Two plants were raised in each pot and the vines trained to wires attached to the top of the greenhouse. The experimental treatment was as follows:

1. Flower buds were removed as soon as they could be separated from the plant without injury. This was done several days after synapsis had occurred in microgametophyte formation.
2. Flowers were removed at anthesis.
3. Flowers were pollinated and fertilization occurred. The young fruit were removed as soon as signs of development were apparent.
4. Flowers were pollinated and fertilization occurred. Five-tenths per cent indolebutyric acid in lanolin was applied when the stigma had dried. Young fruit were removed as in 3.
5. Flowers were induced to form parthenocarpic fruit by application of five-tenths per cent indolebutyric acid in lanolin to the stigmas. Fruit were removed as in 3.
6. Controls. Plants were allowed to fruit normally, the fruit being produced by pollination and subsequent fertilization.
7. Plants fruited normally except that five-tenths per cent indolebutyric acid was applied to the very young fruit two to three days after pollination.
8. Plants were induced to form parthenocarpic fruit by application of five-tenths per cent indolebutyric acid to the stigmas.

The plants were dismantled upon yellowing of fruit of the control plants. Fresh weights were taken immediately and the material thoroughly dried to a constant weight in a well ventilated drying oven at 75 degrees centigrade. Total dry weights were then determined. After cutting in a Wiley mill and additional grinding in a Merker mill, the material was stored in air tight containers for future chemical analyses.

Ten year old Montmorency sour cherry trees were used as the source material for the twigs. About one hundred twigs were selected which had made a uniform growth in length, of fifteen centimeters, the previous season. Since vegetative and flower buds are separate and fairly distinguishable all leaf buds were removed except one terminal at the beginning of the experiment. The following treatments were applied.

1. Flower buds were removed before synapsis in formation of microgametophytes.
2. Flowers were removed just prior to anthesis.
3. Young fruit were removed ten to fifteen days after full bloom.
4. Controls. Normal fruiting was allowed.

The twigs were removed at the time of growth cessation in early summer and the amount of current new growth was used as an index for determining the effects of bud, flower, and fruit removal. Fresh and dry weights were estimated in the usual manner. The material was finely ground and preserved for future analyses.

In the strawberry experiment, the Blakemore variety was used, the young plants being propagated from runners during the early summer. After some development, the individual plants were transferred to five inch pots. They were carefully protected during the early winter and then moved into the greenhouse in the month of February. With the beginning of growth and first appearance of flower buds, experimental treatments identical with those described above for the sour cherry twigs were applied.

All plants were dismantled after fruiting of the controls had ceased. Methods used in weight determinations, drying, and grinding were similar to those already described for the cherry and cucumber.

The time of bud removal, when buds were removed before synapsis as in the sour cherry and strawberry, was ascertained by microscopic examination of the young anthers within the bud. Smear preparations, using aceto-carmine as a stain, were prepared. By the use of such a technique the approximate time of synapsis in the microspore mother cells was readily determined. It is the general rule that microsporogenesis precedes the development of the female gametophyte in floral differentiation. By examining large numbers of buds, one can judge with a high degree of accuracy the internal developmental stages of the flower bud, by observing visibly its external size and appearance.

In all cases of young fruit removal, the plants were defruited only after the ovaries had shown definite external signs of development following pollination or chemical treatment. The time interval between anthesis and fruit removal varied from a few days to two weeks.

It must be emphasized that, in all experiments conducted, the specimens representing the various groups were grown side by side in similar containers, thus insuring as much uniformity in environment as possible.

Chemical Analyses

All plant material was analyzed for total nitrogen, sugars, starch, and hemicelluloses. Determinations for nitrogen and the various carbohydrate fractions were carried out according to the adopted procedures in the Department of Horticulture at the University of Missouri. These have been published in detail by Murneek and Heinze (95), and Heinze and Murneek (49).

Results

Tables 1, 2, and 3 present the dry weights in grams of the vegetative and reproductive constituents, along with the total amount of dry matter produced for each treatment applied to the cucumber, strawberry, and cherry, respectively. Fresh weights were determined for all plants, as was the total vegetative extension of the cucumber and sour cherry, but are not presented since they displayed the same differences as did the dry weights.

In Table 1, the difference in growth response between disbudded and deflorated plants in the cucumber is interesting, though to a certain degree unexplainable. Removal of flower buds before synapsis was physically impossible without undue injury hence, it was not attempted. The excessive growth of plants with buds removed over those with flowers removed would indicate a general vegetative inhibition due to flower formation. A flower inhibition is not so hard to visualize in view of their abundant production and the lack of food reserves in the vegetative tissue.

The effects of gametic union in the cucumber are evident. In examining treatment 3, one can observe that the amount of dry matter produced is significantly greater than that in treatment 5. In the former, fruit were produced which contained fertilized eggs; the fruit in the latter group were devoid of embryos. A like growth stimulation occurred in treatment 4 where gametic union also took place. Conversely, the dry weights of plants in treatment 2 agree very closely with those in 5, fertilization occurring in neither class. A comparison of vegetative extension in the above groups presented differences similar to those for dry weights. Although the results are not recorded here, periodic measurements of height of plants portrayed, as did the results of Dearborn (22), that those in which fertilization was permitted, definitely had a higher rate of vegetative extension for a period of two weeks after pollination than those

TABLE 1

EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON GROWTH OF CUCUMBER PLANTS
(Dry weights in grams per 10 plants)

Treatment	Whole plant	Reproductive parts	Vegetative parts	Comments*
1. Buds removed before anthesis	409.1	3.8	405.3	- Inhibition by flowers
2. Flowers removed at anthesis	258.2	30.0	228.2	+ Inhibition by flowers - Syngamy
3. Fruit removed after fertilization	303.7	23.7	280.0	+ Inhibition by flowers + Syngamy
4. Treatment 3 + I.B.A.**	296.2	27.8	268.4	+ Inhibition by flowers + Syngamy + "Hormone" effect
5. Partheno-carpic fruit removed	250.9	20.1	230.8	+ Inhibition by flowers - Syngamy + "Hormone" effect
6. Controls Normal fruiting	264.4	125.6	138.8	+ Inhibition by seeds and fruit
7. Controls Normal fruiting + I.B.A.	249.0	104.7	144.3	+ Inhibition by seeds and fruit + "Hormone" effect
8. Partheno-carpic fruit produced	309.0	109.0	200.0	+ Inhibition by fruit only

* Minus (-) or plus (+) signs in this and other tables designates the absence or presence of a function or treatment whose effect is considered significant or crucial.

** 0.5% indolebutyric acid.

of groups in which syngamy was prevented. A comparison of the fruiting plants, with respect to the accumulation of dry matter, indicates that inhibition of vegetative growth is much less when seedless fruit are produced.

Tables 2 and 3 show the production of dry matter by plants and plant parts on which the effects both of synapsis and syngamy were eliminated by bud and flower removal. In the sour cherry, there is a steady increase in dry matter accumulation and vegetative extension with the "addition" of each postulated phase of stimulation. The strawberry gives a picture which is not quite so convincing in so far as dry weights are concerned. However, an examination of the data in Table 5 and 8 for this plant and in 4, 6, 7, and 9 for the

TABLE 2
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON GROWTH OF STRAWBERRY PLANTS
(Dry weights in grams per 10 plants)

Treatment	Whole plant	Reproductive parts	Vegetative parts	Comments
1. Buds removed before synapsis	159.3	0.4	158.9	- Synapsis - Syngamy
2. Flowers removed at anthesis	174.2	3.1	171.1	+ Synapsis - Syngamy
3. Fruit removed after fertilization	164.2	9.0	155.2	+ Synapsis + Syngamy
4. Controls Normal fruiting	166.7	82.2	84.5	+ Inhibition by fruit

TABLE 3
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON GROWTH OF SOUR CHERRY TWIGS
(Dry weights in grams per 25 twigs)

Treatment	Whole twig	Reproductive parts	Vegetative parts	Comments
1. Buds removed before synapsis	61.6	4.6	57.0	- Synapsis - Syngamy
2. Flowers removed at anthesis	85.1	12.2	72.9	+ Synapsis - Syngamy
3. Fruit removed after fertilization	94.3	17.8	76.5	+ Synapsis + Syngamy
4. Controls Normal fruiting	105.6	38.1	67.5	+ Inhibition by fruit

cucumber and sour cherry, wherein the accumulation of total nitrogen and the various carbohydrate fractions are presented, will make the picture more convincing. Invariably in those treatments where plants are allowed to flower and fruit there is an increase in the total production of the products of photosynthesis. Some plants, such as the strawberry, may not as a whole show any noticeable vegetative stimulation due to fertilization when only dry matter

TABLE 4
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON TOTAL NITROGEN ACCUMULATION IN CUCUMBER PLANTS
(In grams per 10 plants)

Treatment	Whole plant	Reproductive parts	Vegetative parts	Comments
1. Buds removed before anthesis	8.989	0.224	8.765	- Inhibition by flowers
2. Flowers removed at anthesis	6.564	1.392	5.172	+ Inhibition by flowers - Syngamy
3. Fruit removed after fertilization	7.815	1.157	6.658	+ Inhibition by flowers + Syngamy
4. Treatment 3 + I.B.A.	8.292	1.207	7.085	+ Inhibition by flowers + Syngamy + "Hormone" effect
5. Parthenocarpic fruit removed	6.494	0.906	5.588	+ Inhibition by flowers - Syngamy + "Hormone" effect
6. Controls Normal fruiting	6.225	2.610	3.615	+ Inhibition by seeds and fruit
7. Controls Normal fruiting + I.B.A.	6.363	2.298	4.065	+ Inhibition by seeds and fruit + "Hormone" effect
8. Parthenocarpic fruit produced	7.626	2.023	5.603	+ Inhibition by fruit only

accumulation is considered. This may be because the synthetic products of metabolism, in a concentrated form, are diverted into the reproductive organs at an early stage. Chemical analyses reveal such a relationship. Of interest, in the cucumber and strawberry and to a lesser degree in the cherry is the almost complete mobilization of available carbohydrates in the reproductive organs of the fruiting plants.

TABLE 5
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON TOTAL NITROGEN ACCUMULATION IN STRAWBERRY PLANTS
(In grams per 10 plants)

Treatment	Whole plant	Reproductive parts	Vegetative parts	Comments
1. Buds removed before synapsis	1.914	0.012	1.902	- Synapsis - Syngamy
2. Flowers removed at anthesis	2.114	0.087	2.027	+ Synapsis - Syngamy
3. Fruit removed after fertilization	2.282	0.210	2.072	+ Synapsis + Syngamy
4. Controls Normal fruiting	1.968	0.969	0.999	+ Inhibition by fruit

TABLE 6
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON TOTAL NITROGEN ACCUMULATION IN SOUR CHERRY TWIGS
(In grams per 25 twigs)

Treatment	Whole twig	Reproductive parts	Vegetative parts	Comments
1. Buds removed before synapsis	0.615	0.063	0.552	- Synapsis - Syngamy
2. Flowers removed at anthesis	1.090	0.386	0.704	+ Synapsis - Syngamy
3. Fruit removed after fertilization	1.333	0.554	0.779	+ Synapsis + Syngamy
4. Controls Normal fruiting	1.132	0.499	0.633	+ Inhibition by fruit

TABLE 7
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON CARBOHYDRATE ACCUMULATION IN CUCUMBER PLANTS
(In grams of glucose per 10 plants)

Treatment and Plant part	Total sugars	Starch	Hemi-cellu-loses	Total Carbo-hydrates	Comments
1. Buds removed before anthesis					
a. Vegetative	45.25	43.48	78.11	166.84	- Inhibition
b. Reproductive	0.11	0.23	0.65	0.99	by flowers
c. Total	45.36	43.71	78.76	167.83	
2. Flowers removed at anthesis					
a. Vegetative	21.27	17.37	44.62	83.26	+ Inhibition
b. Reproductive	3.66	1.42	4.95	10.03	by flowers
c. Total	24.93	18.79	49.57	93.29	- Syngamy
3. Fruit removed after fertilization					
a. Vegetative	23.12	18.51	55.28	96.91	+ Inhibition
b. Reproductive	1.61	1.08	4.10	6.79	by flowers
c. Total	24.73	19.59	59.38	103.70	+ Syngamy
4. Treatment 3 + I.B.A.					+ "Hormone" effect
a. Vegetative	19.28	16.12	54.53	89.93	+ Syngamy
b. Reproductive	3.18	1.65	4.63	9.46	+ Inhibition
c. Total	22.46	17.77	59.16	99.39	by flowers
5. Parthenocarpic fruit removed					+ Inhibition
a. Vegetative	21.91	16.97	43.44	82.32	by flowers
b. Reproductive	1.43	1.23	3.39	6.05	- Syngamy
c. Total	23.34	18.20	46.83	88.37	+ "Hormone" effect
6. Controls Normal fruiting					
a. Vegetative	9.02	4.17	17.34	30.53	+ Inhibition
b. Reproductive	86.71	7.48	11.31	105.50	by seeds
c. Total	95.73	11.65	28.65	136.03	and fruit
7. Controls Normal fruiting + I.B.A.					
a. Vegetative	7.26	5.63	21.88	34.77	+ Inhibition by
b. Reproductive	72.81	6.24	9.87	88.92	seeds and fruit
c. Total	80.07	11.87	31.75	123.69	+ "Hormone" effect
8. Parthenocarpic fruit produced					
a. Vegetative	11.30	8.79	28.73	48.82	+ Inhibition
b. Reproductive	72.02	6.47	14.88	93.37	by fruit
c. Total	83.32	15.26	43.61	142.19	

TABLE 8
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON CARBOHYDRATE ACCUMULATION IN STRAWBERRY PLANTS
(In grams of glucose per 10 plants)

Treatment and Plant part	Total sugars	Starch	Hemi-cellu-loses	Total Carbo-hydrates	Comments
1. Buds removed before synapsis					
a. Vegetative	17.63	10.27	32.29	60.19	- Synapsis
b. Reproductive	0.01	0.03	0.08	0.12	- Syngamy
c. Total	17.64	10.30	32.37	60.31	
2. Flowers removed at anthesis					
a. Vegetative	18.13	9.97	34.70	62.80	+ Synapsis
b. Reproductive	0.74	0.24	0.59	1.57	- Syngamy
c. Total	18.87	10.21	35.29	64.37	
3. Fruit removed after fertilization					
a. Vegetative	17.90	11.28	32.89	62.07	+ Synapsis
b. Reproductive	0.41	0.76	2.02	3.19	+ Syngamy
c. Total	18.31	12.04	34.91	65.26	
4. Controls Normal fruiting					
a. Vegetative	8.53	4.82	15.06	28.41	+ Inhibition
b. Reproductive	59.15	3.80	8.62	71.57	by fruit
c. Total	67.68	8.62	23.68	99.98	

TABLE 9
EFFECT OF BUD, FLOWER, AND FRUIT REMOVAL ON CARBOHYDRATE ACCUMULATION IN SOUR CHERRY TWIGS
(In grams of glucose per 25 twigs)

Treatment and plant part	Total sugars	Starch and Hemi-celluloses	Total Carbo-hydrates	Comments
1. Buds removed before synapsis				
a. Vegetative	2.994	21.589	24.583	- Synapsis
b. Reproductive	0.271	1.108	1.379	- Syngamy
c. Total	3.265	22.697	25.962	
2. Flowers removed at anthesis				
a. Vegetative	4.011	26.438	30.449	+ Synapsis
b. Reproductive	0.817	2.974	3.791	- Syngamy
c. Total	4.828	29.412	34.240	
3. Fruit removed after fertilization				
a. Vegetative	4.104	28.127	32.231	+ Synapsis
b. Reproductive	0.783	4.228	5.011	+ Syngamy
c. Total	4.887	32.355	37.242	
4. Controls Normal fruiting				
a. Vegetative	3.183	22.210	25.393	+ Inhibition
b. Reproductive	11.239	7.144	18.383	by fruit
c. Total	14.422	29.354	43.776	

EXPERIMENTS ON PERIODIC GROWTH MEASUREMENTS

Material and Methods

Spinach, the Long Standing Bloomsdale variety, was chosen as the test plant. The seed was planted in large wooden boxes during the winter, and developed a nice rosette in the cool weather. As longer days and warmer weather approached, the plants became reproductive and, with the first signs of bud development, measurements of the flower stalks were begun and continued at weekly intervals throughout the life of the plant. Because of the great variation in growth and reproduction, the data for each specimen were recorded separately. This was necessary since the male and female plants could not be distinguished at the time the measurements were initiated. Only the data obtained from male plants were utilized because female plants exhibited prolific branching during flowering and their internal developmental changes were much more difficult to ascertain histologically.

The occurrence of synapsis in the male inflorescence was readily determined microscopically, using anther smears and staining with aceto-carmine.

Results

A summary of the growth and development of the male spinach flower stalk is presented in Table 10 and Figure 1. It should be noted that no appreciable extension is evident in the flower stems prior to synapsis, noticeable growth occurring only after the initiation of this phase of sexual reproduction. It is interesting that, in spinach, pollen grains were differentiated over a period of about three weeks, as various portions of the inflorescence developed. In general, the

TABLE 10
WEEKLY ELONGATION INCREMENTS OF THE FLOWER STALK OF MALE SPINACH PLANTS

Age in weeks	Description	Length of flower stalk (cm.)	Weekly increase in elongation (cm.)
5	Rosette stage	1.14	
6	Rosette stage Inflorescence visible	1.55	0.41
7	Synapsis beginning	2.08	0.53
8	Synapsis continuing	6.84	4.76
9	Synapsis continuing	20.45	13.61
10	Synapsis completed Beginning of flowering	38.74	18.29
11	Flowering continuing Full bloom	56.50	17.76
12	Flowering continuing Full bloom	61.53	5.03
13	Senescence	63.32	1.79

maximal elongation of the flower stalk followed the most intensive period of synapses associated with microgametophyte formation.

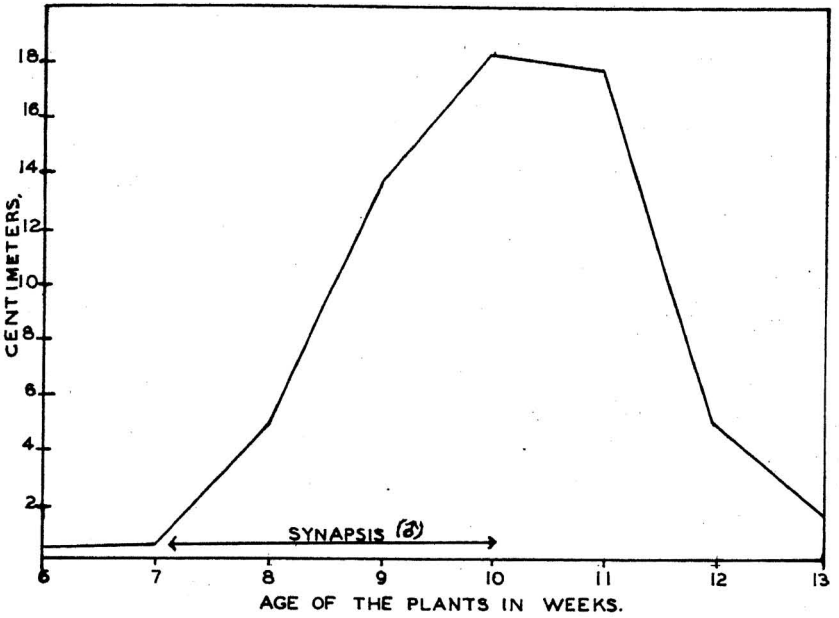

Fig. 1.—Weekly elongation increments in the flower stalks of male spinach plants.

CHANGES IN CATALASE ACTIVITY OF DEVELOPING FLOWER BUDS

Material and Methods

The material used in the catalase studies of developing inflorescences consisted of the flower buds and young fruit of two pear varieties, Kieffer and Vermont Beauty, and the male and female reproductive parts of a single cross of two self fertilized lines of yellow field corn (R136 x F6).*

Periodic collections of flower parts, and determinations of the enzyme activity were made of the reproductive structures, beginning several days before synapsis was initiated in the microspore mother cells, and continuing until well after fertilization had occurred in the ovules. In so far as the author is aware, similar studies have not previously been reported in the literature except for a few general relationships presented by Camp (16), Lopriore (74), Loustalot (75), and Miller (79).

*This seed was supplied, and convenient methods for pollen collection and for pollination were suggested by Dean C. Anderson, Research Associate in Field Crops, University of Missouri.

The method adopted for the determination of catalase activity was that suggested by Heinicke (48) and used successfully by him in estimating the activity of the enzyme in dormant apple twigs.

The mixed buds of the pear varieties studied, which subsequently developed into flowers and fruit, were separated into flower buds on the one hand and vegetative tissue on the other. The latter included the embryonic leaves and cluster bases. This separation of two tissue types was continued even at full bloom and during fruit development. With flower parts of the pear, all determinations were made on freshly collected material, and each figure, derived at, was an average of duplicate determinations of three separate samples each containing ten buds.

In corn, staminate (tassels) and pistillate (kernels) flower parts were collected at various stages of development. All material was frozen, immediately upon collection, at —14 degrees centigrade. At a later date, after all samples had been assembled, catalase determinations were run on material of the entire series the same day. With the frozen corn parts, each value obtained was an average of duplicate determinations of two separate five gram samples. The existing variability of the plant parts used necessitated several determinations for each figure derived.

By means of the smear method already described, the time of synapsis was ascertained in the developing anthers. In all instances syngamy was assumed to follow closely pollination. Prevention of fertilization was accomplished by covering the silks with "bags", especially designed to eliminate pollination.

Results

Tables 11, 12, and Figures 2, 3, depicting the results for the two pear varieties, present some intriguing data. Two maxima and two minima are evident in the flower bud. The results for the cluster bases, however, do not present such a definite relationship, yet the general trends are quite similar. It is particularly striking that in the flower bud, catalase activity is relatively low just before and at the time of synapsis in microspore mother cells. Similarly, a second minimum is reached with full bloom, at the time of, or just before fertilization. Especially of interest is the remarkable increase in enzyme activity following the two minima, and corresponding precisely with the post-synaptic and post-fertilization periods. A difference of nearly a week in time of blooming existed between the two pear varieties during the season the experiment was conducted. This provides additional evidence that the changes in catalase activity are probably due to "internal" factors, related to reproductive development as outlined above, rather than caused by variable environmental conditions.

TABLE 11
CATALASE ACTIVITY OF FLOWER BUD AND CLUSTER BASE PORTIONS OF MIXED BUDS IN KIEFFER PEAR DURING THEIR DEVELOPMENT
(In seconds required to release 10 ml. of O_2 from 3% H_2O_2)

No.	DESCRIPTION	CATALASE ACTIVITY	
		Flower buds	Cluster bases
1.	Buds, 15 days before synapsis	175	145
2.	Buds, 7 days before synapsis	190	180
3.	Synapsis occurring in microspore mother cells	225	136
4.	Microgametophytes in tetrad 3 days after synapsis	112	135
5.	Pollen grains present, separate from tetrad, 7 days after synapsis	125	127
6.	Buds, 10 days after synapsis and 10 days before full bloom	112	105
7.	Buds, 6 days before full bloom	141	195
8.	Buds, 2 days before full bloom	166	150
9.	Full Bloom	265	110
10.	Petals dropping Fertilization has occurred	120	155
11.	Young fruit, 10 days after full bloom	95	110
12.	Young fruit after first drop, 15 days following full bloom	73	118
13.	Young fruit, 22 days after full bloom	220	163

TABLE 12
CATALASE ACTIVITY OF FLOWER BUD AND CLUSTER BASE PORTIONS OF MIXED BUDS IN VERMONT BEAUTY PEAR DURING THEIR DEVELOPMENT
(In seconds required to release 10 ml. O_2 from 3% H_2O_2)

No.	DESCRIPTION	CATALASE ACTIVITY	
		Flower buds	Cluster bases
1.	Buds, 18 days before synapsis	167	114
2.	Buds, 15 days before synapsis	167	115
3.	Buds, 7 days before synapsis	160	110
4.	Synapsis occurring in microspore mother cells	150	96
5.	Microgametophytes in tetrad, 5 days after synapsis	102	95
6.	Pollen grains present, separate from tetrad, 10 days after synapsis	81	80
7.	Buds, 15 days after synapsis and 12 days before full bloom	75	93
8.	Buds, 7 days before full bloom	131	100
9.	Part to full bloom	185	155
10.	Petals beginning to drop, 3 days after full bloom	180	95
11.	Young fruit, 8 days after full bloom	67	121
12.	Young fruit, 12 days after full bloom	57	95
13.	Young fruit after first drop, 17 days following full bloom	40	96
14.	Young fruit, 21 days after full bloom	100	110

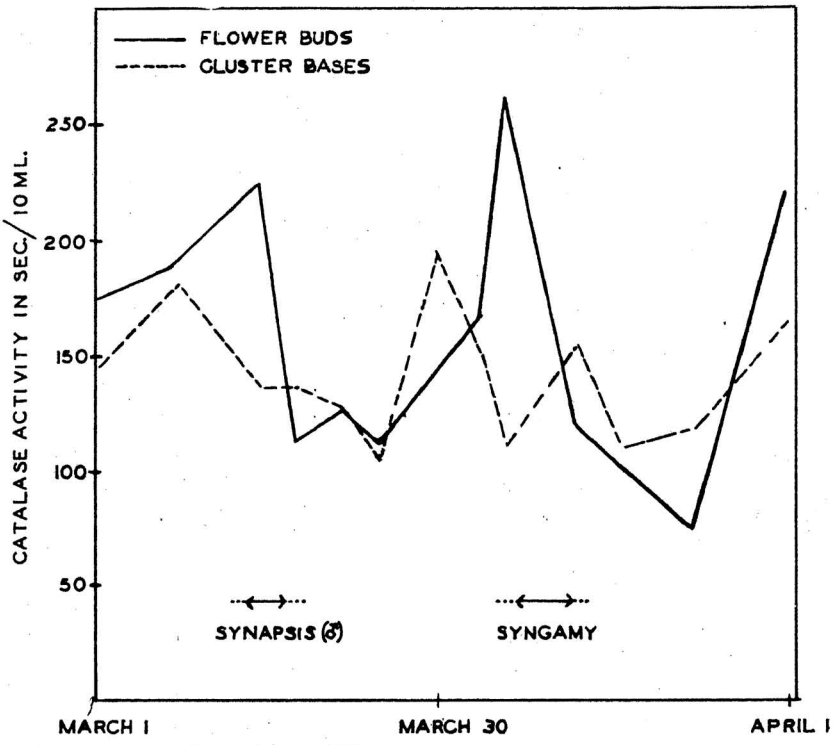

Fig. 2.—Catalase activity in flower buds of Kieffer pear during its development.

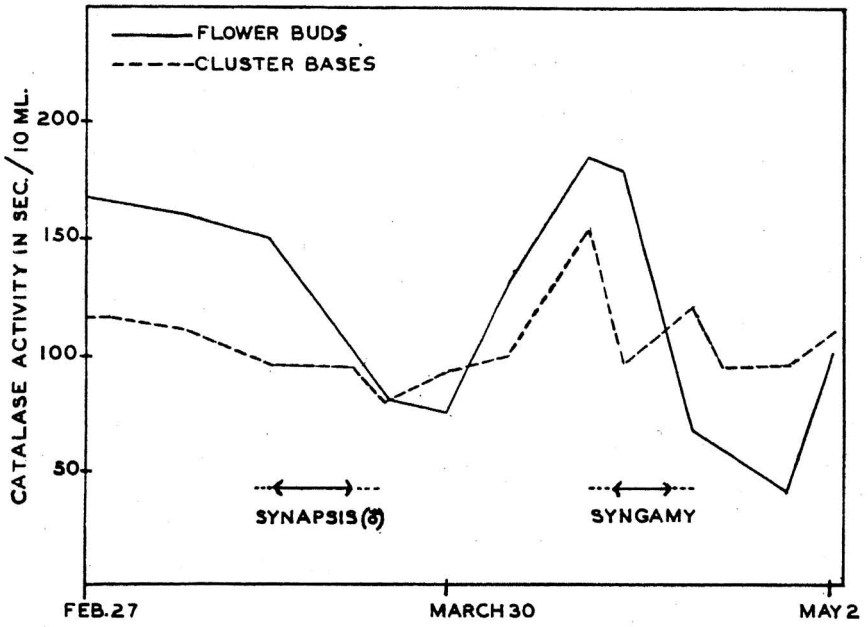

Fig. 3.—Catalase activity in flower buds of Vermont Beauty pear during its development.

The results with corn, shown in Table 13 and Figure 4, are equally convincing and notable though the staminate and pistillate inflorescences are treated separately. Here, as with the pear flower buds, tassels show an increase in catalase activity following chromosome conjugation in the anthers. In a like manner, the ovules depict a remarkable increase following fertilization. The difference in catalase trends of the fertilized eggs and those not fertilized is illustrated in Figure 4. Whether the pre-syngamic increase in catalase activity in these organs is correlated with the internal development of the embryo sac was not determined, but it is highly suggestive that synapsis in macrogametogenesis may be responsible for the increments noted.

TABLE 13
CATALASE ACTIVITY IN PISTILLATE AND STAMINATE INFLORESCENCES IN CORN DURING ITS DEVELOPMENT
(In seconds required to release 10 ml. of O_2 from 3% H_2O_2)

PISTILLATE			STAMINATE	
No.	Description	Catalase activity	No.	Description	Catalase activity
1.	Ovules, 8-10 days before pollination	440	1.	Tassels, 7-10 days before synapsis	326
2.	Ovules, 5-7 days before pollination	305	2.	Tassels, 5 days before synapsis	303
3.	Ovules, before appearance of silks	285	3.	Tassels, 2 days before synapsis	295
4.	Ovules, silks appearing	185	4.	Tassels, synapsis beginning near apex	456
5.	Ovules, controls, collected with No. 6	375	5.	Tassels, 3 days after No. 4, synapsis in basal part	308
6.	Grains, 3-5 days after pollination	64	6.	Tassels, 3-5 days after synapsis	266
7.	Grains, 10-15 days after pollination, "milk" stage	53	7.	Tassels, 5-7 days after synapsis	142
8.	Grains, 15-20 days after pollination, late "milk" stage	35	8.	Tassels, 7-8 days after synapsis	126
9.	Grains, 25-35 days after pollination, "dough" stage	69	9.	Tassels, 8-12 days after synapsis, pollen shedding	210
10.	Grains, mature corn	168			

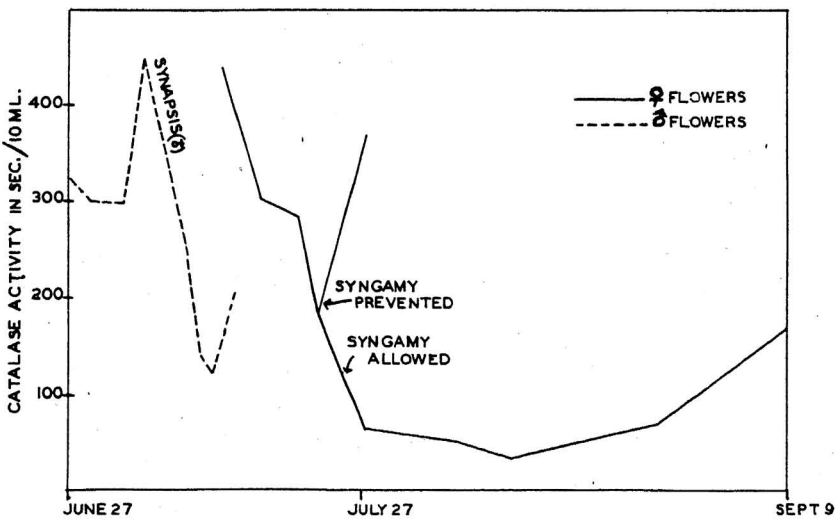

Fig. 4.—Catalase activity in the reproductive organs of corn during their development.

EXPERIMENTS ON EXTRACTION OF GROWTH STIMULATING SUBSTANCES FROM PLANT REPRODUCTIVE ORGANS

Considerable reference has already been made regarding the presence of growth stimulating substances in reproductive organs of plants. The high concentration of growth hormones in certain structures seems to have been accepted as a matter of course with little or no explanation, by investigators in the field of plant growth hormones, as to the possible origin of such factors in the reproductive development of the plant.

Preliminary Observations

During the early phases of this investigation, interesting preliminary observations were made and experiments conducted on extracts obtained from reproductive organs of the corn and tomato plants. An ether extract of young corn grains in the milk stage proved very effective in stimulating parthenocarpic fruit development in the sweet pepper, using the lanolin paste method. The fruit were larger and had a superior shape compared to those produced in a similar manner by indolebutyric acid (Figure 5). This extract also caused proliferations and curvatures on bean seedlings, when applied unilaterally to the first internode.

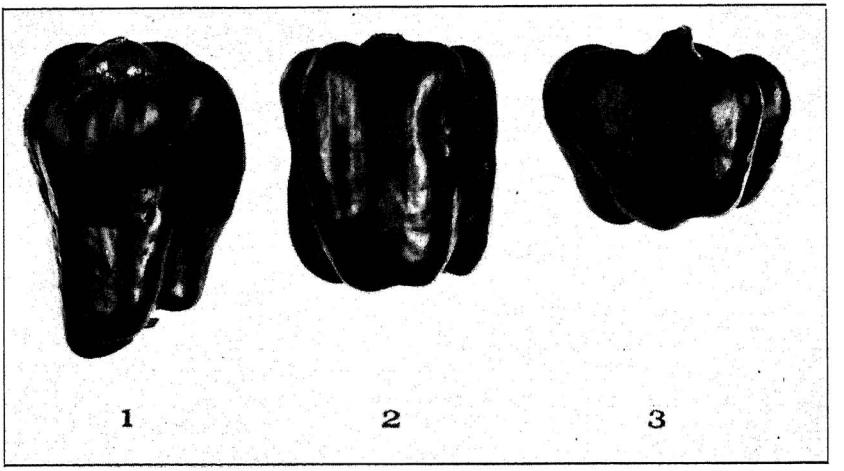

Fig. 5.—Effect of corn kernel extract on seedless fruit induction in pepper: (1) normal pollination; (2) corn kernel extract; (3) 0.5% indolebutyric acid.

Extracts of various reproductive organs in tomato were also tested. Ether was added to equal quantities (by weight) of (a) freshly collected tomato flowers, (b) very young fruit, (c) half grown, and (d) nearly mature tomatoes. After the mixture stood for twenty-four hours, the liquid was filtered off and the ether evaporated. To the remaining crude residue was added an approximately equal

quantity (by weight) of lanolin, such that the total weight of the residue plus the lanolin was equal for all samples. The prepared lanolin pastes were applied on one side of the first internode of young bean seedlings. The subsequent bending and elongation of the internodes were noted and recorded in Table 14. It is significant that only the extract from young fruit collected shortly after fertilization produced noticeable effect on the growth response of the bean seedling.

TABLE 14
GROWTH EFFECTS OF EXTRACTS FROM REPRODUCTIVE ORGANS OF THE TOMATO ON BEAN STEM INTERNODES

TREATMENT	NEGATIVE CURVATURE (degrees)	INTERNODAL ELONGATION OF STEMS (cm.) After 3 days	After 7 days
1. Lanolin only	None	0.98	1.04
2. Indoleacetic acid, 0.005%	18	1.61	1.70
3. Tomato flower extract	None	1.02	1.03
4. Tomato fruit (Diam. 0.5-1.0 cm.) extract	17.5	2.13	2.14
5. Tomato fruit (Diam. 2-3 cm.) extract	None	1.06	1.26
6. Tomato fruit (Diam. 4-5 cm.) extract	None	0.94	1.11

Material and General Methods

After due consideration of different plants for use as source material, in the collection of reproductive structures for growth hormone studies, field corn (*Zea mays*) was chosen. The corn plant was found to be particularly desirable because of its monoecious character and the especially abundant production of staminate and pistillate structures.

A single cross (R136 x F6) of two self fertilized lines of yellow field corn was grown under field conditions during the summer of 1942. Beginning at the time the corn stalks were about three feet high, periodic collections of the male and female reproductive structures were made, continuing until the plants matured in autumn. For the analyses of the synaptic stage of reproduction, corn tassels were collected in large quantities at different stages of development. The earlier collections necessitated the complete dismantling of many plants. At the time of tassel maturation, large amounts of the fresh pollen were acquired.

Pistillate reproductive parts were gathered periodically for the analyses of the syngamic phase of sexual reproduction. By means

of especially prepared "shoot bags", pollination and subsequent fertilization were controlled at will. For the analyses of pollen tube growth, silks pollinated six to eight hours previously were gathered. Controls consisted of silks not pollinated. Collections of corn kernels were made periodically from the time of pollination until the grain had completely matured.

The time of synapsis in the microspore mother cells of the tassels was determined microscopically using the smear technique. Syngamy was assumed to occur subsequent to pollination, the drying of the silks and swelling of the kernels being indicative of the post-fertilization period.

Upon collection all plant material was prepared for preservation immediately. Most samples were divided into two portions. One was subjected to a quick freezing treatment and later kept in a frozen condition at about 10 degrees Fahrenheit. The second lot was thoroughly dried in a well ventilated oven and then stored in air tight containers.

Methods of Extraction

The current literature on methods for the extraction of plant growth hormones is voluminous. Kögl, Haagen-Smit, and Erxleben (60) (61) (62) used ether, alcohol, and water as solvents in the isolation of auxin a, auxin b, and heteroauxin. According to Thimann and Skoog (115) and Gustafson (41), ether is an effective solvent for the extraction of growth substances from fresh or frozen tissues; whereas Avery (3), and Avery, Creighton, and Shalucha (4) reported alcohol to be an excellent solvent for the extraction of auxin from corn endosperm.

More recently and during the course of the present study, several additions have been made to our knowledge of plant growth hormone extraction. Avery, Berger, and Shalucha (5) (6) (9) report the use of alkaline hydrolysis for total extraction of auxin and auxin precursor in corn endosperm. A similar solvent was utilized for the extraction of heteroauxin from corn meal by Haagen-Smit, Leech, and Bergen (44) (45). Link, Eggers, and Moulton (72) suggest frozen vacuum-dried material. The auxin yield from *Lemna* was found to be increased by incubation with chymotrypsin or trypsin, according to Thimann, Skoog, and Byer (116).

The procedure adopted by the author and used in the following studies did not and could not utilize all the knowledge now available pertaining to the extraction of plant growth hormones. The methods selected at the beginning were rigidly adhered to throughout the course of the present study, regardless of new methods and techniques currently appearing in the literature. The isolation and estimation of growth hormones in plant material is still to a large degree in the experimental stage.

In this investigation, di-ethyl ether and ninety-five per cent ethyl alcohol were used as solvents. Where possible both were utilized on dried as well as frozen material.

The extraction procedure used was as follows: To the finely ground plant material (frozen or dried) was added ten times its weight of solvent. The mixture was allowed to stand twenty-four or forty-eight hours at room temperature, except for periodic shaking. Then the solvent was filtered off and the filtrate evaporated to dryness under vacuum distillation. No further purification of the crude extract was attempted. The remaining residue, mixed with an approximately equal quantity of anhydrous lanolin, was applied in the paste form to the test material. All samples in a comparable series were prepared in a like manner, using the same quantity of material (50 grams of dried and 200 grams of frozen), an equal volume of solvent, and the same extraction period. They were tested on uniform biological material. Considerable variation was frequently encountered in the quantity of crude residue remaining after the removal of the solvent. Where such differences existed, variable amounts of lanolin were added, such that the total weight of the paste plus the residue was equal for all specimens in a series. Comparisons of growth stimulating activity were made on the basis of the fresh and dry weights of plant material.

Plant growth hormones are almost universally estimated by biological methods. The general use and merits of the *Avena* coleoptile technique are appreciated by the writer. However, because preliminary investigations had indicated that the reproductive organs of corn were exceedingly rich in growth hormones, the refinements and sensitivity of the coleoptile procedure were not considered essential. Accordingly a simpler, perhaps much cruder, yet accurate, method of estimating the growth substance was adopted, as follows.

Bean seedlings, grown under greenhouse conditions with moderate control of light and temperature, were used as test material. A procedure and technique were developed quite similar to that reported by Mitchell and Whitehead (81). The Stringless Green Pod variety of bean was planted in large wooden boxes. When the first internode was approximately fifteen millimeters long, and the heart shaped leaves only partially developed, uniform and vigorous plants were treated with the various extracts by an application of a small quantity of the paste mixture to one side of the internode. Subsequent bending, after a three to four hour interval, was noted. Marked proliferation of internodal tissue sometimes developed after a week. A negative curvature following the treatment was taken as indicating growth hormone activity in the extract applied. Internodal or stem proliferation was suggestive of an extremely high hormone concentration. When several or all members of a series exhibited a growth response on the bean seedling, the relative and comparative activity was determined by successive dilutions of the original crude extract with lanolin. The usual dilutions employed were one part of the crude extract to one, five, ten, twenty-five, fifty, one hundred, and one thousand parts of lanolin paste, respec-

tively. It must be emphasized that before conclusions were reached regarding the growth stimulating activity of any of the extracts, each series of experiments was repeated at least once, sometimes three to four times. For any one reproductive structure, both alcohol and ether extracts of frozen and dried material were prepared, and several dozen bean seedlings treated. Controls of untreated plants and others using lanolin alone were grown with every experimental series. The degree of curvature or bending of the internode was measured by means of a protractor.

Results

The relative effectiveness of the various corn extracts in the bending of bean seedling internodes is presented in Tables 15 and 16, and Figure 6. The data indicate a complete absence of growth hormone in the male inflorescence prior to synapsis. A similar condition was found in the ovule before fertilization. However, a few days subsequent to chromosome conjugation in the tassel, and the union of gametes in the ear, growth hormones in considerable quantities made their appearance in these structures.

TABLE 15
COMPARATIVE ACTIVITY OF EXTRACTS FROM STAMINATE REPRODUCTIVE ORGANS OF CORN ON BENDING OF BEAN SEEDLING INTERNODES
(Limit in number of times the crude extract can be diluted and retain activity)

No. DESCRIPTION	SOLVENT Alcohol	SOLVENT Ether	PRESERVED BY Drying	PRESERVED BY Freezing	ACTIVITY
1.Tassels, 2-3 days before synapsis					
a.	x			x	Not active
b.		x		x	Not active
2.Tassels, synapsis occurring					
a.	x			x	Not active
b.		x		x	Not active
3.Tassels, 3-5 days after synapsis					
a.	x			x	Not active
b.		x		x	Not active
4.Tassels, 7-8 days after synapsis					ACTIVE WHEN DILUTED:
a.	x			x	5 times
b.		x		x	10 times
5.Tassels, emerging					
a.	x			x	5 times
b.		x		x	10 times
c.		x	x		Not active
6.Corn pollen					
a.	x			x	10 times
b.		x		x	50 times
c.		x	x		Questionable
7.Corn silks. Controls					
a.	x			x	Not active
b.	x		x		Not active
c.		x		x	Not active
d.		x	x		Not active
8.Silks pollinated 6-8 hrs. previously					
a.	x			x	Not active
b.	x		x		Not active
c.		x		x	Not active
d.		x	x		Not active

TABLE 16
COMPARATIVE ACTIVITY OF EXTRACTS FROM PISTILLATE REPRODUCTIVE ORGANS OF CORN ON BENDING OF BEAN SEEDLING INTERNODES
(Limit in number of times the crude extract can be diluted and retain activity)

No.	DESCRIPTION	SOLVENT Alcohol	SOLVENT Ether	PRESERVED BY Drying	PRESERVED BY Freezing	ACTIVITY
1.	Ovules, 8-10 days before fertilization					
	a.	x			x	Not active
	b.		x		x	Not active
2.	Silks, 8-10 days before fertilization					
	a.	x			x	Not active
	b.		x		x	Not active
3.	Ovules, 5-7 days before fertilization					
	a.	x			x	Not active
	b.		x		x	Not active
	c.		x	x		Not active
4.	Ovules, just before emergence of silks					
	a.	x			x	Not active
	b.		x		x	Not active
	c.		x	x		Not active
5.	Ovules. Controls, collected with No. 6					
	a.	x		x		Not active
	b.	x			x	Not active
	c.		x		x	Not active
	d.		x	x		Not active
6.	Grains, 3-5 days after pollination					ACTIVE WHEN DILUTED:
	a.	x		x		25 times
	b.	x			x	25 times
	c.		x		x	25 times
	d.		x	x		10 times
7.	Grains, 10-15 days after pollination					
	a.	x		x		500 times
	b.	x			x	500 times
	c.		x		x	500 times
8.	Grains, 15-20 days after pollination					
	a.	x		x		100 times
	b.	x			x	100 times
	c.		x		x	100 times
9.	Grains, 25-35 days after pollination					
	a.	x			x	25 times
	b.		x		x	25 times
10.	Mature corn					
	a.	x		x		20 times
	b.		x	x		20 times

An accurate estimation of the hormone concentration in immature pollen was not attempted because of the difficulties encountered in its separation from the anthers. Tassels began to exhibit considerable activity approximately seven days after synapsis, which was three to five days before their emergence.

Results obtained from the pistillate flower parts were indeed striking. No detectable growth hormone was present in the developing ovule. The comparison between sample 5 and 6 in Table 16 is both interesting and informative. Both groups were collected the same day. The presence of growth hormones in the young grain

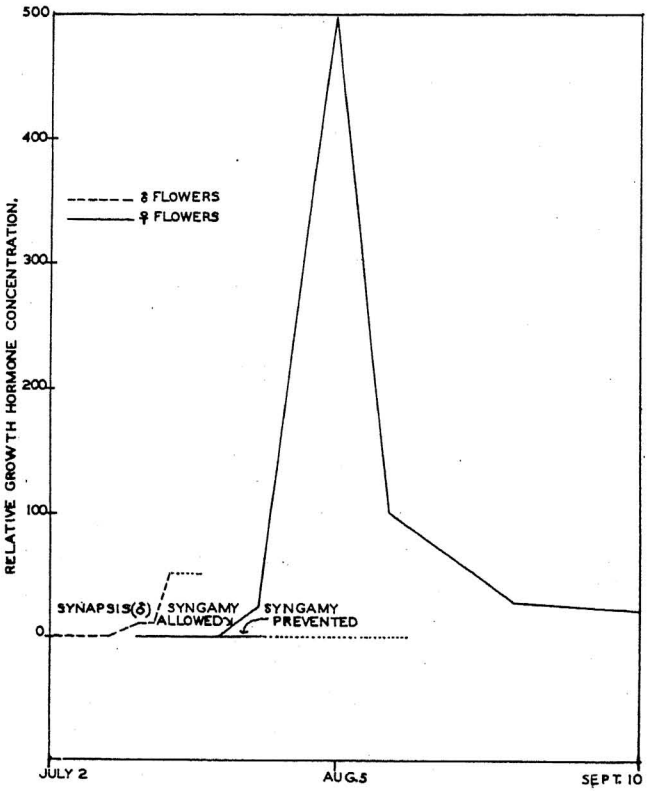

Fig. 6.—Changes in growth hormone content of the reproductive organs of corn during their development.

following fertilization and their complete absence prior to syngamy is strong evidence for their appearance only after gametic union had occurred. These substances suddenly become evident following fertilization and reach a peak ten to fifteen days after the initiation of this crucial phase. As is indicated in Table 16, the crude extract obtained from grains at this peak may be diluted with five hundred parts of lanolin and still bend the internodes of bean seedlings. Extensive proliferation and aerial root formation were also observed in the more concentrated combinations. (Figure 7).

With maturation of the grains, a rapid decrease in hormone content occurs. The mature corn, on the basis of fresh weight, has only four per cent of the activity possessed by young immature kernels in the "milk" stage.

In Figure 6, one may notice that the relative growth hormone concentration, of the respective reproductive structures involved, is

much greater following the syngamic phase than that subsequent to synapsis.

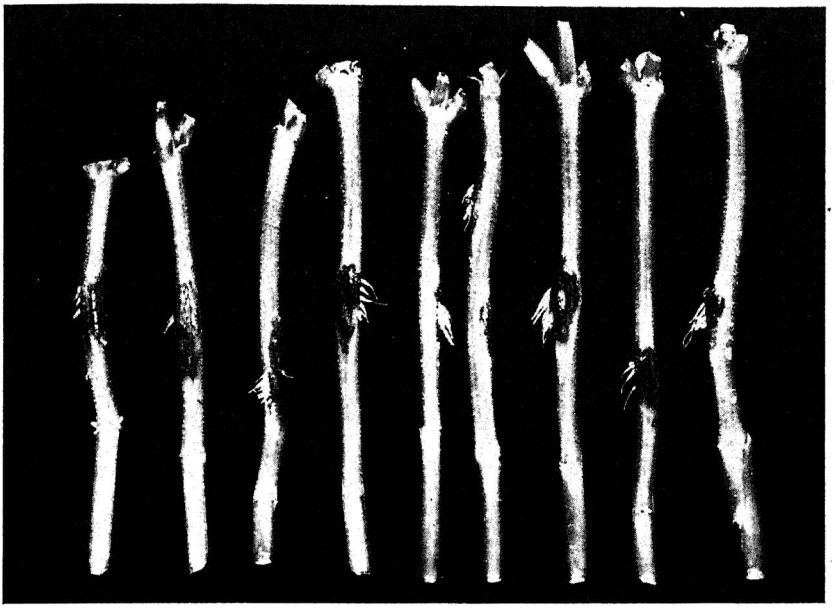

Fig. 7.—Proliferation and aerial rooting of bean seedling internodes from application of corn kernel extract.

EFFECTS OF EXTRACTS FROM REPRODUCTIVE ORGANS OF CORN ON FRUIT SETTING AND DEVELOPMENT IN TOMATO

The relationship between the periodic occurrence of growth hormones in plant reproductive organs and their significance to fruit setting and development in horticultural plants, is more than coincidental. In a preliminary investigation already mentioned in this paper, an ether extract from immature corn grains was effective in inducing parthenocarpic development of pepper ovaries. It was of interest to determine, first, from what corn reproductive parts, extracts could be prepared which would be capable of inducing seedless fruit development; second, how, with normal pollination, the corn kernel extract and the synthetic growth substances would compare in fruit setting; and third, how the above treatments would correspond in their effect on fruit size and yield.

Material and Methods

This phase of the investigation was conducted with the Marglobe tomato. The plant is well adapted to greenhouse experimental work and has been extensively utilized in growth hormone studies concerned with seedless fruit production.

Large numbers were grown in ten and twelve inch clay pots in greenhouse potting soil which is ideal for vigorous growth and fruit setting. During the short winter days, supplementary illumination was provided five hours each day by two hundred watt Mazda lamps operated by a time switch.

Some of the extracts obtained from corn reproductive organs, previously described and tested on bean internodes, were applied, in the lanolin paste form, to the young tomato ovaries just prior to anthesis. Only four buds per cluster were treated. All others were removed. For each treatment in a series of tests, a group of six to twelve plants were used and they were distributed at random among the other treatments in the same experiment. In all cases, controls of lanolin only were run side by side with the other groups.

Corn tissue extracts were considered effective in inducing seedless fruit production if, at any concentration, noticeable development of the ovaries was evident three weeks after application.

In the fruit setting experiments, a tomato flower was considered "set" if, three weeks after application of the lanolin paste to the ovary, the young fruit had attained a size of one centimeter in diameter or larger. Where "per cent fruit set" was determined, all tomatoes were removed while young in order to prevent any inhibition of set on the same plant by maturing fruit.

Size of the tomato fruit was ascertained by averaging the weight of all ripe specimens in each treatment, and yield by calculating the total weight of all marketable fruit produced during the six week interval following ripening of the first fruit.

An alcohol extract was taken from corn grains ten to fifteen days after fertilization, mixed with equal parts of lanolin and applied to the ovaries just before anthesis. In the second and third experiments, the efficiency of this extract was compared with normal pollination, and with the synthetic growth substances, namely, indoleacetic and indolebutyric acids.

Results

The effect on seedless fruit induction of the various corn extracts is given in Table 17. Of all the preparations used, the young corn grains alone are effective. Considerable activity is exhibited by corn kernels ten to fifteen days subsequent to syngamy. The crude alcoholic residues, even when diluted five times with lanolin still retain enough activity to produce a seedless fruit of normal size. Complete ineffectiveness of ovules and even pollen extracts is noted. Corn silks collected six to eight hours following their pollination show no activity.

According to the data in Table 18, the crude extract procured from young corn grains is superior for the induction of fruit setting to

TABLE 17
COMPARATIVE ACTIVITY OF EXTRACTS FROM REPRODUCTIVE ORGANS OF CORN ON SEEDLESS FRUIT INDUCTION IN TOMATO
(Limit in number of times the crude extract can be diluted and retain activity)

No.	DESCRIPTION	SOLVENT Alcohol	SOLVENT Ether	ACTIVITY
1.	Ovules, pollination prevented, collected with No. 2			
	a.	x		Not active
	b.		x	Not active
2.	Grains, 3-5 days after pollination			
	a.	x		Not active
	b.		x	Not active
3.	Grains, 10-15 days after pollination			ACTIVE WHEN DILUTED:
	a.	x		5 times
	b.		x	5 times
4.	Grains, 15-20 days after pollination			
	a.	x		3 times
	b.		x	3 times
5.	Grains, 25-35 days after pollination			
	a.	x		Not active
	b.		x	Not active
6.	Mature corn grains			
	a.	x		Not active
	b.		x	Not active
7.	Corn pollen			
	a.	x		Not active
	b.		x	Not active
8.	Corn pollen + ovules in No. 1			
	a.	x		Not active
	b.		x	Not active
9.	Corn silks, no pollination			
	a.	x		Not active
	b.		x	Not active
10.	Corn silks, 6-8 hrs. after pollination			
	a.	x		Not active
	b.		x	Not active

TABLE 18
EFFECT OF CORN KERNEL EXTRACT ON PERCENTAGE OF FRUIT SET IN TOMATO

Treatment	Cluster 1	2	3	4	Total
1. Indolebutyric acid, 0.5%	55	75	75	85	75.00
2. Indoleacetic acid, 0.5%	35	37.5	60	55	48.64
3. Normal pollination	75	32.5	15	57.5	40.71
4. Corn kernel extract*	80	75	100	100	90.00
5. Lanolin only	0	0	0	0	0

* Residue from an alcoholic extract of corn grains, 10-15 days after fertilization, mixed with equal parts of lanolin.

the synthetic growth substances, indolebutyric and indoleacetic acids. It is interesting that even the synthetic materials gave a better fruit set than normal pollination when the total of all treated flower buds is considered.

The effect of the kernel extract on fruit size and fruit yield is presented in Table 19. The yield from this extract equals and slightly surpasses that obtained from any of the other treatments. The most noticeable differences occurred in fruit size. Here the corn extract definitely produced the largest fruit.

TABLE 19
EFFECT OF CORN KERNEL EXTRACT ON FRUIT SIZE AND YIELD IN TOMATO
(Yield and no. of fruit per 10 plants)

Treatment	No. of fruit	Yield, in grams	Average weight, per fruit
1. Controls			
Normal pollination	58	6344	109.4
2. Corn kernel extract*	40	7012	175.3
3. Indolebutyric acid, 0.5%	59	6613	112.1
4. Indoleacetic acid, 0.5%	50	4028	80.6

*Residue from an alcoholic extract of corn grains, 10-15 days after fertilization, mixed with equal parts of lanolin.

DISCUSSION

General Growth Correlations

The concept, first introduced by Murneek, that synapsis and syngamy initiate two periodic growth stimulations in higher plants, opens a new field in studies of plant metabolism and development. Heretofore, emphasis has largely been focused on the type of vegetative growth which is conducive to flower and fruit formation. In studies concerned with the reverse of this phenomenon, namely, the effects of developing reproductive organs on vegetative increase, the inhibiting effects of fruits and seeds were first emphasized. That developing buds and very young fruit may stimulate growth beyond the reproductive organs, has been fully demonstrated in this investigation. Careful analyses of presented facts indicate that such stimulations arise concomitantly with synapsis and syngamy in the reproductive organs and extend, in a measure, through the rest of the plant. It has not been previously introduced or recognized that the increased growth of the plant subsequent to nuclear fusion and chromosome conjugation at synapsis may be in part or fully accounted for by the periodic production of plant growth hormones within the reproductive organs.

A close analysis of the foregoing data will disclose certain salient relationships. It appears from the results reported that, in general, plants and plant parts utilize their environment more efficiently if germ cell formation and fertilization are permitted. This is evidenced by increased growth and nutrient accumulation, although considerable differences exist among plants in the magnitude of the synaptic and syngamic affects.

Periodic catalase determinations show, in one way at least, the changes in growth and metabolic activity of flower parts and adjacent tissue during their development. Whether or not the analogy is complete between growth activity and the above enzyme response is not known, but the data are useful in providing additional evidence that a period of rapid growth acceleration follows the crucial phases of synapsis and syngamy.

Growth hormone concentrations in the reproductive organs of the corn plant are characterized by two peaks, one occurring in the tassels following microgametophyte differentiation in the male flower buds, and the other in the kernels shortly after embryo initiation.

In the disbudding and deflowering experiments performed on the cucumber, strawberry, and sour cherry, the causes of these peaks in hormone production were eliminated by the removal of reproductive organs. The result was a decrease in total vegetative extension, dry weight, and nutrient accumulation in the treated plants. The decline in growth response was greatest with bud removal, since both synapsis and syngamy were eliminated. In the male spinach plants, growth of the flower stalks became appreciable only after synapsis had commenced. With the flower buds of pear and corn, changes in catalase activity appear to have been directly connected with the internal changes of reduction division in gametophyte formation and gametic union. The correlation between the variations in catalase activity and the growth hormone changes in the same reproductive organs of corn is indeed striking.

These results throw new light upon the interpretation of a series of papers published over sixty years ago, by Kreusler and his coworkers (65) (66), on the growth of the corn plant. According to these investigators, a measurement of the weekly increments in dry weight during the growth period portrayed two noticeable peaks in dry matter increase separated by a period of lesser growth. Such changes were independent of the leaf area and occurred during the formation of the male flowers and the ears, respectively. They were not due to external environmental conditions or to sampling errors. A summary of their data, with the present writer's interpretation is presented in Figure 8. The similarity among these curves for corn growth, those in Figure 4, depicting changes in the catalase activity of the reproductive organs of the same plant, and the growth hormone data in Figure 6 represents more than a chance correlation among all these records directly concerned with growth. The

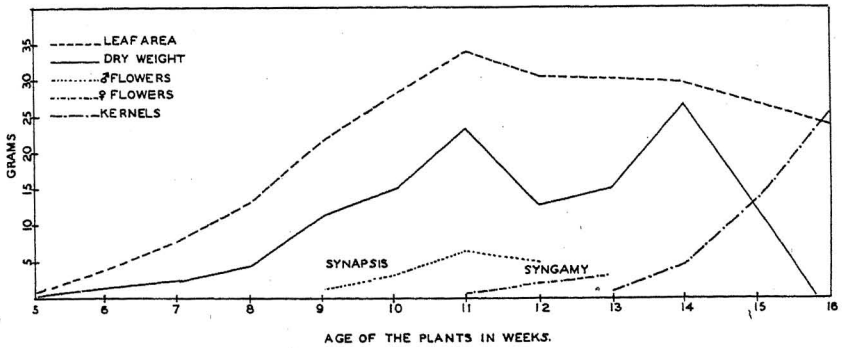

Fig. 8.—Weekly increment in dry weight of corn during its growth period. (Figure after Kreusler, Prehn, and Hornberger (66). Interpretation by Wittwer.)

writer's interpretation of Laibach and Meyer's (69) results, in Figure 9, also for corn, adds additional proof that these factors are interrelated. All of them indicate apparently that the processes of synapsis and syngamy are the causes of inception of the changes.

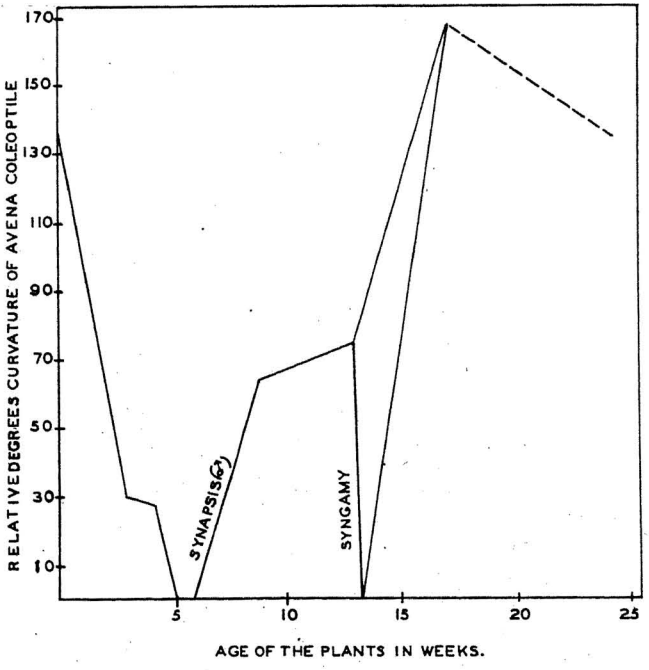

Fig. 9.—Changes in auxin content during the growth period of corn. (Figure after Laibach and Meyer (69). Interpretation by Wittwer).

The sequence of events, as regards reproduction of corn in relation to acceleration of metabolism, would seem to be as follows. With the development of the male inflorescence, synapsis occurs in microgametophyte differentiation. This process causes the production of growth stimulating hormones which in turn stimulate plant growth. Shortly, however, the tassels mature and the pollen is shed, leaving the plant for the time being without a source of activating factors. This period, which occurs at anthesis, is evidenced by a temporary but general reduction in growth. Concomitant with syngamy, a rapid accumulation of growth substances again takes place, this time in the pistillate inflorescence. A marked increase in dry matter production follows, exceeding in magnitude the previous maximum. As the grains develop, their hormone content gradually decreases. Presumably this second diminishing supply of catalytic substances again affects the plant, resulting in a drastic abatement of growth. This, in turn, is followed by senescence and final death of the organism, save the fruit (grain).

A more thorough study of the corn plant by Hornberger (52) revealed that increases in uptake of mineral nutrients, in synthetic products of metabolism, and in general dry weights, all were associated with the appearance of the male and female flowers. He assumed the causes of the two maxima in growth to be internal but of unknown nature.

Briggs, Kidd, and West (14), in a review of the results of Kreusler, Prehn, and Hornberger, summarized them in the generalized growth curve (Figure 10), wherein, the weekly percentage increase in dry weight is plotted against the leaf areas. The author's interpretation of this curve with respect to sexual reproduction is also presented here. Suggestions as to the significance of the two subsidiary maxima and minima were made by Murneek (91).

In their discussion of the general growth curve, Briggs *et al.* point out that in those instances where there is only one prominent subsidiary growth maximum, the male and female flowers appear together. It would seem, under such conditions, that the interval between synapsis and syngamy is decreased and the two maxima would blend into one. Undoubtedly in many plants this very condition exists and a separation of the growth responses arising from these processes is more difficult.

The condition of increased growth and nutrient uptake associated with sexual reproduction is also evidenced in the recent results of Olson and Bledsoe (98), who worked with cotton. The first maximum occurred during the "square" stage and the second, a larger one, during early boll formation. The relation of these maxima to bud and fruit development can hardly be accidental. Wilfarth, Römer, and Wimmer (124) report a rise in nutrient uptake for several species of plants during flowering and fruiting.

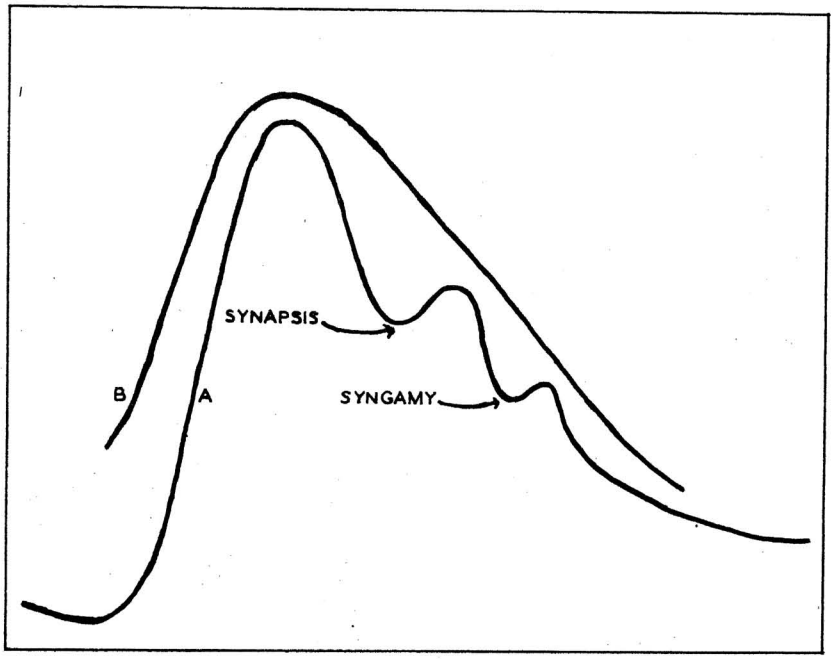

Fig. 10.—Weekly percentage increase in dry weight of corn during its growth period. (A) dry weight; (B) leaf area. (Figure after Briggs, Kidd, and West (14). Interpretation by Wittwer).

The work of Gill (31) on cambium activity in relation to flowering, indirectly provides excellent proof of a growth stimulation initiated by synapsis. Four catkin bearing genera were studied. In *Salix* and *Populus*, the inflorescences are inclosed in buds during the winter and the microgametophytes develop (synapsis occurs) in the spring. Concomitant with catkin differentiation in these genera, the cambium is activated immediately below the buds. Quite a different condition exists in *Corylus* and *Alnus*, whose floral parts are exposed throughout the winter and pollen grain differentiation (synapsis) is complete in autumn before the beginning of the winter rest. In the springtime with the elongation of the catkin and the shedding of pollen, there was no stimulation of vascular tissue. The contrast is significant indeed.

Additional evidence of a growth stimulation arising during both bud and early fruit formation is given by Weilung (122) and Spaning (109). The assimilation intensity (carbon dioxide intake) varied with the development of the flower parts. Fertile, sterile, as well as dioecious species of plants were studied. Weilung, in an investigation of the latter type, found in *Rumex, Spinacia,* and

Mercurialis an interesting situation. In the male specimens, only one maximum in assimilation occurred, that being during the bud stage. The female plants showed two maxima separated by a minimum. The first rise in carbon dioxide utilization occurred at bud formation, the second followed anthesis and was correlated with early fruit development. Spaning investigated the variations in the assimilation intensity of fertile hermaphroditic plants, and found the two regular maxima, one associated with bud, the other with fruit development, and the minimum at flowering. Sterile plants of the same species displayed no maxima or minima in their carbon dioxide intake. Moreover, their assimilation in aggregate was much less than plants carrying buds and fruit.

Katunsky (58), in an indirect way, has similarly shown that plants utilize carbon dioxide most efficiently during flower and fruit development.

To aid the reader in becoming more cognizant of the stimulating role that synapsis and syngamy play, a further analysis of existing data may prove helpful. In higher plants, the rate of growth is largely determined by (a) the photosynthetic activity of the leaves and (b) absorption of mineral nutrients by the roots. That these two functional processes show a marked acceleration immediately after embryo and microgametophyte initiation, has been verified by the results presented herein and by other investigators previously mentioned (52) (66) (98) (109) (122).

Another feature representative of the post-synaptic and post-syngamic periods is the production of growth promoting hormones within the reproductive organs. Cholodny and Gorgovsky (18) have reported that heteroauxin may increase the rate of photosynthesis when applied externally to the leaf. A significant rise in dry matter accumulation followed the application of the same substance to bean seedlings, according to Mitchell and Hamner (80).

From the above evidence one might reasonably postulate that the hormones produced in conjunction with synapsis and syngamy are directly responsible for several growth responses, associated with sexual reproduction in higher plants, of which the following may be emphasized: (a) Periods of rapid cell divisions in the flower bud and young fruit. (b) Initiation of cambium activity. (c) Periodic growth and movements of the flower stalk. (d) Induction of fruit development. (e) Two separate stimulations extending throughout the plant, each arising, presumably, from increased metabolic efficiency of roots and leaves.

The above conceptions have an interesting parallel in animal reproduction. Bogart, *et al.* (11), and Asdell, Bogart, and Sperling (1) report that pregnancy in rats is a definite stimulus to growth. In animals that breed normally, growth was significantly greater than in virgins. In a recent communication in Science (28), stimulation of wound healing and regeneration of tissue is reported by the

use of an animal embryo extract taken from guinea pigs. The period of puberty in man is correlated with a marked increase in height and weight, as has been shown in the human growth curve (19) (21). That synapsis and syngamy may also accelerate growth in animals as well as plants by the production of special substances seems highly probable. It would be unwise, however, to carry the analogy too far.

Pollen and Kernel Hormones

Their probable role in plant development.—Observational as well as experimental evidence indicates that the growth stimulating substance(s) obtained from the staminate reproductive organs following reduction division in the male flowers, are quite different in their physiological behavior from those produced in the young kernel.

In corn, pollen and tassel extracts seem to be specific for stimulating cell, stem, and internode elongation. The most rapid increase in height of the corn plant is during tassel development (65) (66). The growth of the flower stalk in spinach, and "bolting" in many other species of plants is correlated with flower bud formation. Internodal elongation in bean seedlings, after the application of corn pollen extract gave two and six-tenths times the extension that

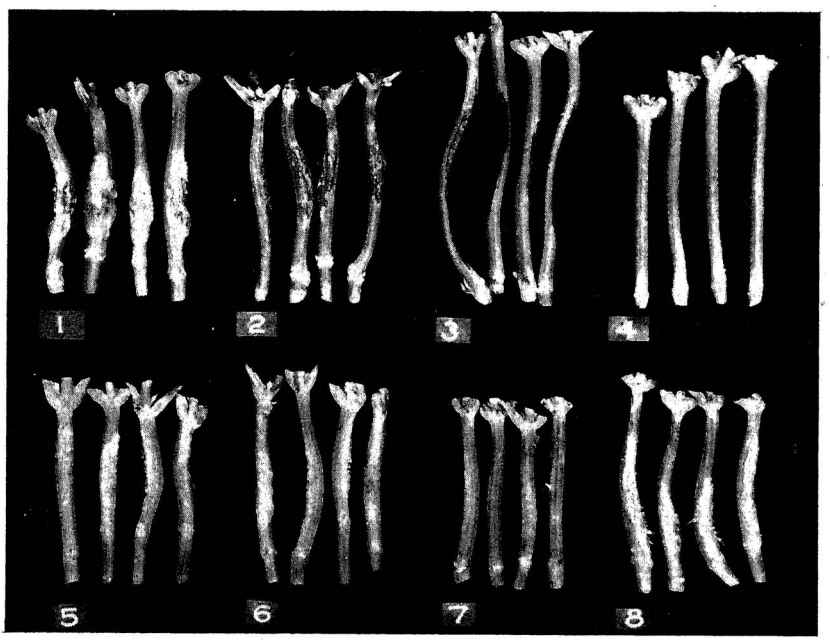

Fig. 11.—Comparative effects from application of corn extracts to bean seedling internodes. (1) alcohol extract of immature grain; (2) ether extract of immature grain; (3) ether extract of pollen; (4) controls, no treatment; (5) indoleacetic acid, 1%; (6) indoleacetic acid, 0.5%; (7) indoleacetic acid, 0.1%; (8) indolebutyric acid, 0.5%.

resulted from applying 0.002 per cent indoleacetic acid (81). Comparing pollen with kernel extracts and heteroauxin, the author also demonstrated an elongation due to the pollen to be at least twice that obtained from either of the others.

The crude residue extracted from the immature corn grain appears specific for fruit setting and fruit development. (As has been mentioned, it was effective in seedless fruit production only from grains gathered during a very limited period following fertilization). There is a complete absence of any stimulating effect on tomato ovaries when the active material in pollen is applied. The kernel extract is not capable of inducing the degree of elongation on bean seedlings that pollen is, but instead produces marked stem proliferation and aerial rooting. The effect of the active fraction in pollen is one of elongation even at the highest concentration used. (Figure 11.)

It would logically seem that the function of the pollen growth hormone, in so far as sexual reproduction is concerned, is one of stimulating pollen tube growth. Especially would this be true in corn where pollen tubes of extraordinary length are usually developed. In contrast, the function of the growth factor produced in young kernels would reasonably be one of accelerating seed and fruit development.

The above postulations are just as applicable when one considers plant development in general. During flower bud formation, the greatest increase in vegetative extension generally occurs. In the post-floral and fruiting stage, plant growth is not characterized so much by increase in length and height as by gain in thickness or enlargement of parts already present, and by increased synthesis of organic matter (food supply).

Effects in fruit setting.—Since Gustafson's report (34) on the inducement of parthenocarpy in fruit by growth promoting chemicals, the close relationship between fruit development and growth substances, which have come to include both synthetic and naturally occurring compounds, has been recognized. Generally speaking, synthetic growth materials have been found equal to, if not surpassing, normal pollination for fruit setting in the tomato and other plants. The per cent set and size of the seedless fruit obtained by the use of such compounds are at least equal to the normal seeded ones (39) (42) (53). A chemical stimulation by the same synthetic substances of ovules in *Datura* has been demonstrated by Overbeek, Conklin, and Blakeslee (100), while Gustafson (43) has indicated that a correlation exists between fruitfulness and the auxin content in ovaries of the sour cherry.

Certain extracts of pollen have been reported effective for induction of seedless fruit (35) (128). In these cases, however, the results are none too conclusive. Facts seem to indicate that the growth hormones in pollen grains are generally not sufficient to account for the stimulation of the ovaries following fertilization.

That the mere growth of the tubes in the style may result in fruit production (127) (129) cannot be denied, but such examples provide exceptions to the general rule that fertilization is essential for fruit growth. Muir (85), in a study of pollen and pollen tube extracts, concluded that such structures were not the source of growth hormones found in embryos after fertilization.

In complete contrast to pollen, the kernel extract produces a truly remarkable stimulatory effect upon young ovaries. Perhaps it is due to the naturally occurring auxins already found and identified in the corn grain (45) (61). An existing combination of the three known auxins in the kernel may account for its demonstrated superiority to the synthetic substances in fruit development. It may likewise be just as possible that some "auxin" yet unidentified, specific for fruit setting, is produced in the kernel subsequent to fertilization. Logically, at least, it might be the source of such material.

Chemical nature.—Very little is known as to the chemical nature of the active materials in the extracts secured from corn pollen and immature grains. The growth stimulating activity in neither case seems to be destroyed by prolonged heating. Solubilities of the two are quite different. Ether is the only known solvent capable of extracting appreciable quantities of the growth factor from the pollen, whereas alcohol, ether, acetone, and water are all very effective in removing it from the young grains.

Crude extracts of plant parts, as used in this investigation, may contain a variety of factors affecting growth. Stimulating and inhibiting substances seem to be abundantly distributed in the plant kingdom. Recent evidence indicates that there is considerable variability in the biological and chemical properties of auxins obtained from different plants and their parts (17) (55) (70) (71) (99) (116). The presence of plant growth inhibitors has also been demonstrated (32) (73) (110). Auxins in divers tissues of various plants do not respond alike to the same extraction methods (6) (7) (41) (55) (72) (115) (116). A difference in the physiological effect of auxin a and heteroauxin is noted by Overbeek (99). Cholodny (17) observed a dissimilarity in the action of indoleacetic acid and a water extract of corn endosperms on the development of oat seedlings.

Concentration.—The concentration of the growth "hormone" in the developing embryos is exceedingly high. In a typical extraction procedure, using ninety-five per cent ethanol, approximately six grams of the alcohol soluble residue were obtained from one hundred grams of the fresh corn. To this crude residue may be added thirty grams of lanolin. The resulting mixture is still effective in causing proliferation and callus formation in bean seedling internodes. What is perhaps more important is that this diluted extract will induce parthenocarpic fruit development in the tomato. Such material is

equal in its physiological effects on tomato ovaries to at least a concentration of 0.05 - 0.1 per cent indolebutyric acid in lanolin. This was found to be the lower range of effectiveness of this synthetic material for the induction of seedless tomato fruit, which is approximately a concentration of growth hormone in the immature corn grain equivalent to 0.015 - 0.03 per cent of the fresh weight, using indolebutyric acid as a standard. These values represent, with two possible exceptions (8) (73), the highest amount of growth hormones known to occur in plant tissues.

In so far as the writer is aware, this investigation presents the first instance in which crude plant extracts have been used with a large measure of success for the induction of parthenocarpic fruit development in horticultural plants. A possible value of the corn kernel extract for the improvement of fruit set, size, and yield on a practical basis would suggest itself, in view of the experimental results obtained.

SUMMARY

Two periodic stimulations in growth are associated with sexual reproduction in higher plants. They have their origins in the process of fertiliation, with its two cytologically important phases, union of chromosomes (synapsis) and union of nuclei (syngamy). The first is initiated within the partially developed flower bud concomitant with chromosome conjugation in the micro- and macrospore mother cells. The second occurs during gametic union in the embryo sac at the approximate time of fertilization. Their existence and operation have been disclosed by the following experimental and observational evidence.

1. Removal of reproductive organs (disbudding, deflowering, defruiting) at different stages of development, has demonstrated that a period of renewed growth, which arises, presumably, from increased metabolic efficiency of the roots and leaves, follows each of the above two crucial phases of fertilization. These intervals of accelerated activity in the plant are made manifest by increases in the vegetative extension, the production of fresh and dry matter, the absorption of soil nutrients, and the accumulation of photosynthetic products.

2. A rise in elongation increments and a change in movements of the flower stalk (pedicel) characterize the post-synaptic and post-syngamic periods in developing inflorescences. Generally two spurts in growth and alterations in movement occur, the one during bud development, the other following pollination. The two growth maxima are usually separated by a minimum, which occurs at full bloom.

3. Catalase activity in developing flowers and accessory reproductive tissue of the pear tree and corn plant exhibited two prom-

inent peaks. The first increase followed synapsis in the microspore mother cells, the second occurred after nuclear fusion in the embryo sac.

4. By alcohol extraction and bean seedling internode assay, an estimation of growth hormones in the reproductive organs of the corn plant during its development, disclosed two maxima in their accumulation. Following the initiation of chromosome conjugation in the staminate and fertilization in the pistillate flowers, substances possessing marked growth stimulating properties were produced, the greatest concentrations being attained within the tassels ten days to two weeks after the beginning of synapses, and in the immature kernels an equal period subsequent to syngamy. The pre-synaptic stage in the male and the pre-syngamic period in the female inflorescences were distinguished by a complete absence of growth substances in the respective reproductive organs.

It was revealed that the two maxima representing hormone accumulation correspond quite precisely, (a) with the peaks in catalase activity and (b) with the spurts in growth following gametophyte and embryo inception.

5. Crude extracts of the immature corn kernels, applied in lanolin paste, were active in fruit setting and parthenocarpic fruit induction in the tomato. The percentage set, yield, and size of fruit were superior to that obtained by means of normal pollination and by the application of synthetic growth substances to flowers not pollinated.

6. A critical examination and new interpretation of data published in germane investigations revealed a striking correlation between (a) periods of greatest vegetative extension, accumulation of dry matter, absorption of soil nutrients, and carbon dioxide utilization, and (b) peaks in the production of growth stimulating hormones in the reproductive organs. These intervals of maximal increase followed closely in all cases the beginning of synapses in the flower bud and embryo initiation in the young fruit.

Brief suggestions are made as to the different roles the growth substances found in pollen and immature fruit may play in plant development.

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