THE DEVELOPMENT OF THE EMBRYO, ENDOSPERM, AND PERICARP OF THE PEACH (PRUNUS PERSICA, SIEB. ZUCC.) AS RELATED TO FRUIT THINNING WITH PLANT REGULATORS

By

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AN ABSTRACT

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Approved Arthur E. Mitchell

A series of studies were conducted during the period of years from 1955 through 1958 to determine the time of application and the value of naphthaleneacetic acid (NAA), N-1-naphthylphtalamic acid (NPA), and 3-chloro-isopropyl-N-phenyl carbamate (3-chloro IPC) for blossom and fruit thinning of peaches.

Applications of NPA during 1955 through on Redhaven and Halehaven peach trees at 200, 250, 300 and 400 ppm three days after full bloom did not result in sufficient thinning to eliminate hand-thinning, except for the results from the applications of NPA at 300 ppm on Redhaven trees in 1956 and 1958. The use of 3-chloro IPC at 400 ppm on Redhaven and Halehaven trees four weeks after bloom in 1956 and 1957 resulted in hastening fruit ripening and in unfavorable "beaked" peaches, but did not thin sufficiently to eliminate hand-thinning. NAA at 30 ppm applied 42 days after bloom, two weeks after "shuck-off", in 1957 and 1958 resulted in significant fruit thinning on Redhaven and Halehaven trees. An application of NAA on Redhaven 35 days after bloom at 30 ppm resulted in significant thinning, whereas an application of NAA 49 days after bloom resulted in no apparent thinning.

An application of NAA at 30 ppm 42 days after bloom on Redhaven and Halehaven trees in 1957 significantly reduced the number of flower buds in the spring of 1958.

A study was initiated in 1958 to compare the natural drop of blossoms and young fruits from Redhaven peach trees with the drop resulting from the use of NAA and NPA as thinning agents.

The daily blossom drop of the control trees was heaviest during a seven day period one week after bloom. Most of the drop from the Redhaven peach trees, which received an application of NPA at 300 ppm three days after bloom, took place during this same period. The "June" drop of the control trees appeared in two periods of heavy fruit drop, the first period occurred approximately 35 days after bloom and remained constant for 14 days. The second period of "June" drop occurred about 49 days after bloom and lasted for 11 days. An application of NAA at 30 ppm 42 days after bloom appeared to delay the second period of "June" drop by three days.

Anatomical investigations made in 1958 on Redhaven peaches revealed that syngamy took place between seven and 14 days after bloom. The peach embryo of both the Halehaven in 1957 and 1958 and Redhaven in 1958 continued in the filamentous stage, linear in shape, for a 28-day period. This form developed immediately after the first division of the zygote. The embryo became bulbous in shape, the spherical stage, approximately 42 days after bloom and continued for about seven days. Rapid growth of the peach embryo was observed about 49 days after bloom during the transitional stage, when the

embryo changed to a triangle shape. Cellular formation of the endosperm occurred about 42 days after bloom. The seed and pericarp of the peach continued to grow at a rapid rate for a period of 49 days after bloom when a sharp decline in growth occurred.

Diethyl ether extracts from peach seeds collected from Redhaven trees from June 2, 1958, four weeks after bloom and at week intervals thereafter for a period of four weeks, revealed that the natural hormone content of the acid and neutral fractions reached a maximum in quantity six weeks after bloom. From various qualitative tests, it appeared that the natural hormone present in the acid fraction was not IAA, however, there was a strong indication that the natural hormone present in the neutral fraction was EtIA.

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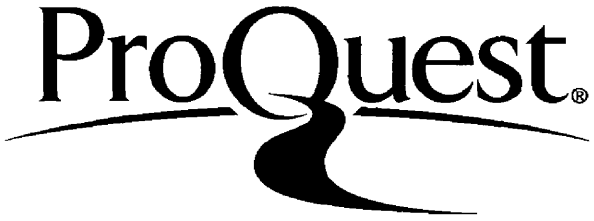

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INTRODUCTION

Blossom and fruit thinning is a necessity in commercial peach growing, as over productivity is common for the peach tree. Since this method of thinning presents a means of eliminating an expensive procedure of removing part of the blossoms and young fruit with hand labor, many investigations of chemical thinning of peaches have been stimulated. Thus far the results of these studies have been contradictory primarily because of the erratic response of peach trees to the chemical thinning treatments.

The plant regulator, naphthaleneacetic acid (NAA), used successfully for thinning apples when applied as a foliar spray during and shortly after bloom, was found to result in effective thinning of peaches at low concentrations when it was applied 30 to 42 days after bloom (33, 44). Certain investigators have attributed its failure to reduce the fruit set of peaches when applied shortly after bloom to lack of absorption of the thinning agent because of the limited leaf surface of the trees (6). Murneek (74) believed that the peach embryo which plays a functional role in thinning, was resistant to inhibition by NAA during its inactive stage immediately after fertilization.

The induction of embryo abortion appeared to Murneek and Teubner (76) and Luckwill (62) as the mechanism action in which thinning of apples was accomplished with post-bloom applications of NAA. The embryo of the apple

is in a very elementary stage of development, 8- to 16-celled, two weeks after full bloom. This has been found to be one of the most sensitive periods for thinning apples with NAA (96). Tukey and Young (102) stated that the early growth and development of the peach embryo appeared to take place more slowly than that of the apple. This could account for the ineffectiveness of NAA as a thinning agent for peaches when applied close to bloom.

If the concept of embryo abortion is entirely accountable as a cause of fruit thinning, the early development of the peach embryo should be more closely investigated in an attempt to recognize what may account for the late period of sensitivity of peaches to NAA as a thinning agent in contrast to the early period of sensitivity for the apple.

Another plant regulator, N-1-naphthylphthalamic acid (NPA) has been found to effectively reduce blossom set of peach trees, if applied within a seven-day period immediately after full bloom (29). Therefore, the thinning action of NPA appears to be of a different nature than that of NAA. Contrary to NAA, the thinning action of NPA is related to the early development of the peach flower and its component parts.

Because of the varying results reported on chemical thinning of peaches, and the apparent variation in time of application for the different chemicals resulting in effective thinning, a study was undertaken with the following objectives in mind: (1) to determine the time of application,

concentration, and effects of NPA, NAA and 3-chloro IPC for effective chemical thinning of peaches, (2) to compare the natural drop of peach blossoms and fruits to the drops from NPA and NAA treated trees to determine the reason for the drop, (3) to relate the development of the embryo, endosperm, and pericarp of the peach with the time of application of the plant regulators, NPA and NAA, for more effective thinning, and (4) to determine the relative concentration and content of natural hormones occurring in peach seeds for the period four weeks after bloom through "June" drop.

LITERATURE REVIEW

Chemical Thinning of Fruit

Chemical thinning as related to tree fruits results in the removal of a portion of the flowers or fruits by the application of a chemical during or after bloom to increase the size of the fruit at harvest time and to reduce annual bearing. The materials used can be divided into two classes, "caustic" chemicals and plant regulator compounds.

Working with a dinitro spray as a "caustic" material in 1933, Auchter and Roberts (3) found it possible to reduce the blossom set of apple trees without undue injury to the tree. Watson (105) showed that the dinitro compounds reduce set by injuring the style of the pistil preventing possible fertilization. Since the report of Auchter and Roberts (3, 4), many papers have been presented on the use of caustic chemicals for blossom thinning of tree fruits and Batjer and Hoffman (6) have made an excellent review of these presentations.

Because of the required critical timing of dinitro sprays and the erratic results from their use, considerable attention has been focused on plant regulator materials for thinning fruit.

In 1941 Burkholder and McCown (15) reported that naphthaleneacetic acid (NAA) would reduce fruit set when applied as a spray to apple trees in full bloom. This was substantiated shortly afterward by Schneider and Enzie (84, 85), and Greene (31).

Development of post bloom thinning began with a report by Davidson et al. in 1945 (22). Stebbins, Neal and Gardner (91) confirmed these results using naphthaleneacetic acid (NAA) effectively from petal-fall to two weeks after petal-fall.

A number of investigations followed which were concerned with the time of application and concentration of NAA for the various apple varieties (1, 6, 23, 24, 25, 34, 35, 60, 62, 72, 74, 88, 89, 90).

Murneek (74) stated that the range of NAA concentration for fruit thinning of apples in Missouri was between 10 and 30 ppm depending upon the variety and the time of application. Batjer (6) in Washington, and Luckwill (60) in England, found that earlier the applications of NAA increased the effectiveness of any single concentration in post-bloom thinning of apples. Luckwill (62), though, found that the greatest thinning effect obtained from NAA on Crawley Beauty was at the time the endosperm changes from the free-nuclear condition to the cellular stage.

NAA was the first plant growth regulator suggested as a possible thinning agent for peaches. Southwick, Edgerton and Hoffman (87) reported little success in reducing the fruit set on Golden Jubilee, Elberta and Valiant in 1947 with either the sodium salt or the methyl ester of naphthaleneacetic acid at concentrations of 10 to 40 ppm. The applications were made during the period of full bloom to 20 days after bloom. Murneek and Hibbard (75)

also reported unsuccessful thinning of peaches with NAA at the same concentrations and period of application.

Three years later, Hibbard and Murneek (33) found that NAA applied 35 days after full bloom on Elberta at 40 to 60 ppm, significantly reduced fruit set. Their explanation of the significant thinning was that the embryo is resistant to inhibition by NAA during its inactive stage. They implied that the application must be made after the inactive stage to be effective.

Batjer and Hoffman (6) reported favorable peach thinning with 10 to 30 ppm of NAA applied 30 days after full bloom when earlier applications were found to be unsuccessful They attributed the ineffective thinning with NAA when applied shortly after bloom to the limited leaf surface for absorption of the thinning agent.

Kelley (40, 41, 42, 44) also reported favorable peach thinning with NAA when applied two weeks after "shuck-off", about 42 days after full bloom. From experience, he found that the date of the "shuck-off" stage was much better than full bloom for calculating the optimum time of application (44).

A second material, 3-chloro-isopropyl-N-phenyl carbamate (3-chloro IPC) was reported by Marth and Prince in 1953 (66) as an effective thinning agent of peaches when used at concentrations of 200 to 500 ppm 30 and 42 days after full bloom. They also reported that treated fruits matured earlier and were softer than those not treated. And, no foliage injury occurred from the

use of 3-chloro IPC which was not true for naphthaleneacetic acid. Kelley (41) observed effective thinning with 3-chloro IPC applied two weeks after "shuck-off", 42 days after full bloom, at 200 ppm on Elberta and at 400 ppm on Halehaven, but extensive fruit injury resulted. Horsfall and Moore (36) found satisfactory thinning of Halehaven with 3-chloro IPC at 200 to 300 ppm applied 35 to 48 days after full bloom, and of Elberta at 300 ppm applied 38 to 40 days after full bloom. In all cases hand thinning had to supplement the chemical thinning, but no fruit injury was reported.

Recently, Edgerton and Hoffman (29) trying various growth regulating chemicals reported thinning of Redhaven, Halehaven, Elberta and July Elberta with N-1-naphthylphthalamic acid (NPA). This material was found to be most effective when used approximately three days after full bloom. They suggested a concentration range of 150 to 400 ppm depending on the variety. Burkholder and Armstrong (14) using this same material had results varying from no thinning to favorable thinning, to over-thinning. Westwood (106) successfully used NPA at 200 and 300 ppm one week after full bloom on Elberta and Redhaven. Westwood favored the later application in order to eliminate the danger of thinning before a possible late killing frost.

Natural Abscission of Young Fruit

Many investigators have studied the occurrence of natural fruit drop which takes place early in the growing season. This drop appears to be closely

associated with the removal of flowers and young fruit by the application of various thinning chemicals.

Abscission of apple fruits early in the growing season as described by Howlett (37) takes place as two primary "drops". He considered that the first drop usually consisted of "unenlarged" flowers and "enlarged" flowers, while the second drop consisted of only the latter. Murneek (71) pointed out that abscission of young apple fruits consisted of four periodic "waves". The first drop was actually two over-lapping "waves". The first one consisted of abnormal and of unpollinated flowers. This drop also included pollinated flowers when fertilization had failed to take place. The second "wave" consisted of flowers in which the torus had enlarged. The second drop or "June" drop Murneek divided into third and fourth abscission "wave" although the evidence for this division was not too strong. The last two "waves" of fruit abscission, he pointed out, consisted of fruit where fertilization was followed immediately by embryo abortion.

Detjen (26) suggested only two periods of drop following bloom for peach trees. The first drop shortly after bloom consisted primarily of developing pistils, but may contain some fruits with aborted pistils and with "undeveloped" pistils. The "June" drop, the second period, he stated, consisted entirely of fruit with developing embryos. The "June" drop, he described, was much heavier than the first.

Bradbury (7) found three periods of abscissed immature cherry fruit following the initial drop of "undeveloped" pistils. She found some unpollinated flowers dropping as late as two and one-half weeks after bloom. She observed a degeneration of the embryo sac of the abscissing fruits caused by incompatibility of gametes with fruit drop reaching a peak three and one-half weeks after bloom. "Drops" from the third period occurring six and one-half weeks after bloom, consisted of fruits with degenerated embryos and/or endosperms.

The cause of young fruit abscission according to Howlett (37) and Bradbury (7) appears to be nutritional competition. However, Detjen (26) and Detjen and Gray (27) considered genetic factors of greater importance than the environmental one of nutrition. Kraus (46) observed that the earliest drops of immature apples, which fell shortly after the abscission of fertilized flowers, showed slight ovular development, but with evidence of less endosperm and embryo development in those ovules as compared to those in the fruit remaining on the tree. Detjen (26) and Murneek (71) stressed that embryo abortion was primarily responsible for the abscission of the immature fruits of the "June" drop. Bryant (13) making a study of the factors affecting embryos in the McIntosh, stated "The vigor of the apple embryos resulting from effective pollination continued to be manifested in endosperm development."

Studying the Carman variety of peach, Harrold (32) observed that the zygote nucleus divided 12 days after bloom. This was preceded by the first

endosperm nucleus division by two or three days. The endosperm remained in a free-nuclear state until the end of the sixth week. He agreed with Bradbury (7) in observing three periods of fruit drop, the first during a three-week period beginning one to three days before bloom, the second lasting a period of seven days at five weeks after bloom, and the third during a seven-day period about the seventh week after bloom. The drops of the first period consisted mostly of fruits with ovules which degenerated between the megaspore mother cell stage and the early megagametophyte stage. The ovules of other fruits in this group gave evidence that fertilization had taken place. The dropped fruits of the second and third periods were found to have ovules in which the nucellus, endosperm, and embryo lacked normal development, as measured by size. The only structural difference he could observe between the developing fruit and the abscissed fruit was the degeneration of the nucellus in the chalazal region of the abscissed fruit. From this observation he believed that such disorder in the chalazal region would disarrange the vascular system and might cause disintegration of the endosperm and abortion of the embryo because of the failure of food conduction.

Dorsey (28) found that the first division of the endosperm in Elberta peaches occurred about 20 days after full bloom, and in the zygote a week later. The embryo had reached only a six-to eight-celled stage as late as 40 days after full bloom. For the J. H. Hale, a variety in which retarded fruits or

"buttons" are common, he found the development of the embryo and endosperm greatly retarded as compared to the Elberta. He believed that "single fertilization", referring to a single gamete fusion of either the egg or the endosperm nucleus, was the most probable cause of this condition. From this he inferred that embryo and endosperm development are interdependent.

Tukey (100, 101) by artificially destroying cherry and peach embryos at various periods of growth, demonstrated that the embryo has a definite bearing on fruit formation. Destruction of the embryo early in the development of the fruit, during the arrested pericarp development or earlier, resulted in abrupt check in fruit growth, and eventual abscission of the fruit. Destruction of the embryo after this stage did not influence fruit development.

Seed and Fruit Development

Many investigators have studied the development of the embryo and endosperm in relation to the development of the pome and stone fruits. However, there is still information to be derived. Maheshwari (65) stated that after fertilization the zygote remains inactive. The duration of inactivity depends on the species and environmental conditions. The first division of the zygote is followed almost always by the formation of a transverse wall, forming a terminal cell at the interior side of the embryo sac and basal cell at the exterior side. Six principal types of embryos among Dicotyledons have been recognized by Johansen (39) based on whether the terminal cell divides

longitudinally or transversely and on the contribution of the basal cell to the developing embryo. These six embryo types are Piperad, Onagrad, Asterad, Solanad, Cryophyllad and Chenopodiad.

Teubner and Murneek (96) stated that the apple embryo can be closely compared to that of Geum urbanum which has been classified by Maheshwari (65) as an Asterad type. There was a single transverse division in this zygote which was followed by an oblique division in the terminal cell and a second transverse division in the basal cell.

In contrast to the findings of Teubner and Murneek (96), Meyer (67) observed that the distil cell of the four-celled embryo of the McIntosh apple which is formed from a transverse division of the distal cell of the two-celled embryo, produces all the tiers of the embryo proper. He suggested that the apple embryo development was of the Solanad type from Johansen's (39) classification. The embryo of the peach has not been classified as to type.

Meyer (67) studying the early development of the McIntosh embryo from the zygote to the beginning of the cotyledonary stage, proposed three stages based on the broad patterns of meristematic activity. The first, the "filamentous" stage, began with the zygote and continued as long as, according to Meyer, "The embryo grows and divides as a linear object." The "spherical" stage, the second, began when the distal tiers of cells increase in size in three dimensions. The third, the "transitional" stage, was marked by a change in

the distribution of growth, giving a triangle appearance to the embryo, and closed when the two cotyledonary primordia protrude from the surface of the embryo.

Maheshwari (65) stated that in most cases the first division of the primary endosperm precedes the first zygote division. Three types of endosperm development were recognized by Maheshwari as nuclear, cellular, or helobial.

Pechoutre (81), Osterwalder (79), Tukey (97), and Luckwill (56) have found the endosperm development of the apple and stone fruits to be nuclear. In the nuclear type, the first division and a few of the subsequent divisions do not form walls, but the nuclei may either remain free or in later stages become separated by walls. As Brink and Cooper (10) have an excellent review of embryo and endosperm development in dicotyledonary plants, only the literature relative to the development of the component parts of the peach seed will be discussed here.

Osterwalder (79) reported that the first cell division in the zygote of hand-pollinated apple flowers occurred nine days after pollination. This, he stated, was followed by a period of rapid growth 12 to 16 days after full bloom when the embryo was in the four- to eight-cell stage. Knight (45) confirmed the observations of Osterwalder of the early stages of embryo development.

From Osterwalder's findings (79) the division of the endosperm

nucleus preceded that of the zygote. He showed that one of the first two endosperm nuclei migrated toward the micropylar end of the embryo sac; and, it was from this nucleus that the cellular endosperm was derived. The remaining nucleus formed the large-nuclear endosperm in the chalazal region. Osterwalder (79) described also the isthmus of constriction which divided the embryo sac into two regions. He found that the cell walls of the endosperm were not laid down beyond the isthmus toward the chalazal region and suggested that this was due to the chemical make-up and nutritive function of that region.

Pechoutre (81) apparently made the first observations of embryo development in the Prunus species. Although the details were not definite from a time relationship, he implied that the embryo development of the Prunus species was much slower than the embryo development of the apple.

Tukey (97) found the embryogeny of Prunus avium to be similar to Pyrus malus. He observed that the first division of the sweet cherry zygote occurred about four days after full bloom when it produced a suspensor cell. Subsequence embryo development was very slow until about 24 days after full bloom, when a sudden rapid development of the embryo takes place. The primary endosperm nucleus of the sweet cherry as described by Tukey (97) divided prior to the division of the zygote. Cell walls began to appear on the ninth day after full bloom at the micropylar end of the embryo sac. Cell wall formation continued progressively from the micropylar to the chalazal end

and from the periphery inward until it became a solid mass. He found that the chalazal region of the embryo sac never became multicellular. The rapid development of the cellular endosperm of the sweet cherry commenced about seven days before the rapid development of the embryo.

Tukey (98, 99) proposed three stages of fruit development for Prunus persica and Prunus cerasus. The first stage (Stage I) was characterized by a period of rapid increase of the pericarp following fertilization. Coinciding with this was the rapid development of the nucellus and integuments. For the peach, Stage I continued for about 40 to 50 days during which the growth of the embryo was extremely slow. In Stage II there was a marked decline in the rate of growth of the pericarp and a cessation of growth of the nucellus and integuments. He found that the embryo commenced to grow rapidly at the beginning of Stage II and reached maximal size by the end of Stage II. The endocarp began to harden during this stage. It was suggested by Tukey (97), Harrold (32), and Luckwill (57) that the initiation of this period of rapid embryo development in both the stone fruits and the apple may coincide with the change of the endosperm from a free-nuclear to a cellular state. The beginning of Stage III was coincident with the second rapid increase in rate of growth of the pericarp, and continued until the fruit was mature. Tukey (98) found that in early ripening varieties of peaches Stage III began when the embryo was in a period of rapid growth, which, in turn, sometimes caused embryo abortion.

Other investigators who have emphasized the three definite stages of

growth for the stone fruits are Connors (16), Lilleland (48), Lott (52, 53), Harrold (32), and Lott and Ashley (54) on peaches; Lilleland (46, 50) on apricots; Lilleland and Newsome (51) on cherries; Lilleland (49) on plums; and Brooks (12) on almonds.

Tukey and Young (102) suggested a similar periodicity in the growth of the apple indicating that the carpel of the apple developed similarly to the pericarp of the Prunus fruits. They found that the carpel blades and the nucellus and integuments reached full length early in the development of the fruit, while the embryo remained microscopic for some time after full bloom. However, unlike the embryo of Prunus, the embryo of the apple began rapid development before the nucellus and integuments and the carpel blade have heached maximum length. They believed that there was greater similarity between the development of the embryo, nucellus and integuments, and the carpel of the apple and of the Prunus fruits than their findings revealed.

Mitchell (68) found a close similarity in fruit development between the pear and the drupe fruits. He noted that the enlargement on the entire fruit of the Bartlett pear was very rapid just after full bloom and lasted up to eight weeks when growth declined noticeably. He reported that the nucellus and integuments acquired almost their full length during this early period of rapid fruit growth, while the embryo remained microscopic. Related to the decline of fruit growth eight weeks after full bloom was the rapid increase in length

of the embryo which reached its maximum size about four weeks later. Resumption of rapid growth of the entire pear fruit 10 weeks after full bloom was found to be related to a decline in embryo development.

As reported by Tukey and Young (102) and Mitchell (68), the early development of the cortex of the apple and the enlargement of the carpel, pith, and cortex of the pear, involved an appreciable amount of growth by both cell division and cell enlargement. However, cell division ceased at about the time of rapid embryo development. By contrast, cell division in the fleshy pericarp of the sour cherry (99) and of the cling peach (5) did not cease until about the first half of Stage II, during rapid embryo development. Fruit enlargement of the apple, pear, and stone fruits after the period of rapid embryo growth was brought about almost entirely by cell enlargement.

Suggested Mechanisms for Fruit Thinning with Naphthaleneacetic Acid

Many investigations have been conducted regarding the possible mechanism by which NAA may reduce fruit set. Van Overbeek (103) suggested from indirect evidence that NAA may accelerate abscission of young fruits by accelerating growth in the abscission zone of the pedicel, which functions in the decomposition of the middle lamella. This condition led eventually to the mechanical fracture of the abscission zone causing the fruit to drop.

Recently Luckwill (62) observed that NAA sprayed on open apple flowers before pollination induced incompatibility between pollen tubes and

stylar tissue, thus accounting for thinning action of NAA when applied at this stage. When NAA was applied on apple trees during post bloom, an obvious delay in immature fruit dropping has been observed by four investigators (62, 76, 89, 93). Therefore, one of the effects of thinning sprays of NAA appeared to be a temporary delay of abscission for one or two weeks, which is similar to the effect produced by a preharvest application of NAA to prevent fruit drop (93). Struckmeyer and Roberts (93) proposed a possible mechanism of NAA thinning after recognizing this effect. Their hypothesis considered the plant regulator thinning of fruit as an extension of immature fruit drop, resulting from the nutritional competition caused by the temporarily increased set.

A fourth possible mechanism of fruit thinning using NAA has been proposed by Murneek (71, 73) by an explanation of natural fruit drop involving the functional importance of the embryo as a natural source of hormone to prevent fruit abscission. Murneek (73) found that with NAA thinning sprays on apples, large fruits tended to remain on the tree, whereas small fruits abscissed. He believed that this was due to the presence of larger number of seeds in the larger fruits with the embryos supplying the required amount of hormone to prevent fruit abscission. He mentioned the occurrence of seed abortion in fruits sprayed with NAA. Based on this, he suggested that NAA used at relatively high concentrations for apple and peach thinning might

disturb the embryo and/or endosperm during the critical period of early seed development.

By removing immature fruits from the apple tree, but leaving the pedicel intact with the spur, Murneek and Teubner (76) found that an application of NAA at 30 ppm, immediately after the removal of the fruits, delayed the abscission of the pedicels. In another experiment, after examining abscissed fruit of NAA sprayed trees, they observed embryos which were inhibited in growth as compared with those in seeds of apples which had set and were developing. The two investigators emphasized the control invested in the embryo on the abscission of the pedicel. From these series of experiments, Murneek and Teubner believed that the extent of fruit thinning by NAA was partially dependent upon its temporary positive effect on the abscission layer of the pedicel (30) and its strong negative effect on the hormonal function of the embryo.

Luckwill (61) and Teubner and Murneek (76) have added much support to the hypothesis that NAA induces embryo abortion. The later found that the most susceptible stage during apple embryo development to this induced abortion was when the embryo was in the 8- to 16-celled stage. They believed the addition of an exogenous supply of a plant regulator to the already natural hormone saturated embryo resulted in inhibition of this organ.

Further evidence that the embryo may control fruit abscission as a

mechanism of fruit thinning has been supported by the "somatoplastic sterility" theory. Cooper, Brink and Albrecht (8, 9, 17, 18, 19, 20, 21) found much evidence in support of this theory.

"Somatoplastic sterility" as explained by Brink and Cooper (8) is the hyperplasia of the somatic tissues of the fertilized ovule, such as the nucellus and integuments. Over-growth of the nucellus and integuments occurred as a result of a lag in the normal development of the endosperm, thereby leading to the disintegration of the endosperm. Subsequently, the embryo became disorganized and eventually the complete ovule collapsed.

The condition as found in the alfalfa ovule after self-fertilization first appeared whenconductive tissues between the apex of the vascular bundle and the chalazal pocket fail to differentiate (8). Cytoplasm of the nucellus cells adjacent to the embryo sac became finelly vacuolated and extensive meristematic activity occurred at the chalazal portion of the inner integument during this time. After self-fertilization of the alfalfa, endosperm disorganization developed, first at the chalazal end and then progressed toward the micropyle. Subsequently, the cytoplasm of the cells of the embryo became dense and the embryo collapsed after the complete disintegration of the endosperm.

These phenomena were later found to occur in unfruitful interspecific crosses of Nicotiana (9). Brink and Cooper (9) believed that the over-growth

of the maternal tissue as a fore-runner may, more or less completely obstruct the immediate line of food supply to the endosperm. They emphasized the nutritive role which the endosperm plays in the development of the embryo.

The "somatoplastic sterility" theory has been brought forth also as a reason for natural fruit drop. As stated earlier, Harrold (32) in 1935 believed that immature fruit drop of peaches may be caused by the degeneration of the nucellus in the chalazal region thereby affecting endosperm disintegration and embryo abortion. Dorsey (28) made the same observation in 1939 as described by Brink and Cooper in 1940 (9) when he stated that the growth of the embryo was dependent on endosperm development in normal fruit of the peach variety J. H. Hale.

Britten in 1947 (11) and Teubner and Murneek in 1955 (96) investigated the possibility of a plant regulator function in "somatoplastic sterility". Britten (11) found that a naphthaleneacetic acid treatment on the corn ear at the time of fertilization produced a temporary stimulation of the ovaries for eight to nine days, but lagged behind the normal ovaries in development afterwards. Treated caryopsis as compared to those receiving no treatment were found to consist of endosperm and embryo retarded in development when observed nine days after treatment. The endosperm of treated ovaries at this stage appeared to lack the absorptive layer of cells at the base. Endosperm and embryo disintegration was found to occur in treated caryopsis in

12 to 18 days. Even though a condition approaching "somatoplastic sterility" appeared, Britten believed the resemblance to this condition may have been only superficial.

Studying the inhibition of embryo development by naphthaleneacetic acid, Teubner and Murneek (96) working with the pepper plant observed some degeneration of the endosperm 12 days after treatment. Endosperm and embryo degeneration was present in the oldest inhibited ovules when examined 90 days after treatment. The degenerating embryo sacs at this late stage were found to exhibit a hyperplasia at the chalazal region.

Hormone Production During Seed and Fruit Development

The release of natural hormones by the physiological processes associated with fertilization, appears to play an important role in the growth and development of the fruit.

Murneek (70) suggested that the action of gamete union may be a second source of stimulation to the development of the fruit and seed. Wittwer (107) working with corn found two periodic stimulations in growth associated with the process of fertilization, one occurred at the time of the synapsis phase, and the other at the time of the syngamy phase. Two major peaks of hormone accumulation in the corn caryopsis followed these two phases. Redemann, Wittwer and Sell (81) have since identified theis hormone found in corn kernels as ethyl indoleacetate.

In 1942, Muir (69) working with tobacco, Nicotiana tobacuum, found large quantities of the hormone in pollinated pistils, but very little in non-pollinated pistils. He observed no hormones in pollen grains or pollen tubes, but he believed the pollen tubes may have released an enzyme which activated a hormone from inactive compounds in the style and pistil. Nitsch (77) studying the production of natural hormones during the growth of the pollen tube down the style of the strawberry flower also indicated that these natural hormones may have accounted for the initial stimulation of fruit development and temporary prevention of abscission.

In 1946, Luckwill (55) isolated a substance from the apple endosperm which he found to be active in setting parthenocarpic tomato fruit. Luckwill and Woodcock (64) could only partially identify this substance, but maintained that the apple hormone was not indoleacetic acid. Teubner (94) apparently isolated the same substance from apple seeds and identified the compound as ethyl indoleacetate.

Luckwill (56) used the capacity of the extracted substance to set parthenocarpic tomato fruit for a quantitative measurement of the natural hormone present in the apple seeds. He was able to correlate the periods of high hormone content of apple seeds with corresponding periods of reduced fruit drop using the apple variety Beauty of Bath (57). In further studies he found the peaks of hormone accumulation to correlate with the cessation of

the first drop and the "June" drop (59). The hormone content of the seeds was found to be lowest just prior to the first drop, the "June" drop, and pre-harvest drop. These peaks of hormone accumulation were very similar for the apple varieties, Lane's Prince Albert (59) and Crawley Beauty (61).

Luckwill (57, 58) found a close correlation between the time of cellular formation of the endosperm and the appearance of high hormone content in the apple seeds of Beauty of Bath. He suggested that the endosperm itself might be the location of the hormone production. By separating the embryo from the endosperm and nucellus he found that the concentration in the outer layers, the endosperm and nucellus, was eighteen times as great as in the embryo. Since the nucellus was almost completely absorbed by the endosperm soon after the time the hormone concentration was measured, it seemed probable to him that the greater concentration was in the endosperm.

Luckwill (57) reported that immediately after the cellular formation of the endosperm of the apple, the embryo entered a period of rapid growth during which the hormone content of the seed decreased to a low level. He suggested as a possible explanation, that the hormone played a part in embryo development during the period of its rapid growth, and thus the hormone was depleted more quickly than was produced by the endosperm.

MATERIALS AND METHODS

I. Field Studies on Chemical Thinning of Peaches using Naphthaleneacetic Acid, N-1-naphthylphthalamic Acid, and 3-chloro IPC.

A series of studies were conducted during the period 1955 through 1958 to determine the value of naphthaleneacetic acid[1] (NAA), N-1-naphthylphthalamic acid[2] (NPA), and 3-chloro-isopyl-N-phenyl carbamate[3] (3-chloro IPC) for blossom and fruit thinning of peaches. The studies were performed at the Bailey farm in the area of Pontiac in 1955 and 1956, at the Michigan State University Horticultural farm, East Lansing in 1956, 1957 and 1958, and at the Michigan State University Graham Experiment Station, Grand Rapids in 1957.

Each treatment included five replicated mature bearing trees of medium vigor, except for certain studies in East Lansing in 1958, when five-year-old early bearing trees were used. Three to four branches were selected in different areas of each tree, and approximately 500 blossoms or fruit per tree were used for thinning observations. Final fruit set counts were made each year the middle of July.

The thinning treatments made are recorded in Table 1.

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[1] App-L-Set, a product of Dow Chemical Company, Midland, Michigan was used for NAA.

[2] Peach-Thin, a product of American Chemical Paint Company, Ambler, Pennsylvania, was used for NPA.

[3] Chloro IPC Miscible, a product of Niagara Chemical Division, Middleport, N. Y., was used for 3-chloro IPC treatments.

Table 1

Thinning Treatments on Redhaven and Halehaven Peach Trees Used in 1955, 1956, 1957 and 1958

Material	Season	Location	Time of application (days after full bloom)	Conc. (ppm)	Comments
NPA	1955	Pontiac	3	200	
NPA	1955	Pontiac	3	400	
NPA	1956	East Lansing	3	300	
NPA	1956	East Lansing	3	300	Twilight application
NPA	1957	Grand Rapids	3	200	
NPA	1957	Grand Rapids	3	250	
NPA	1957	East Lansing	3	300	
NPA	1958	East Lansing	3	250	Halehaven only
NPA	1958	East Lansing	3	300	Halehaven only
NPA	1958	East Lansing	3	250	Five-year-old Redhaven trees
NPA	1958	East Lansing	3	300	Five-year-old trees
3-chloro IPC	1956	East Lansing	28	400	
3-chloro IPC	1957	Grand Rapids	28	400	
NAA	1957	East Lansing	42	30	
NAA	1958	East Lansing	35	30	Redhaven only
NAA	1958	East Lansing	42	30	
NAA	1958	East Lansing	49	30	Redhaven only
NAA	1958	East Lansing	42	30	Five-year-old Halehaven trees

All fruit thinning treatments were applied with a hand gun using 500 pounds pressure. The trees were sprayed to the drip point. All applications were made under fast drying conditions, except for one twilight application of NPA in 1956. This treatment was made at approximately 8:30 p. m. to assimilate slow drying conditions.

Firmness of the fruit was evaluated in 1956 and 1957 at the beginning and during harvest. The readings were made at four locations on the fruit, on the dorsal and ventral sutures, and in the middle of the two cheeks. The pressures were taken with a Magness-Taylor pressure tester equipped with a 7/16-inch plunger, as suggested by Rood (83). The size of the harvested fruit was recorded only in 1956 using standard fruit sizing rings.

Studies were conducted in 1958 using plots established during 1957. The plant regulator thinning treatments applied in 1957 at the Michigan State University Horticultural farm, East Lansing were used to evaluate the influence of NPA and NAA on the number of Redhaven and Halehaven flower buds the following year. Flower buds were counted in April 1958 on six unbranched terminals, two terminals 2 to 5 1/2 inches in length, two terminals 5 1/2 to 9 1/2 inches in length, and two terminals 9 1/2 to 15 inches in length, selected on each tree. This conformed with the technique used by Kelley of Illinois in 1955 (43).

II. Field Studies Comparing Natural Blossom and Fruit Drop with that Resulting from the Use of Naphthaleneacetic Acid and N-1-naphthylphthalamic Acid.

In order to observe the pattern of fruit drop, a study was initiated in 1958 at the Michigan State University Horticultural farm, East Lansing to compare the natural drop of blossoms and young peach fruits with the drop resulting from the use of NAA and NPA for blossom and fruit thinning. Twenty uniform mature bearing Redhaven trees were selected for this investigation to provide five trees per treatment, of which five trees were treated and five were used as a control. A minimum of three branches were selected in different areas of each tree and approximately 500 blossoms per tree were observed at specific periods to evaluate fruit development and drop.

An application of 30 ppm of naphthaleneacetic acid (NAA) was made on five of the ten selected trees two weeks after "shuck-off", June 14, when conditions were favorable for fast drying. The blossoms and developing fruits on the selected branches of all ten trees were counted at weekly intervals from bloom (May 3) to two weeks after "shuck-off" (June 14). After this time dropped fruits of the NAA treatment and the control treatment from the selected branches of all ten trees were counted twice weekly until immature fruit drop was completed by July 4. An additional count was made the middle of July to check for possible additional fruit drop occurring after July 4. It should be noted that the ten Redhaven trees received uniform

insecticide and fungicide applications during this period of observation.

Each time drop was counted the number of blossoms and fruit remaining on each branch was recorded. The drop per 100 blossoms or fruits for each branch at each time interval was found by dividing the number of blossoms or fruits hanging at the time of previous counting date into the number that had dropped during this interval (94). The daily drop per 100 blossoms or fruits was then computed for each interval by dividing by the number of days between the record dates.

In a similar study counts were made on tagged branches of NPA treated and control Redhaven trees at bloom and at 14 days after bloom in order to determine the approximate time the blossoms or fruit dropped. Final counts were made July 12 on these same trees.

The peach blossoms which fell during the 14-day period after bloom were examined macro- and microscopically. For the microscope study the ovules of the peach blossoms which dropped were killed and fixed in 50 percent FAA (38) for 24 hours. The ovules were rinsed three times in 50 percent ethyl alcohol for 30 minutes each, frozen in a 10 percent gelatin medium, and then cut into sections of 20 microns in thickness with a Model 880 American Optical Company freezing microtome. The sections were placed on a glass slide and stained three minutes with Delafield's Hematoxylin. After the stain was removed from the slide, the sections were mounted in glycerin on microscope slides and cover slips were placed on them. The sections were then studied under a Zeiss Nr. 250190 microscope.

III. Anatomical Investigations of the Developing Embryo and Endosperm of Halehaven and Redhaven Peach.

Flowers and fruit were gathered from a single mature bearing Halehaven peach tree in 1957 and from a single mature bearing Redhaven and Halehaven peach tree in 1958 to study the developing embryo and endosperm of young peach fruits. The trees located on the Michigan State University Horticultural farm, East Lansing, were representative of those treated with NAA. They received the same insecticide and fungicide applications as those of the NAA treatments.

At each time of sampling, fruits were taken from one branch in order to localize the thinning effect to only that branch on the tree. Blossom and/or fruit collections were made twice weekly on the Halehaven tree during 1957 from four days after bloom (May 6) to eight weeks after bloom (June 28). In 1958, blossom and fruit collections were made on the Redhaven tree at full bloom (May 3); the fourth day after full bloom; one week after full bloom; and at weekly intervals thereafter until five weeks after full bloom (June 7), after which time the frequency of collections was increased to twice weekly through the eighth week after bloom (June 28). Halehaven fruits were collected twice weekly beginning five weeks after bloom (June 7) and ending the eighth week after bloom. A final fruit collection of Redhaven and Halehaven was made nine weeks after bloom, 1958, for macro-measurements.

Approximately 25 persisting blossoms and/or fruit were collected

at each sampling from each variety. The ovaries were dissected through the ventral and dorsal sutures with a razor blade keeping the seeds[1] intact. Five of the 25 fruits in 1957 and ten of the 25 fruits in 1958 collected during the period of two weeks after full bloom to eight weeks after bloom which appeared to have only one viable seed were used for growth measurements. All Redhaven blossoms and fruits collected from the time of full bloom to two weeks after bloom contained two viable ovules[1].

Measurements taken were as follows:

Pericarp length: distance between the pedicel attachment and the line of demarkation where the style arises from the ovary.

Pericarp transverse diameter: greatest cross-sectional diameter of the fruit.

Seed length: longest longitudinal distance of the seed.

Seed width: greatest cross-sectional diameter of the seed.

Measurements were made with a metric ruler to the nearest 0.5 millimeter. Length of the pericarp and seed was used in order to measure the increments of growth (98). Width was taken only to substantiate the growth increments.

The seeds used for measurements were killed and fixed in 70 percent FAA[2] and then dehydrated, using the tertiary butyl alcohol method described by Johansen (38). The material was then embedded in Fisher tissuemat with

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[1] In this study the term "ovule" was used before syngamy and the term "seed" afterward.

[2] A solution of 5 milliliters of 37 to 40 percent formaldehyde, 5 milliliters of glacial acetic acid and 90 milliliters of 70 percent ethyl alcohol.

a melting point of 53-55° C. The paraffin embedded material was cut longitudinally into sections 10 to 12 microns in thickness with a Model 820 American Optical Company rotary microtome. The sections were then mounted in series on standard microscope slides, using Haupt's adhesive (38). The paraffin was removed from the sections with xylol and the sections were stained five seconds with fast green (38). The sections were mounted in Clarite and allowed to dry for four days before microscopic examination.

All microscopic examinations were made with a Zeiss Nr. 250190 microscope. A calibrated optical lens was used for all microscopic measurements. Length and width of the embryo, the endosperm, and the embryo sac of five seeds were observed for each time of sampling. The ten fresh seeds from both Halehaven and Redhaven fruits of the final collection, July 5, 1958, were used only for macro-measurements.

In addition to the microscopic examinations of the material embedded in paraffin in 1958 ten fruits were selected daily from each Redhaven and Halehaven tree from June 11 (five and one-half weeks after bloom) to June 20, 1958, to study the development of the endosperm. The seeds of these fruits were removed, killed and fixed in 50 percent FAA (38). After 24 hours the ovules were rinsed thoroughly with three changes of 50 percent ethyl alcohol. Each seed was then cut with a sharp razor blade longitudinally through the micropyle to expose the embryo sac. The embryo sac was removed from

the seed by pulling the nucellus tissue away from the embryo sac with dissecting needles under a dissecting microscope. The embryo sac was placed on a glass microscope slide and stained three minutes in Delafield's hematoxylin. After the excess stain was removed, the material was mounted in glycerol and then examined under a microscope. These examinations were made primarily to determine when the endosperm changed from a free-nuclear condition to a cellular state.

Photomicrographs were taken with an Exakta VX camera using a microscope adapter. The light was obtained from a type 31-33-26 Bausch and Lomb Optical Company microscope light with a ground glass filter (39375) and a blue glass filter (39370) placed between the light source and sub-stage mirror of the microscope. Kodak Plus-X film was used for taking the black and white photo-micrographs.

IV. Natural Hormone Content and Concentration of Young Redhaven Peach Seeds as Related to Early Growth and Development.

Luckwill (57) found that natural hormone content of apple seeds was related to natural fruit drop. On the basis of this finding, a study was initiated to determine the relative concentration and content of the natural hormone in developing peach seeds.

Fruit collections were made June 2, 1958, four weeks after full bloom, and at weekly intervals thereafter for a period of four weeks from five Redhaven trees at the Michigan State University Horticultural farm, East Lansing. Five hundred fruits were collected on each of the first two sampling dates, but only 300 fruits were gathered each time thereafter. Equal numbers of fruits were taken from each of the five trees by stripping individual branches. Fruit with aborted seeds were discarded. The fruits were split with a razor blade and the seeds removed. The seeds were then weighed, with a separate count and weight made for the fruit containing one or two seeds. Following this operation the fresh single and double seeds were placed together in a plastic bag and transferred immediately to storage at -10° C.

When time permitted, the frozen plant material was next extracted with cold, peroxide-free diethyl ether as described by Van Overbeek et al. (104). To carry out this procedure, the seeds were ground in a mortar and pestle with sand added to aid in the grinding. About 50 milliliters of diethyl ether

were added to the mixture during the grinding process. The contents were emptied into a 500-milliliter Erlenmeyer flask and placed in storage at 2° C for eight hours. The supernatant was decanted from the flask. The ground seeds were washed three times with 50 milliliters of diethyl ether and added to the original supernatant so as to extract the remaining natural hormone. This extract was then separated into acid and neutral fractions with 200 milliliters of five percent sodium bicarbonate solution by means of a separatory funnel (96). The neutral substances remained in the ether layer, while the acid substances passed into the sodium bicarbonate layer. The bicarbonate solution was acidified to pH 2.8 with hydrochloric acid and extracted with 200 milliliters of diethyl ether. Both fractions, acidic and neutral, were concentrated to five milliliters under reduced pressure.

Three dilution quantities, 0.01, 0.05 and 0.10 milliliters of each acid and neutral fraction of the diethyl ether extract were placed with a milliliter syringe into individual porcelain boats to which had been added small strips of filter paper. After the ether extracts had evaporated, one milliliter of a phosphate-citric acid buffer solution[1] as used by Nitsch and Nitsch (78) was placed in each boat. Standards of 0.01, 0.10 and 1.00 micrograms of indoleacetic acid (IAA) and ethyl indoleacetate (EtIA) were placed also in the boats preceding the addition of the buffer solution. The control treatment consisted of the buffer solution alone.

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[1]Double concentration of phosphate-citric acid buffer solution is made up of 0.359 grams of K_2HPO_4, 0.204 grams of citric acid, 4.0 grams of sucrose in 100 milliliters of water solution.

The avena straight growth test was used as part of the bioassy study as described by Nitsch and Nitsch (78) and modified by Teubner (96). This method of biological assy was chosen instead of the tomato ovary test as used by Luckwill (55) because of the simplicity of its procedure. The procedure for obtaining oat coleoptiles was as follows: Husked seeds of the Brighton variety of oats were placed in distilled water and evacuated in a suction flask. The seeds were removed from the suction flask and soaked for two hours. The soak water was discarded and the seeds were rinsed twice with distilled water. The seeds were then placed groove side down on a wet paper towel covering a dessicator plate with the visible radicals slightly over the edge of the dessicator plate. The plate with the seeds was placed in a culture dish with sufficient distilled water to keep the paper towel wet. The culture dish with its contents was placed in a dark incubator for three days. The germinating seeds were exposed to two hours of red light 24 hours after they were placed in the dark incubator in order to inhibit elongation of the first internode. After three days in the incubator, the germinating seeds were removed and the coleoptiles were cut in uniform sections of five millimeters, discarding the apical three to four millimeters. The coleoptile sections were then threaded on thin glass rods five centimeters long, three or four to a rod. The leaf inside the coleoptile cylinder was punched out during the threading operation. All cutting and threading operations were done under red light. Three rods with a total of 10 sections were placed in each boat. In turn, the

boats were put into petri dishes and kept in a dark incubator for 24 hours. Measurements of the coleoptile sections following the incubator treatment were made with a ruler to the nearest 0.5 millimeter.

Standard curves showing the rate of growth of the coleoptile sections as influenced by the different concentrations of IAA and EtIA were made on semi-logrithmic graph paper with the concentrations in parts per million charted logrithmically. The quantity of the natural hormones was based on microgram equivalents of IAA for the acid fraction and microgram equivalents of EtIA for the neutral fraction. The hormone content of the seeds was expressed as microgram equivalents per 100 fruits; and hormone concentration in the seeds was expressed as microgram equivalents per gram of fresh weight of the seeds.

Paper chromatography was utilized for a qualitative analysis of the acid and the neutral fractions of the peach seed extract (92). Using a one-milliliter syringe, 0.05 milliliters of the acid and 0.05 milliliters of the neutral extract were spotted on different strips of three-quarter inch Whatman No. 1 filter paper. Both fractions of the extract were partitioned by a solvent mixture composed of 2-propanol, ammonia, water as a ratio of 8:1:1 volume to volume (92).

The dried chromatograms with the partitioned extracts were scanned for ultraviolet fluorescent spots with a 2537 Angstrom "Mineralight". The dried chromatographed paper was then cut into small sections and bioassayed by the avena straight-growth method previously mentioned. Ehrlicher's spray reagent was applied to similar treated chromatograms for further qualitative analysis as described by Sen and Leopold (86).

RESULTS

I. Field Studies on Chemical Thinning of Peaches Using N-1-naphthylphthalamic, Naphthaleneacetic Acid, and 3-chloro IPC.

The findings from the use of N-1-naphthylphthalamic acid (NPA), naphthaleneacetic acid (NAA), and 3-chloro IPC as thinning agents for Redhaven and Halehaven peaches under field conditions are given in Tables 2 and 3.

In 1955 fruit set on Redhaven and Halehaven trees in the Pontiac area, sprayed with NPA at 200 and 400 ppm, was not significantly different from the control treatments (Table 2). However, NPA applied at 300 ppm in 1956 in East Lansing on the same varieties resulted in significant thinning. There was no significant difference between day and twilight applications of NPA made in 1956, even though it had been suggested that slow drying conditions would make NPA more effective. Because of the over thinning at 300 ppm in 1956, NPA was used at 200, 250 and 300 ppm in 1957. No significant thinning occurred at the lower concentrations in 1957 (Table 2) and even though there was significant thinning at 300 ppm, the fruit on both the Redhaven and Halehaven treated trees had to be hand thinned.

In 1958, significant reduction of fruit set occurred when 300 ppm of NPA was applied to five-year-old Redhaven and Halehaven trees and when 250 ppm of NPA was applied to five-year-old Redhaven trees (Table 2). However,

Table 2

Fruit Set Resulting from the Use of N-1-Naphthylphthalamic Acid (NPA) as a Chemical Thinning Agent on Redhaven and Halehaven Peach Trees

Material	Season	Location	Time of Application (days after full bloom)	Conc. (ppm)	Redhaven (Set per 100 blossoms)	Halehaven (Set per 100 blossoms)
NPA	1955	Pontiac	3	200	6.8	20.8
NPA	1955	Pontiac	3	400	5.1	13.7
Control	1955	Pontiac			12.9	18.7
NPA	1956	East Lansing	3	300 (day)	3.3**	8.3**
NPA	1956	East Lansing	3	300 (night)	4.2**	7.1**
Control	1956	East Lansing			20.4	37.6
NPA	1957	Grand Rapids	3	200	13.3	23.7
NPA	1957	Grand Rapids	3	250	12.2	26.0
Control	1957	Grand Rapids			17.0	26.7
NPA	1957	East Lansing	3	300	31.8**	41.6**
Control	1957	East Lansing			46.0	59.9
NPA	1958	East Lansing	3	250		34.1
NPA	1958	East Lansing	3	300		31.1
Control	1958	East Lansing				33.1
					(five year-old trees)	
NPA	1958	East Lansing	3	250	16.0**	
NPA	1958	East Lansing	3	300	6.9**	29.3*
Control	1958	East Lansing			41.8	39.9

*Significantly less fruit set than the control treatment at 5 percent level.

**Significantly less fruit set than the control treatment at 1 percent level.

Table 3

Fruit Set Resulting from the Use of 3-Chloro IPC and Naphthaleneacetic Acid (NAA) as Thinning Agents on Redhaven and Halehaven Peach Trees

Material	Season	Location	Time of Application (days after full bloom)	Conc. (ppm)	Redhaven (Set per 100 blossoms)	Halehaven (Set per 100 blossoms)
3-chloro IPC	1956	East Lansing	28	400	15.5*	19.4**
Control	1956	East Lansing			25.4	37.6
3-chloro IPC	1957	Grand Rapids	28	400	18.6	22.8**
Control	1957	Grand Rapids			29.7	43.6
NAA	1957	East Lansing	42	30	27.4**	63.2**
Control	1957	East Lansing			63.3	82.4
NAA	1958	East Lansing	35	30	41.4*	
NAA	1958	East Lansing	42	30	42.4*	25.5*
NAA	1958	East Lansing	49	30	51.0	
Control	1958	East Lansing			56.3 (five-year-old trees)	41.9
NAA	1958	East Lansing	42	30		50.9
Control	1958	East Lansing				48.3

*Significantly less fruit set than the control treatment at 5 percent level.

**Significantly less fruit set than the control treatment at 1 percent level.

no significant thinning was realized in 1958 on 10-year-old Halehaven trees treated with NPA at 250 ppm and 300 ppm. In 1958 the heavy fruit drop of the five-year-old Redhaven and Halehaven trees occurred during the period May 10 to 17, seven to fourteen days after bloom. This was in accord with Edgerton and Hoffman (29) who reported that the heavy drop from NPA treated peach trees occurred about 10 days after bloom.

The reduction in fruit set on Redhaven and Halehaven trees treated with 3-chloro IPC at 400 ppm in 1956 was significant, but when used at 400 ppm in 1957, significant thinning occurred only on Halehaven trees at the Graham Experiment Station, Grand Rapids (Table 3). However, in both 1956 and 1957, all Redhaven and Halehaven trees receiving 3-chloro IPC thinning treatments produced undesirable "beaked shaped" peaches (Figure 1). Not only were the peaches misshapened, but the "beaked" portion became soft when the peaches reached the firm-ripe stage making them easy to bruise, a condition very favorable for the development of brown rot. Because of the undesirable effect from 3-chloro IPC on peaches, the use of the plant regulator for thinning peach trees was terminated in 1957.

The use of NAA 42 days after bloom at 30 ppm on Redhaven and Halehaven trees in 1957 and 1958 gave significant fruit thinning (Table 3 and Figures 2 and 3) except for the five-year-old Halehaven trees. Also, the use of NAA in 1958 on five-year-old and older Redhaven trees, at the same concentration,

Figure 1

Injury on Redhaven peaches from 3-chloro IPC.

Top row: uneven development of the fruit

Middle row: undesirable enlargement of base of the style, "beaked fruit"

Bottom row: desired shape for mature Redhaven peaches.

35 days after bloom resulted in significant thinning. However, when NAA was applied 49 days after bloom, on Redhaven, no significant thinning was accomplished (Table 3). Even when thinning was significant from the use of NAA in 1958, light hand thinning was necessary. Injury to the terminal growth of the Redhaven trees from the application of NAA was apparent, but appeared to have little commercial significance. Foliage injury on Halehaven trees was not as severe as on Redhaven.

Fruit Firmness: As firmness of flesh of peaches has been found to be a favorable index for maturity (83), data of firmness of the fruit from peach trees treated with NPA, NAA and 3-chloro IPC as thinning agents were recorded at harvest time in 1956 and 1957. These data are summarized in Table 4.

Redhaven fruits from trees of the NPA treatments made in East Lansing in 1956 reached maturity, as measured by firmness of flesh, approximately three days earlier than those from the control trees. It was conjectured that this earlier maturity was related to the light fruit set on the treated trees rather than to the NPA treatment. In 1957 there were no significant differences in firmness of fruit treated with NPA and those receiving no treatment (Table 4). Since fruit set was not reduced by the use of NPA at 200 or at 250 ppm in 1957, it seemed apparent that the early ripening of the fruit on the NPA treated trees in 1956 was not an influence of the chemical, but the result of a light set of fruit on the trees at harvest time (Table 2).

Table 4

Firmness of Redhaven and Halehaven Peaches at Harvest as Affected by Various Plant Regulator Thinning Sprays in 1956 and 1957

Treatment	Conc. (ppm)	Redhaven			Halehaven			
		Aug. 11	Aug. 14	Aug. 17	Aug. 28	Aug. 30	Sept. 4	Sept. 11
		Fruit firmness (lbs. per sq. in.)						
1956								
East Lansing								
NPA	300		16.0**	1.2**			7.7**	0.9*
3-chloro IPC	400		10.9**	2.2**			6.3**	2.2
Control			26.1	18.7			20.4	3.2
1957								
East Lansing								
NAA	30	25.4				16.3		
NPA	300	22.9				17.9		
Control		24.1				15.8		
Grand Rapids								
NPA	200		20.3		17.4			
NPA	250		20.5		17.6			
3-chloro IPC	400		16.3**		7.1**			
Control			21.1		12.5			

*Significantly less fruit firmness than control at 5 percent level.

**Significantly less fruit firmness than control at 1 percent level.

Redhaven fruit on trees treated with 3-chloro IPC were less firm in both 1956 and 1957 than those of the control treatment (Table 4).

The use of NAA as a thinning agent in 1957 had no apparent influence on the rate of ripening of Redhaven fruit (Table 4).

As found for the Redhaven in 1956, Halehaven fruits on NPA treated trees matured a few days earlier than the untreated fruit when evaluated by firmness of flesh. Here, also, early maturity appeared to be the result of reduced fruit set on the NPA treated trees, rather than to a direct ripening influence of the NPA compound, as there were no differences in firmness of flesh between the NPA and control treatment in 1957 (Table 4).

The use of 3-chloro IPC on Halehaven as a thinning agent in 1956 and 1957 resulted in earlier ripening of the fruit when compared with the control treatment (Table 4). Possibly that the 3-chloro IPC compound was responsible for the earlier ripening of the treated fruit of both Redhaven and Halehaven as the reduction of fruit set was not excessive (Table 3).

Fruit Size: The size range of 100 peaches picked at harvest time from the trees of the different thinning treatments in East Lansing in 1956 are given in Table 5.

As expected, the degree of thinning resulting from the chemical treatments directly influenced the size of the harvested peaches. The fruit of Redhaven and Halehaven from the NPA treatments were the largest, and these

Figure 2

Typical branches of a Redhaven peach tree sprayed with naphthalene-acetic acid at 30 ppm 42 days after bloom. Picture taken before the occurrence of fruit drop. Note the size of the aborted fruits.

Figure 3

Typical branches of a Redhaven peach tree sprayed with naphthalene-acetic acid at 30 ppm 42 days after bloom. Picture after the occurrence of fruit drop.

trees were thinned the heaviest. The Redhaven peaches from the 3-chloro IPC treatments were intermediate in size, and the peaches from the Halehaven trees treated with 3-chloro IPC and those from the untreated trees were the smallest.

The Effect of NAA and NPA on Flower Buds: Since terminal injury to the Redhaven and Halehaven trees from the applications of NAA in 1957 was very apparent, an evaluation of the effect that NAA and NPA may have on the number of flower buds was made in the spring of 1958. The effect of NPA and NAA on flower bud formation when used on Redhaven and Halehaven trees are summarized in Table 6 as the average number of flower buds per linear foot of terminal growth.

The average number of flower buds on terminal growth of Redhaven trees treated with NAA at 30 ppm 42 days after bloom the previous growing season was significantly reduced to 7.0 when compared to the average number of flower buds on the trees of the NPA and control treatments, 17.0 and 11.9 respectively (Table 5). There was also a significant increase in the average number of Redhaven flower buds on trees treated with NPA at 300 ppm three days after bloom in 1957 when compared with the control trees, 17.0 as compared to 11.9 buds per linear foot of terminal growth. The reduction in flower buds in 1958 from the use of NAA on Halehaven trees at 30 ppm 42 days after bloom in 1957 was significant, 12.8 flower buds per linear foot, as compared to 17.4 buds for the NPA treatment. Although not significantly different, the average of 12.8 flower buds per linear foot in 1958 on peach trees sprayed with NAA in 1957 was less than the average of 16.1 flower buds per foot for the control trees.

Table 5

Size of Redhaven and Halehaven Peaches as Influenced by the Use of N-1-Naphthylphthalamic Acid (NPA) and 3-Chloro IPC as Thinning Sprays, 1956

Variety	Redhaven			Halehaven		
Material	Control	NPA	3-chloro IPC	Control	NPA	3-chloro IPC
Concentration (ppm)		300	400		300	400
	Size range of 100 peaches at harvest					
1 1/8 - 2 in.	89	0	6	33	11	25
2 - 2 1/4 in.	11	0	69	36	33	64
2 1/4 - 2 3/4 in.	0	56	25	31	56	11
2 3/4 - 3 1/2 in.	0	44	0	0	0	0

Table 6

The Effect of Naphthaleneacetic Acid (NAA) and N-1-Naphthylphthalamic Acid (NPA) Applied in 1957 on the Flower Buds on Redhaven and Halehaven Trees During Spring 1958

Variety	Redhaven	Halehaven
Material		
	(Average number of flower buds per linear foot)	
NAA[1]	7.0	12.8
NPA[2]	17.0	17.4
Control	11.9	16.1
L. S. D. 5 percent level	4.0	3.8
1 percent level	5.4	N. S.

[1]NAA was applied at 30 ppm 42 days after bloom in 1957.

[2]NPA was applied at 300 ppm three days after bloom in 1957.

II. Field Studies Comparing Natural Blossom and Fruit Drop with that Resulting from the use of Naphthaleneacetic Acid and N-1-naphthylphthalamic Acid.

The average daily blossom or fruit drop of young Redhaven peaches of the control trees is recorded in Table 7. The average daily fruit drop on naphthaleneacetic acid (NAA) treated trees as observed after the NAA treatment on June 14, is recorded also in Table 6. The average daily fruit drop of the controls and the treated trees is presented as a bar graph in Figure 4.

As shown in Figure 4, the daily blossom drop of the control trees was heaviest during May 10 to May 17. Gross examination of the blossoms and fruit from May 10 to May 17 revealed that 20 percent of the blossoms which dropped contained "developed" ovaries as large as those in blossoms still persisting on the tree. The 80 percent consisted mostly of blossoms with "undeveloped" pistils plus a few with no pistils at all. This first heavy drop appeared to conform with the findings of Detjen (27). Also, it could be divided into two categories as reported by Murneek (71) for apples, consisting of unpollinated flowers and of pollinated flowers where fertilization had failed to take place.

The so-called "June" drop, although showing only one prolonged period of heavy drop, may be divided into a first and second period, Figure 4. A significant increase of daily drops occurred during June 7 to 14, and this rate of daily drop remained relatively constant for two weeks, the first period, when

Table 7

Average Daily Blossom and Fruit Drop of Young Redhaven Peaches of the Control and Naphthaleneacetic Acid (NAA) Treated Trees from Full Bloom to One Month Before Harvest, 1958

Period	Control trees			NAA sprayed trees			"t" test between control and NAA treated trees
	Average daily drop per 100 blossoms or fruits[1]	$S_{\bar{x}}$	"t" test between periods	Average daily drop per 100 fruits[1]	$S_{\bar{x}}$	"t" test between periods	
May 3 to May 10	0.1	0.1					
			7.5**				
May 10 to May 17	2.2	1.0					
			4.1**				
May 17 to May 24	0.6	1.2					
			1.2				
May 24 to May 31	0.2	0.0					
			2.1*				
May 31 to June 7	0.4	0.4					
			3.7**				
June 7 to June 14	1.2	0.7					
			1.0				
June 14 to June 18	1.6	1.4		1.1	1.1		1.1
			0.2			0.2	
June 18 to June 21	1.7	2.0		1.2	1.2		0.8
			2.3*			0.2	
June 21 to June 25	4.1	3.3		1.1	1.5		3.1**
			0.3			4.0**	
June 25 to June 28	4.5	3.7		6.3	4.6		1.1
			2.5*			0.4	
June 28 to July 2	1.9	1.8		5.7	3.7		3.5**
			3.1**			1.9	
July 2 to July 5	0.4	0.5		3.5	2.2		5.0**
			2.1*			4.8**	
July 5 to July 12	0.1	0.0		0.3	1.2		0.7

*Significant at 5 percent level. **Significant at 1 percent level.

[1]Average daily blossom or fruit drop was determined by dividing the number of dropped blossoms or fruits for any one period by the number of blossoms or fruits persisting at the beginning of that period time 100. This in turn was divided by the number of days of the period.

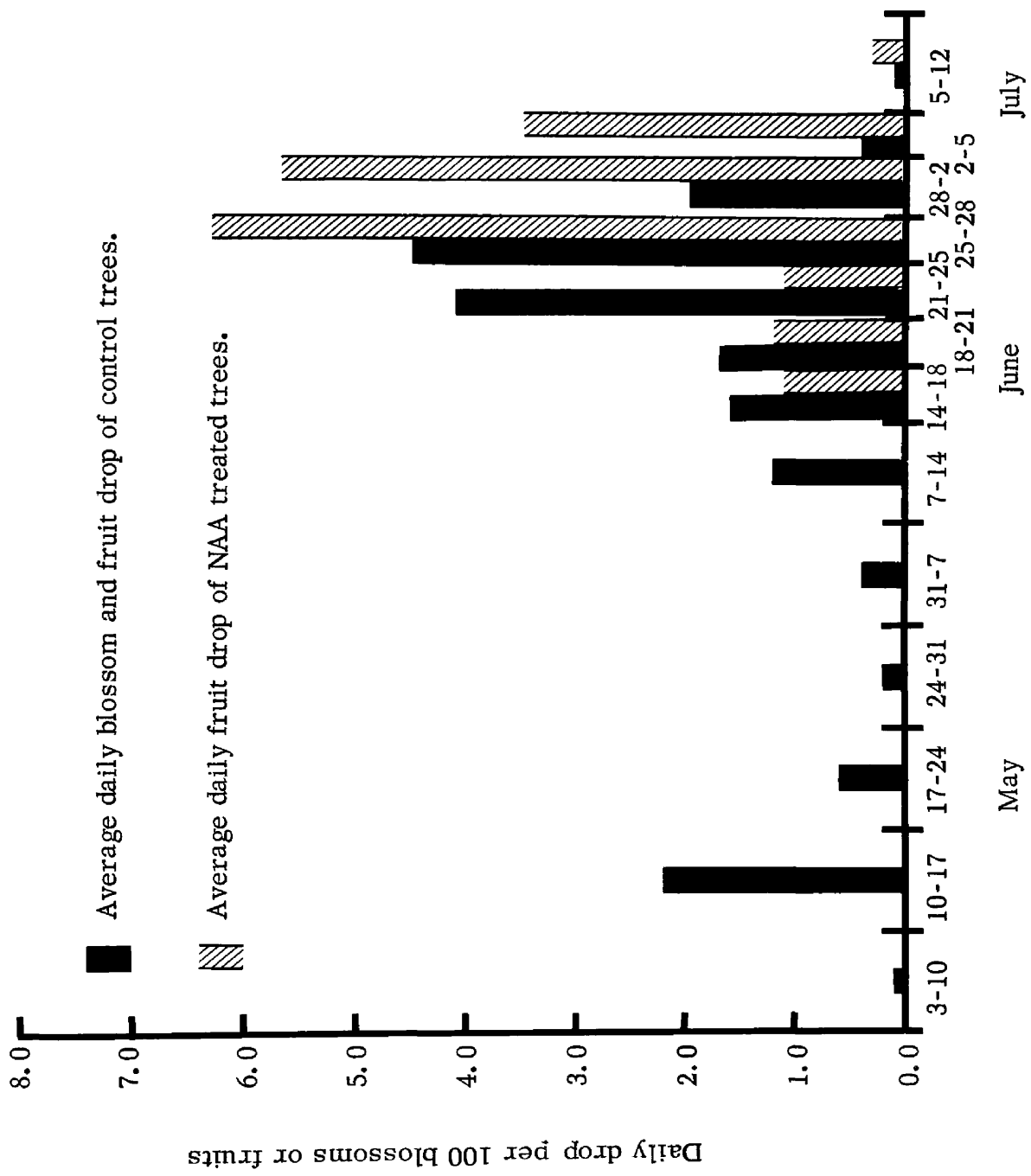


Figure 4. Comparison of average daily blossom and fruit drop of the control and naphthaleneacetic acid (NAA) treated Redhaven peach trees. Significantly different between the control and NAA treated trees at 1 percent level**.

the daily drop increased significantly again, the second period, Figure 4. "June" drop occurred about five weeks after bloom, as reported by Harrold (32) for the peach variety, Carman. The line of demarkation between the first and second period of "June" drop as found in this study was rather hard to determine, but apparently took place approximately 49 days after bloom, June 21, 1958. This appeared to occur about four to five days after the time of cellular formation of the endosperm (Table 10). Harrold (32) found the third period of fruit drop occurred about the seventh week after bloom. The duration of "June" drop in this study appeared longer than reported by Harrold (32) for the Carman variety. The fruits which dropped in June contained developed seeds, indicating that fertilization had taken place. However, the seeds were brown and shrivelled.

The only significant difference between the daily fruit drop of the control trees and the NAA treated trees occurred during June 21 to 25, 4.1 as compared to 1.1 per 100 original fruits; June 28 to July 2, 1.9 as compared to 5.7; and July 1 to 5, 0.4 as compared to 3.5 (Table 7).

NAA applied 42 days after bloom at 30 ppm appeared to delay the second period of "June" drop three to four days (Figure 4). A similar delay in fruit drop was reported by Struckmeyer and Roberts (93) when NAA was used as a thinning agent for apples, but the delay in fruit drop which they reported lasted as long as one or two weeks. The use of NAA on peaches in this study

did not extend the second period of "June" drop for a longer time than was found for the control trees. This was contrary to the findings of Kelley (44) working with peaches, and Teubner and Murneek (96) working with apples.

As the NPA thinning treatments were made three days after full bloom it seemed desirable to determine the approximate time the blossoms or fruits dropped from NPA treated Redhaven trees. To accomplish this, counts were made of blossoms and fruits on tagged branches at the time of bloom, and at two and ten weeks after bloom. It was found that 88.2 percent of the original number of blossoms at full bloom had dropped during the period of 14 days after bloom with the majority of drop occurring in the last seven days. Whereas, during this same period of 14 days, it was found that about 30 percent of the original number of blossoms had dropped from the control trees. This period of heavy blossom drop on both the control and treated trees coincided with the time of the first heavy blossom drop of the control trees of Redhaven in the NAA study (Figure 4).

As stated previously Murneek (71) suggested that the first heavy drop of apples could be divided into two categories consisting of unpollinated flowers and of pollinated flowers where fertilization had failed to take place. On the basis of this, assuming that the pollination should stimulate the growth and development of the pistil (77), the flowers which fell during the first heavy drop from non-treated control trees and the NPA treated trees of Redhaven were examined to

determine the number which had been pollinated and those which were unpollinated. Examinations of the flowers from the NPA treated trees revealed that 65.5 percent of those which had dropped contained a "developed" pistil. Whereas, for the control trees it was found that only 19.8 percent of the flowers which dropped contained a "developed" pistil. This would indicate that the number of pollinated flowers which had abscissed during this period of drop was increased by NPA treatment made three days after bloom on Redhaven peach trees. A similar result was found for NPA treated Halehaven trees as 83.3 percent of the blossoms which had abscissed contained a "developed" pistil as compared to only 20.0 percent for the control trees.

In order to determine if fertilization had taken place in the flowers which had dropped from Redhaven trees treated with NPA, the ovules of ten of these flowers were examined under the microscope. No more than eight nuclei were found within the embryo sac of any one of these ovules. In comparison, 14 nuclei were found in the embryo sacs of flowers still remaining on the untreated control trees. This would indicate that fertilization had not taken place in "developed" pistils of the flowers which had abscissed from the Redhaven trees treated with NPA or that the zygote failed to develop.

III. Anatomical Investigations of the Developing Embryo and Endosperm of Halehaven and Redhaven Peach

The progressive growth and development of the embryo, endosperm, seed and pericarp of the Halehaven peach from full bloom through the period of "June" drop in 1957 and 1958 and of the Redhaven peach in 1958 are recorded in Tables 8, 9 and 10. Growth of the embryo, the endosperm, the seed and the pericarp in length is plotted against days in Figures 5 and 6 for the Halehaven peach in 1957 and 1958, and in Figure 7 for the Redhaven peach in 1958. The data of the time of cellular formation of the endosperm of Halehaven and Redhaven peaches in 1958, as analyzed from whole mounts, are given in Table 11.

Microscopic examinations of ovules of Redhaven peach blossoms collected at full bloom, May 3, 1958, revealed approximately three nuclei within a megaspore (Table 10). Four and seven days after bloom, about six and eight nuclei were located within the megaspore tissue (Table 10). In the collection made seven days after bloom, three nuclei, the antipodals, were observed at the distil end; three nuclei, the synergids and the egg; at the micropylar end and one or two nuclei, the endosperm nucleus, at the center of the embryo sac.

Syngamy apparently took place in the Redhaven peach between May 10 and May 17, 1958, 7 to 14 days after bloom. From observations of certain slides, the zygote had not divided in most cases by May 17, but many endosperm nuclei, about 14 in number, appeared within the embryo sac (Table 10).

Table 8

Growth and Development of the Embryo, Endosperm, Seed and Pericarp of the Young Halehaven Peach from Full Bloom Through "June" Drop, 1957 (Average of Five Samples)

Date of Sampling	Days After Bloom	Pericarp		Seed		Endosperm			Embryo			
		Length (mm)	Width (mm)	Length (mm)	Width (mm)	Stage	Length (mm)	Width	Stage	Cells (no.)	Length (u)	Width (u)
May 6	4	2.1	1.4	0.7	0.3							
May 10	8	3.4	2.0	1.2	0.5							
May 13	11	3.9	1.9	1.3	0.5	Free-nuclear	0.13	0.03	Zygote	1	24.3	14.0
May 17	15	4.3	2.5	1.5	0.6	Free-nuclear	0.26	0.05	Filamentous	5	31.2	18.0
May 24	22	8.2	4.3	3.1	1.0	Free-nuclear	0.44	0.05	Filamentous	8	35.2	20.8
May 31	29	10.2	7.4	3.3	1.3	Free-nuclear	1.92	0.05	Filamentous	15	41.6	26.0
June 7	36	21.8	14.9	8.4	3.6	Free-nuclear	8.37	0.18	Filamentous	31	45.3	25.1
June 14	43	28.3	24.0	12.2	7.0	Early cellular	12.17	0.26	Early spherical	60	65.6	36.0
June 17	46	33.5	30.7	15.3	9.7	Cellular	1.02[1]	0.26[1]	Spherical	300	98.0	59.2
June 21	50	39.2	34.5	16.7	10.7	Cellular	1.21[1]	0.62[1]	Spherical	300	139.1	105.3
June 24	53	40.5	35.5	18.3	11.8	Cellular	2.34[1]	0.75[1]	Transitional		194.4	133.2
June 28	57	40.1	36.0	18.4	11.8	Cellular	4.80[1]	1.69[1]	Cotyledonary	-	680.5	487.1

[1]Measurements of cellular endosperm.

Table 9

Growth and Development of the Embryo, Endosperm, Seed and Pericarp of the Young Halehaven Peach from Four Weeks after Bloom through "June" Drop, 1958 (Average of Five Samples)

Date of Sampling	Days after bloom	Pericarp		Seed		Endosperm			Embryo			
		Length[1] (mm)	Width[1] (mm)	Length[1] (mm)	Width[1] (mm)	Stage	Length (mm)	Width (mm)	Stage	Cells (no.)	Length (u)	Width (u)
June 7	35	24.7	19.5	9.8	5.3	Free-nuclear	9.75	0.16	Fila-mentous	46	54.0	34.6
June 11	39	32.4	25.2	13.7	8.2	Early cellular	13.68	0.23	Early spherical	100	67.7	44.3
June 14	42	32.4	26.4	14.6	8.8	Part cellular	14.60	0.30	Spherical	189	86.4	49.1
June 18	46	34.4	31.3	16.2	10.8	Cellular	1.09[2]	0.34[2]	Late spherical	290	97.9	53.3
June 21	49	35.8	31.4	16.8	11.3	Cellular	1.38[2]	0.59[2]	Transitional	1000	144.7	89.3
June 28	56	37.7	33.6	18.3	12.2	Cellular	2.27[2]	1.15[2]	Transitional	-	222.5	152.6
July 5	63	38.1	34.9	18.4	12.5	Cellular	8.20[1,2]	6.30[1,2]	Cotyledonary[1]	-	2100.0[1]	1890.0[1]

[1]Average of ten samples.

[2]Measurements of cellular endosperm.

Table 10

Growth and Development of Embryo, Endosperm, Seed and Pericarp of the Young Redhaven Peach from Full Bloom through "June" Drop, 1958 (Average of Five Samples)

Date of Sampling	Days after bloom	Pericarp		Seed		Endosperm			Embryo			
		Length[1] (mm)	Width[1] (mm)	Length[1] (mm)	Width[1] (mm)	Stage	Length (mm)	Width (mm)	Stage	Cells (no.)	Length (u)	Width (u)
May 3	0	2.1	1.6	0.7	0.5		0.04	0.01	(megaspore - three nuclei)			
May 7	4	2.4	2.1	0.8	0.5		0.05	0.02	(megaspore - six nuclei)			
May 10	7	3.1	2.4	0.9	0.6		0.05	0.02	(megagametophyte-eight nuclei)			
May 17	14	6.7	5.4	2.4	1.3	Free-nuclear	0.67	0.04	Zygote	1	30.3	24.1
May 24	21	9.7	8.2	4.3	2.4	Free-nuclear	2.78	0.09	Filamentous	15	45.0	27.0
May 31	28	17.2	13.0	6.7	3.4	Free-nuclear	4.56	0.13	Filamentous	27	50.2	31.3
June 7	35	22.1	17.7	8.8	4.8	Free-nuclear	8.80	0.15	Filamentous	46	59.7	31.4
June 11	39	27.6	21.6	11.9	6.9	Free-nuclear	11.92	0.23	Filamentous	56	60.3	31.9
June 14	42	30.8	25.5	12.9	7.8	Early cellular	12.98	0.29	Early spherical	80	61.9	32.4
June 18	46	31.6	26.7	13.9	8.8	Part cellular	13.90	0.30	Spherical	95	72.0	38.9
June 21	49	32.6	30.0	15.0	9.9	Cellular	1.18[2]	0.43[2]	Late spherical	560	106.6	54.0
June 28	56	37.9	32.5	18.2	11.5	Cellular	1.63[2]	0.79[2]	Transitional	-	144.0	94.3
July 5	63	37.7	32.7	18.1	12.0	Cellular	6.33[1,2]	4.99[1,2]	Cotyledonary[1]	-	1000.0[1]	900.0[1]

[1]Average of ten samples.

[2]Measurements of cellular endosperm.

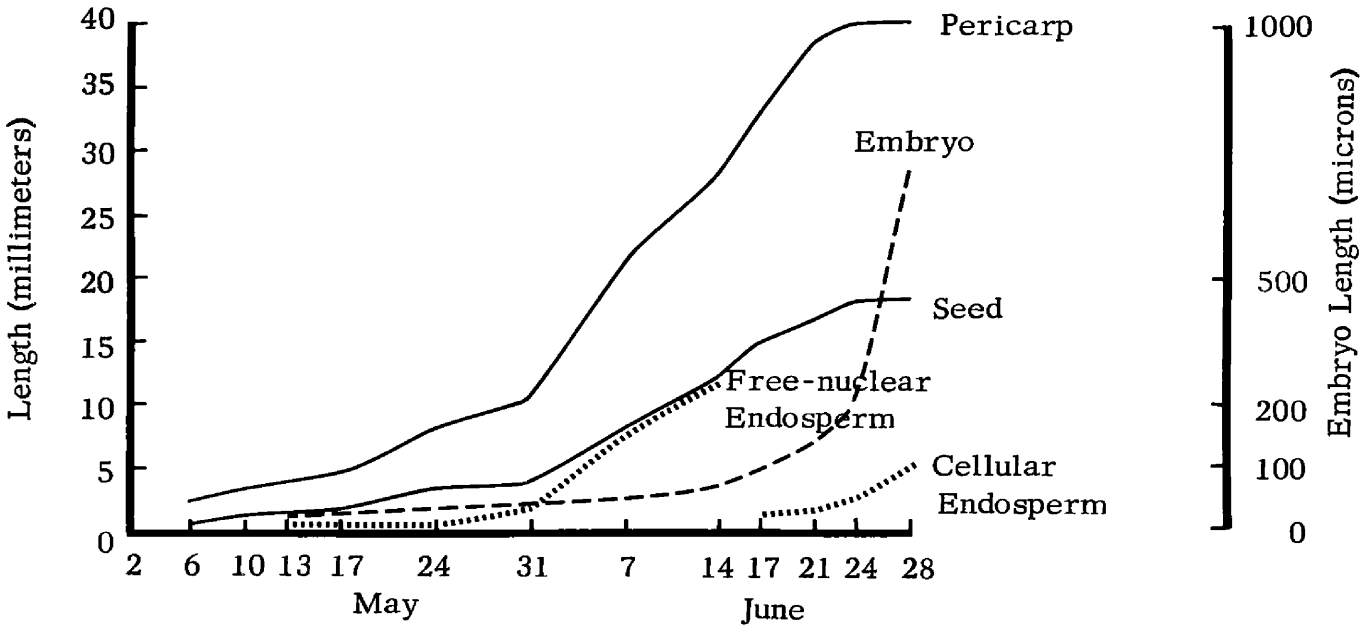


Figure 5. Growth of the embryo, endosperm, seed and pericarp of the young Halehaven peach from full bloom through "June" drop, 1957. Growth was determined by linear measurements in millimeters, except embryo length in microns.

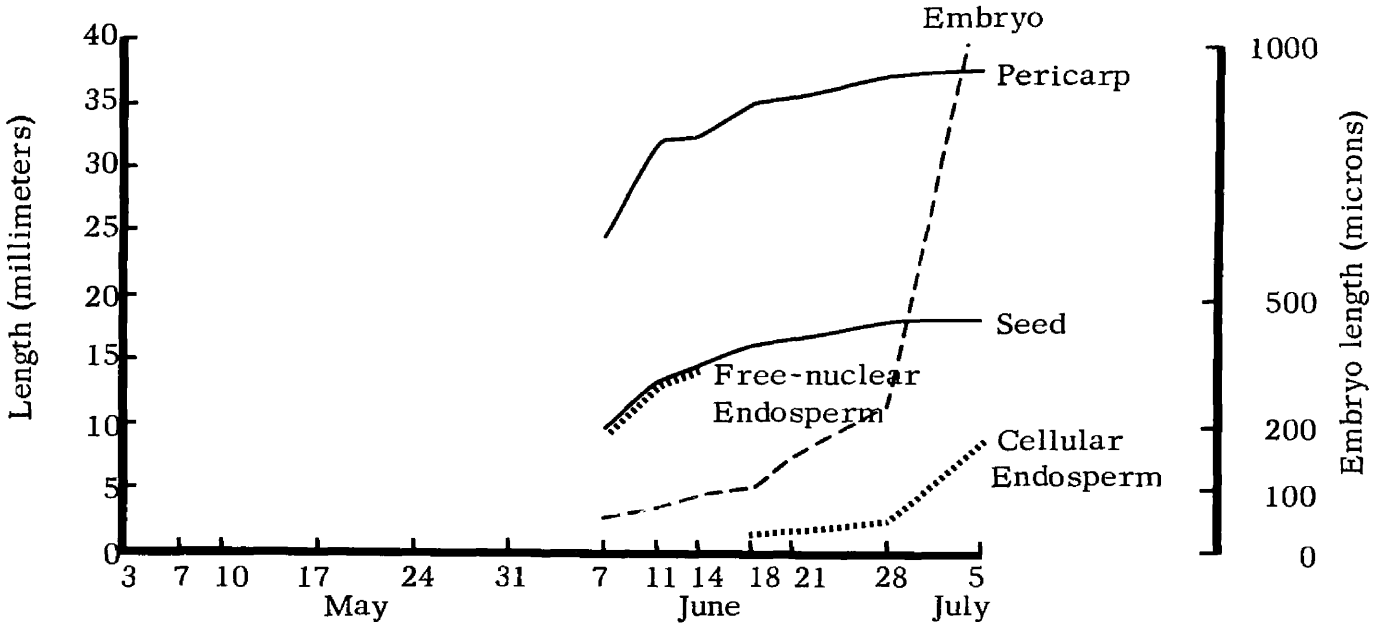


Figure 6. Growth of the embryo, endosperm, seed and pericarp of the young Halehaven peach during "June" drop, 1958. Growth was determined by linear measurements in millimeters, except embryo length in microns.

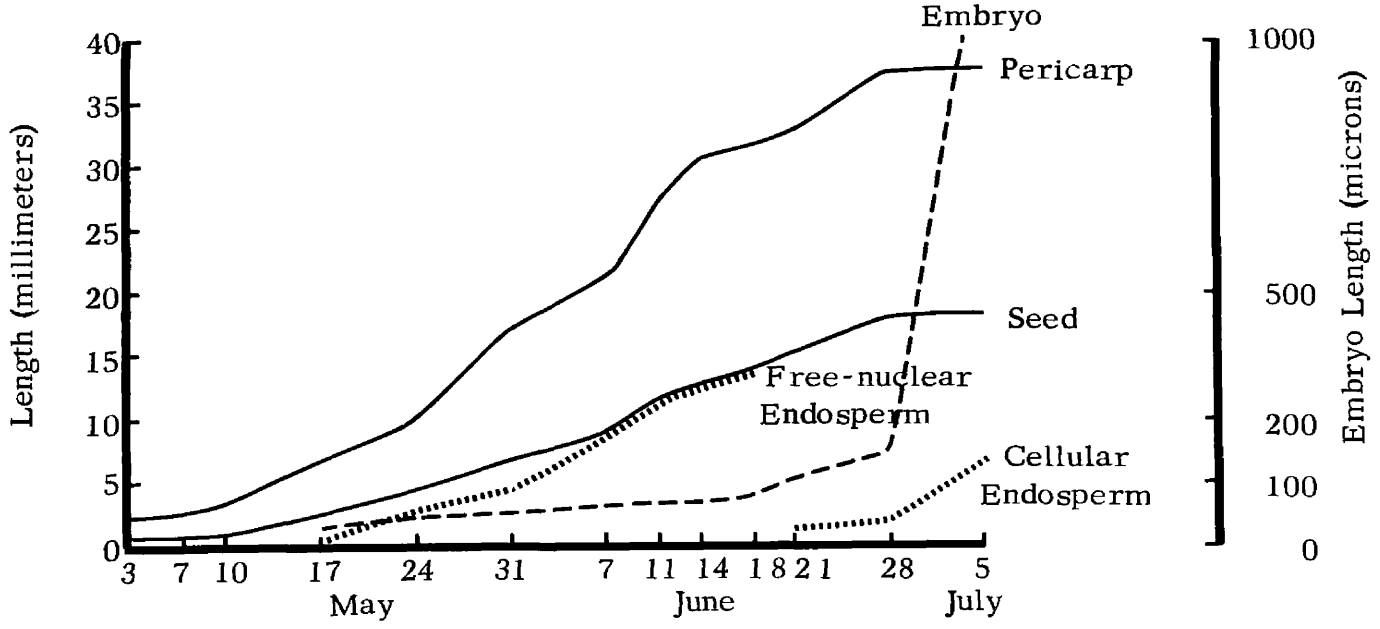


Figure 7. Growth of the embryo, endosperm, seed and pericarp of the young Redhaven peach from full bloom through "June" drop, 1958. Growth was determined by linear measurements in millimeters, except embryo length in microns.

The microscopic sections of Halehaven peach ovules of 1957 collected during the first week after bloom were stained too heavy to study possible megagametogenesis.

The presence of a zygote within the embryo sac was observed first in Halehaven peach seeds collected May 13, 1957, about 11 days after bloom and first division of the zygote occurred within four days (Table 8). In 1958, working with the Redhaven variety the zygote was first observed on May 17, 14 days after bloom (Table 10); and in some cases first division of the zygote had already taken place (Figure 8). Harrold (32) studying the peach variety, Carman, observed zygote division 12 days after bloom, while Dorsey working with the Elberta peach (28) found the zygote division to occur about 27 days after bloom. The peach embryo of both Halehaven and Redhaven varieties grew very slow in length, width and cell number for approximately 42 days after the first division (Figures 5 and 7).

An attempt was made to classify the developmental stage of the peach embryo as reported by Meyer (67) for the embryo of the developing McIntosh apple. Meyer classified three stages of proembryo development from the time of first cell division to the stage of cotyledon development as filamentous, spherical and transitional. He described the filamentous stage as follows: "The embryo beginning with the first cell division grows and divides as a linear object." The filamentous stage of the peach embryo is exemplified in

Figure 8

Two-celled embryo found in the Redhaven peach on May 17, 1958, 14 days after bloom. The embryo is in the early filamentous stage. The endosperm is in the free-nuclear stage. (X510)

Figure 9

A typical embryo found in the Redhaven peach on June 11, 1958, 39 days after bloom. The embryo of approximately 56 cells is the late filamentous stage. The endosperm is in the free-nuclear stage. (X200)

Figure 10

The endosperm found in the Redhaven peach on June 14, 1958, 42 days after bloom. Cellular formation of the endosperm has begun at the micropylar end and the periphery of the embryo sac. (X200)

Figure 11

A typical embryo found in the Redhaven peach on June 18, 1958, 46 days after bloom. The embryo of approximately 95 cells is in the spherical stage. The endosperm has changed from the free-nuclear state to the cellular condition. (X200)

Figure 8

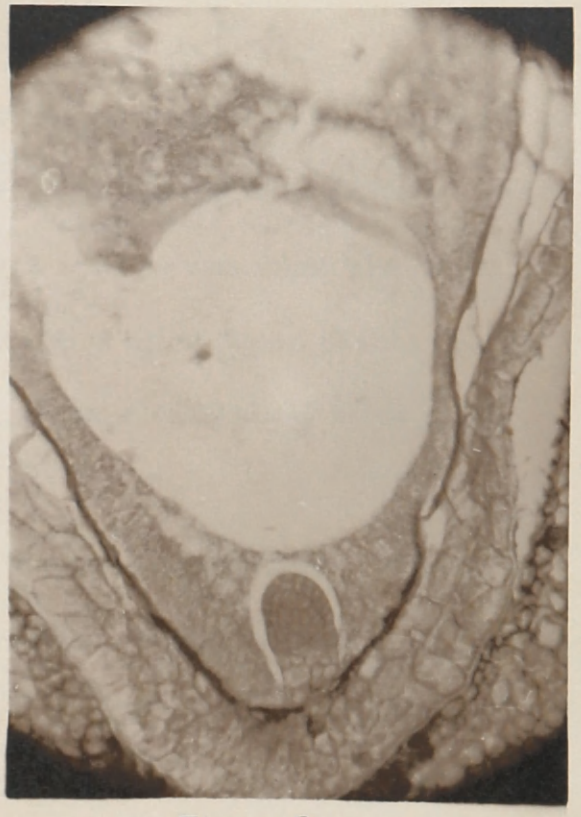


Figure 9

Figure 10

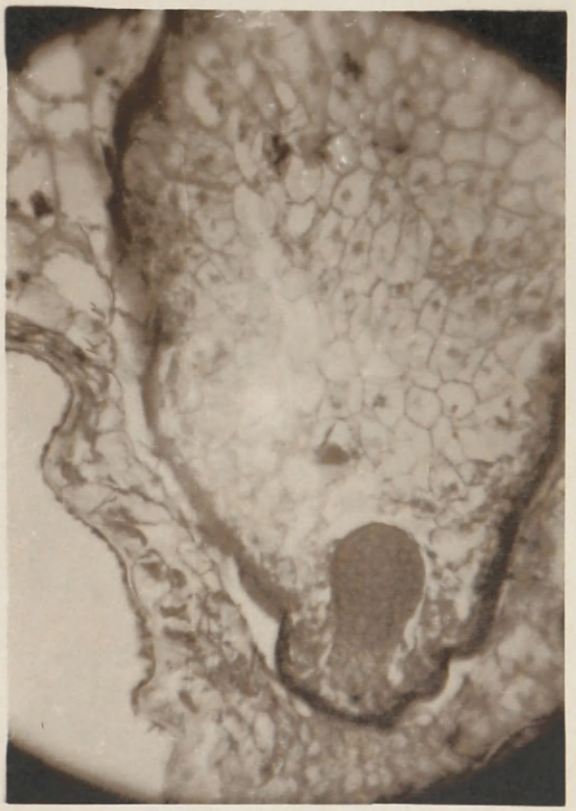


Figure 11

Figures 8 and 9. In 1957 the embryo of the Halehaven peach could be classified as filamentous for approximately 28 days from about 15 to 43 days after bloom (Table 8). In 1958 the Redhaven peach embryo was found also to be filamentous for a 28-day period beginning 14 days after bloom (Table 10 and Figure 9). Meyer (67) found that this embryo stage lasted only 10 days for the McIntosh apple.

The second stage as described by Meyer (67), the spherical stage of the embryo, begins when the distil tiers of cells increase in size in three dimensions. This stage of the peach embryo is shown in Figures 11 and 12. In 1957 this stage was observed about 43 days after bloom for the Halehaven variety and continued for 7 to 10 days, Table 8, and in 1958 this spherical stage was observed about 39 days after bloom and lasted 7 to 10 days (Table 9). For the Redhaven variety, the spherical stage began about 42 days after bloom and continued for 7 to 10 days (Table 10). For both the Redhaven and Halehaven this stage was found to begin about three or four days before endosperm cellular formation. Meyer (67) observed that the spherical stage of the embryo of the McIntosh apple began about 22 days after fertilization and lasted for nine days.

The transitional stage or the third stage of embryo development marked by the elipitical development of the distil portion of the embryo, appeared first when the embryos of the Redhaven and Halehaven reached about the 300- to 500-

celled stage (Figure 13). By this time the embryo of both peach varieties had attained a linear dimension of 100 microns in 1957 and 1958 (Tables 8, 9 and 10). This change took place in the Halehaven variety about 49 days after full bloom in both 1957 and 1958. This change in development of the Redhaven embryo was about 49 to 53 days after bloom comparing closely with that of the Halehaven variety. The change from the spherical to the transitional stage occurred for both peach varieties about three or four days after the complete change of the endosperm from free-nuclear to cellular formation (Tables 8, 9, 10 and 11).

Coinciding with the change to the transitional stage was the gradual increased rapid rate of growth in length, width, and cell division of the Halehaven and Redhaven embryos. However, the highest rate of embryo growth was found to occur during the termination of the transitional stage (Figure 14) and the initiation of the cotyledonary stage (Figure 15). This was about seven days after initial transitional development. To express this in a different way, the rapid growth of the embryo took place about 10 days after cellular formation of the endosperm (Tables 8, 9, 10 and 11).

In general, the time of initiation of the rapid growth of the peach embryo as found in this study agrees with Tukey's observations of various varieties of peaches (98). He classified this change in growth rate as Stage II, which begins with the rapid development of the peach embryo, about 49 days after bloom.

Endosperm development of both the Halehaven and Redhaven fruits

Figure 12

A typical embryo found in the Redhaven peach on June 21, 1958, 49 days after bloom. The embryo is in the late spherical stage. The endosperm has changed from the free-nuclear state to the cellular condition. (X93)

Figure 13

A typical embryo found in the Halehaven peach on June 21, 1957, 49 days after bloom. The embryo is in the early transitional stage. The endosperm has changed from the free-nuclear stage to the cellular condition. (X93)

Figure 14

A typical embryo found in the Redhaven peach on June 28, 1958, 56 days after bloom. The embryo is in the late transitional stage, the cotyledonary primordia showing. The endosperm has changed from the free-nuclear state to the cellular condition. (X46)

Figure 15

A typical embryo found in the Halehaven peach on June 28, 1958, 57 days after bloom. The embryo is in the cotyledonary stage. The endosperm has changed from the free-nuclear state to the cellular condition. (X46)

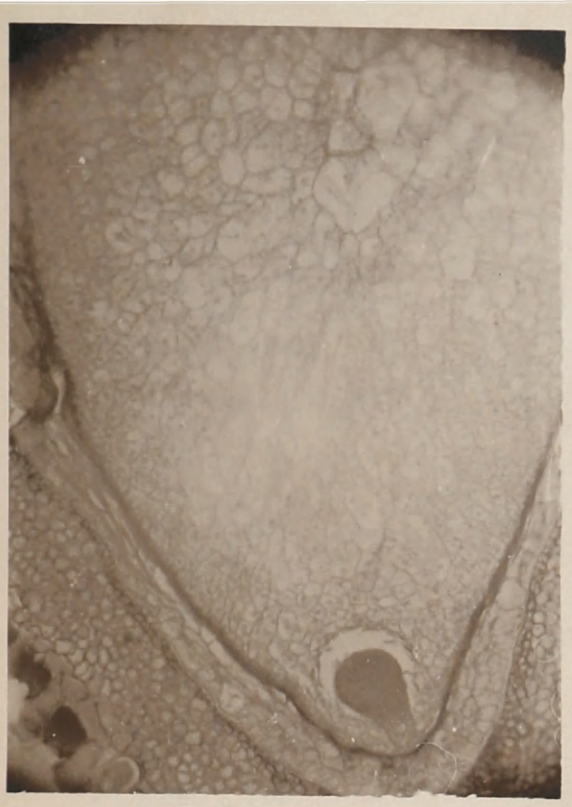


Figure 12

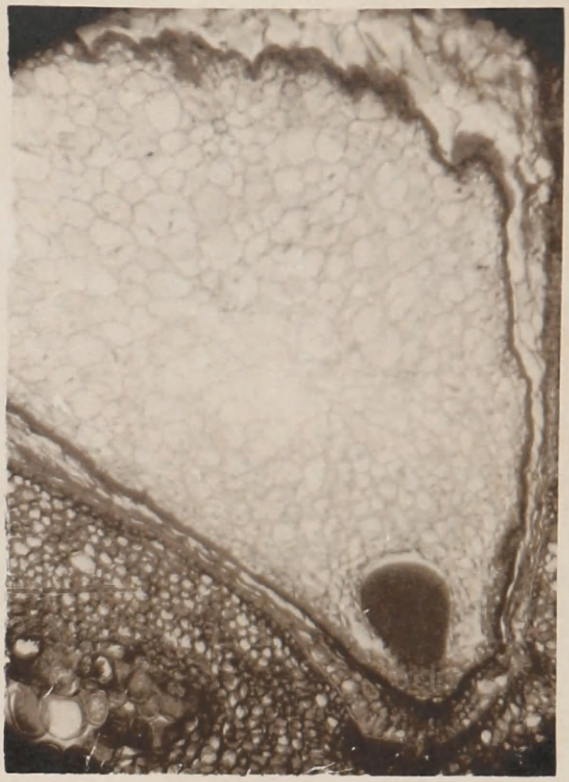


Figure 13

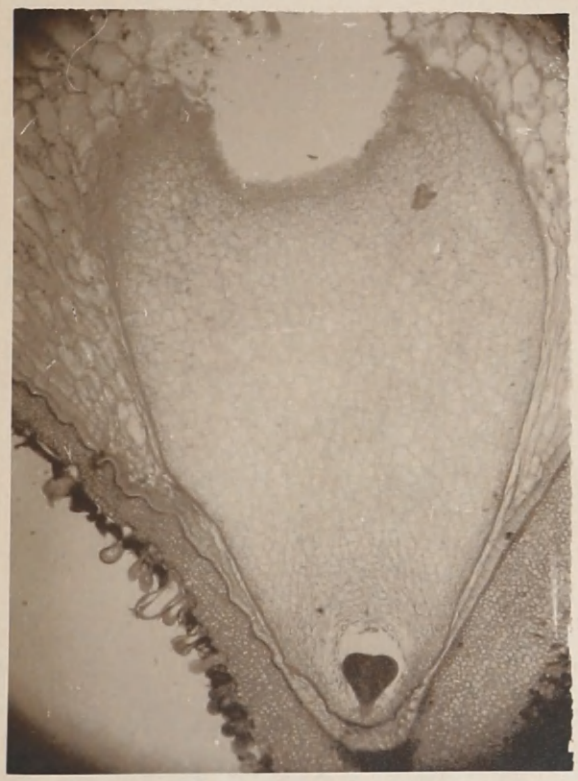


Figure 14

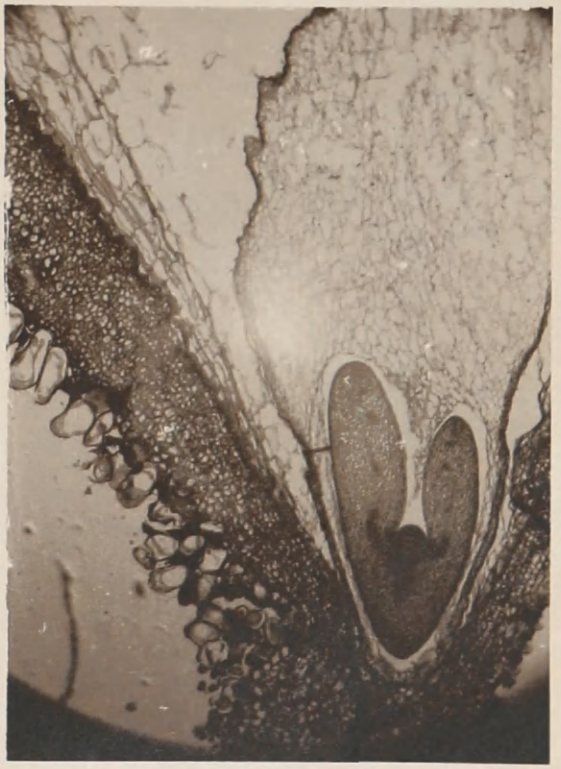


Figure 15

Table 11

Cellular Development of the Endosperm of Redhaven and Halehaven Peach Seeds During "June" Drop, 1958

Date of Sampling	Days after bloom	Days after "shuck-off"	Number of Seeds with Cellular Endosperm per 10 Samples	
			Redhaven peach	Halehaven peach
June 11	39	10	0	0
June 12	40	11	0	1
June 13	41	12	0	2
June 14	42	13	0	4
June 15	43	14	0	10
June 16	44	15	0	10
June 17	45	16	1	
June 18	46	17	5	
June 19	47	18	10	
June 20	48	19	10	

was very rapid immediately after syngamy, the development primarily manifested as many divisions of the free-nuclei. Approximately 14 nuclei were found within embryo sac of the Redhaven peach collected May 17, 1958 (Table 10) at the time of zygote division. The time of the first division of endosperm nucleus, 7 to 14 days after bloom, agrees closely with Harrold's study of the Carman peach.

The rate of cell division of the embryo in the Redhaven variety was higher than the endosperm nuclear division during the period May 17 to 24. The change was one to 15 cells for the embryo as compared to 14 to 90 nuclei of the endosperm (Table 10). Furthermore, 14 days after the first evidence of an endosperm, microscopic studies revealed that the embryo sac contained as many as 400 endosperm nuclei as compared to about 27 cells for the embryo.

During the period of 21 days after the first appearance of the endosperm, the embryo sac of both Halehaven and Redhaven developed rapidly in length toward the chalazal pocket of the ovule (Tables 8 and 10) and the endosperm appeared to occupy the entire area of the embryo sac during this lengthening process. The extension of the embryo sac to the chalazal pocket apparently was complete 35 days after bloom for Halehaven in both 1957 and 1958 and for Redhaven in 1958 (Figures 7, 8 and 9).

The change of the endosperm from the free-nuclear state to cellular formation in the Halehaven peach in both years appeared about 42 days after bloom and was apparently complete within two or three days (Tables 8, 9 and 11).

This change for the Redhaven variety was very similar to the Halehaven, occurring 44 to 45 days after full bloom (Tables 10 and 11). Harrold (32) found also that the endosperm remained in a free-nuclear state until the end of the sixth week, 42 days after bloom.

Long thin strands of cytoplasm appeared throughout the free-nuclear endosperm of Halehaven and Redhaven a week before formation of the cell walls with the greatest concentration of these strands surrounding large vacuolated areas near the micropylar end of the endosperm (Figures 9, 10 and 11). Gradual formation of the cell calls appeared at the micropylar end at the periphery of the embryo sac and continued until the large vacoule in the center became completely occupied with cells. Tukey (97) described a similar development in the cellular formation of the endosperm of the sweet cherry, Prunus avium.

Immediately after endosperm cellular formation, the endosperm of the Halehaven and Redhaven peach grew very rapidly in length and width (Tables 8, 9 and 10) reaching about one-third the volume of the seed 18 days after cellular formation.

Coincident with this, the rate of growth of the seed and pericarp of the Halehaven and Redhaven peach declined sharply. This change in growth rate occurred seven to eight weeks after full bloom, during the 1958 season (Figures 8 and 9) and it followed a very similar growth pattern as found for the Halehaven

in 1957 (Figure 7). These growth relations were described by Tukey (98) in an earlier study of the growth and development of the peach.

The endocarp of both the Halehaven and Redhaven of 1958 were found to begin to harden about 35 days after bloom, making it difficult to break the pericarp in half with the fingers. By 56 days after bloom the endocarp was too hard for penetration of even a razor blade.

The term "shuck-off" is used frequently in relation to peaches and means the shedding of the hypanthium from the young fruit. The hypanthium unites the stamens, chorolla and calyx to the receptacle of a perigynous flower. Most of the shucks were shed from Halehaven peaches in 1957 about 30 to 32 days after bloom. Whereas, the greatest portion of "shuck-off" from both Halehaven and Redhaven peaches in 1958 occurred approximately 29 days after bloom. Endosperm cellular formation occurred approximately 12 days after "shuck-off" for Halehaven in 1957, 15 days after "shuck-off" for Halehaven in 1958, and 18 days after "shuck-off" for Redhaven in 1958. It might be that the time of "shuck-off" for a specific variety could be used to determine the approximate time that the endosperm of the peach changed from a free-nuclear state to cellular form.

IV. Natural Hormone Content and Concentration of Young Redhaven Peach Seeds as Related to Early Growth and Development.

Studies in 1957 revealed that the endosperm of the Halehaven seeds changed from a free-nuclear state to a cellular state approximately six weeks after bloom. Therefore, studies were set up to evaluate the natural hormone content and concentration of the seeds before, during, and after the endosperm became cellular.

The growth responses using the avena straight growth test by treating 5 millimeter sections of Brighton oats with diethyl ether extracts of young Redhaven peach seeds and with various concentrations of indoleacetic acid (IAA) and of ethyl indoleacetate (EtIA) are recorded in Table 12. A summary of the data of natural hormone content and concentration from diethyl ether extracts of young Redhaven peach seeds as determined by the avena straight growth tests shown in Table 12 are recorded in Table 13. The calculated concentration and content of hormones present in the seeds are plotted against times of samplings in Figures 16 and 17. The acid and the neutral fractions of the June 15 diethyl ether extract were chromatographed using a 8:1:1 volume ratio of 2-propanol, ammonia, and water solvent, and the sections of the chromatograms were analyzed by the avena straight growth test. These data are recorded in Table 14. The data of the chromatograms are plotted as histograms in Figures 18 and 19.

The natural hormone content from the acid and neutral fractions of

Table 12

Length of Avena Coleoptile Sections taken 24 Hours after Treatments of Diethyl Ether Extracts of Redhaven Peach Seeds and of known Concentrations of Indoleacetic Acid and Ethyl Indoleacetate. The Initial Length of the Avena Coleoptile Sections was 5 Millimeters (Average of 10 Sections)

Date of Sampling	Fraction of Extract	Dilutions of the Extract			Control Treatment[1]	Known Concentrations of IAA and EtIA			
		0.01 ml	0.05 ml	0.10 ml		0.01 ppm	0.10 ppm	1.00 ppm	Hormone
		(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	
June 2	Acid	7.95	8.20	7.90	7.90	8.45		10.65	IAA
June 2	Neutral	9.05	10.00**	9.70	7.90	9.35	10.80	11.00	EtIA
June 7	Acid	8.30	9.55**	9.85	7.55	9.65	11.40		IAA
June 7	Neutral	9.10	12.15**	13.00	7.65	10.45	12.10		EtIA
June 15	Acid	9.60	10.10**	8.70	7.90	8.46		10.65	IAA
June 15	Neutral	9.75	10.75**	10.10	7.90	9.35	10.80	11.00	EtIA
June 21	Acid	9.20	9.80**	10.20	7.90	8.45		10.65	IAA
June 21	Neutral	9.05	10.05**	9.25	7.90	9.35	10.80	11.00	EtIA
June 28	Acid	8.65	9.90**	8.55	7.80	9.15	11.75		IAA
June 28	Neutral	9.15	10.85**	9.40	7.60	9.90	12.15		EtIA

**Significantly higher than control at 1 percent level.

[1]Distilled water plus phosphate buffer solution.

Table 13

Summary Data of Natural Hormone Content and Concentration from Diethyl Ether Extracts of Redhaven Peach Seeds as Determined by the Avena Straight Growth Test[1]

	Fraction	Date of Sampling				
		June 2	June 7	June 15	June 21	June 28
Average final length of coleoptile (mm)	Acid	8.20	9.55	10.10	9.80	9.90
	Neutral	10.00	12.15	10.75	10.05	10.85
Final length of coleoptile/ 5 mm (its initial length)	Acid	1.64	1.93	2.02	1.96	1.98
	Neutral	2.00	2.43	2.15	2.01	2.17
Hormone equiv. IAA (ppm)	Acid	.002	.011	.280	.130	.022
Hormone equiv. EtIA (ppm) (determined from standard curves)	Neutral	.028	.065	.091	.030	.027
Hormone equiv. IAA (ug)	Acid	0.2	1.0	22.4	10.0	1.7
Hormone equiv. EtIA (ug) (per 5 ml of extract)	Neutral	2.2	5.9	8.2	2.7	2.4
Number of fruit at each sampling		500	498	300	300	300
Hormone content equiv. IAA	Acid	0.04	0.20	7.47	3.35	0.59
Hormone content equiv. EtIA (ug in seeds per 100 fruits)	Neutral	0.45	1.18	2.73	0.90	0.80
Fresh weight of seeds extracted with ether		14.1	28.4	48.7	97.4	139.7
Fresh weight of seeds of 100 fruits		2.8	5.3	16.2	32.5	46.6
Hormone concentration equiv. IAA	Acid	.0147	.0352	.4600	.1031	.0127
Hormone concentration equiv. EtIA (ug per gram fresh weight of seeds)	Neutral	.1587	.2060	.1682	.0772	.0171

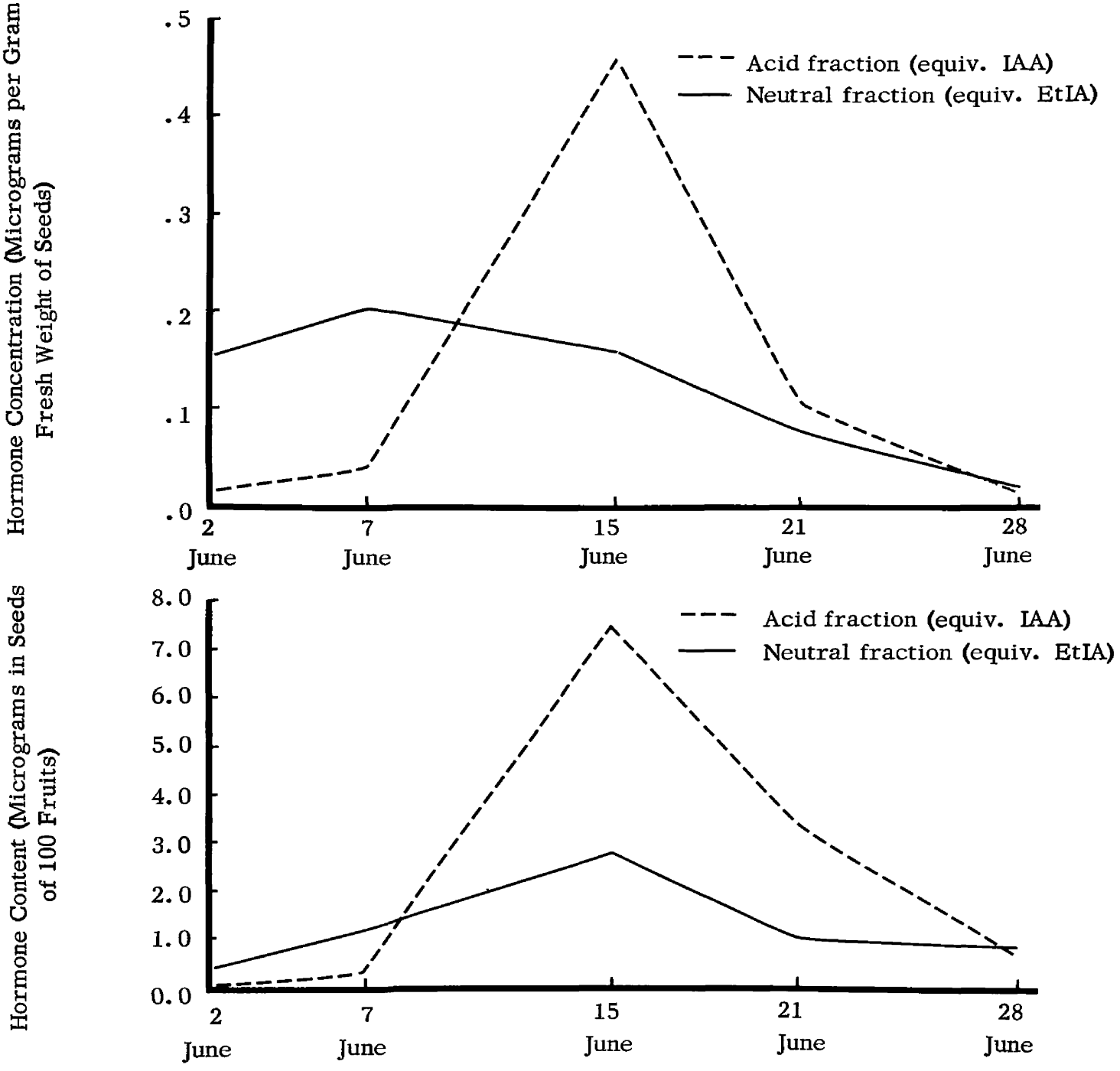
[1] A 0.05 milliliter dilution of each fraction of the diethyl ether extract was used in the avena tests and also in making these tabulated calculations.

Figure 16

Natural hormone concentration from diethyl ether extracts of Redhaven peach as determined by the avena straight growth test, 1958. Hormone concentration of the acid fraction of the extract is computed in equivalent indoleacetic acid (IAA) micrograms per gram fresh weight of seeds. Hormone concentration of the neutral fraction is computed in ethyl indoleacetate (EtIA) micrograms per gram fresh weight of seeds.

Figure 17

Natural hormone content from diethyl ether extracts of Redhaven peach seeds as determined by the avena straight growth test, 1958. Hormone content of the acid fraction of the extract is computed in equivalent indoleacetic acid (IAA) micrograms in seeds of 100 fruits. Hormone content of the neutral fraction of the extract is computed in equivalent ethyl indoleacetate (EtIA) micrograms in seeds of 100 fruits.


Hormone Concentration (Micrograms per Gram Fresh Weight of Seeds)
.5
.4
.3
.2
.1
.0
Acid fraction (equiv. IAA)
Neutral fraction (equiv. EtIA)
2 June
7 June
15 June
21 June
28 June
Hormone Content (Micrograms in Seeds of 100 Fruits)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Acid fraction (equiv. IAA)
Neutral fraction (equiv. EtIA)
2 June
7 June
15 June
21 June
28 June

the extracts of young Redhaven peach seeds sampled on June 2, 7, 15, 21 and 28 as determined by the avena straight growth test was significantly higher than the control treatments of the phosphate buffer solution for all sampling dates with one exception, the acid fraction of June 2 (Table 12). The natural hormone content of the seeds of both the acid and neutral fractions reached a maximum in quantity of June 15 (Figure 11). The concentration of the natural hormone in the seeds was highest on June 15 for the acid fraction of the extract. Whereas, the hormone concentration of the neutral fraction was highest on June 7. The natural hormone concentration of the neutral fraction was relatively the same for the first three dates of sampling, June 2, 7 and 15, after which time it decreased markedly. During the period the weight of the seed of 100 fruits increased from 2.8 grams on June 2 to 46.6 grams on June 28 (Table 13). The hormone concentration and content present in the acid fraction had a greater variation during the period of sampling than was found for the neutral fraction (Figures 16 and 17).

It was observed that the dilution quantities of 0.5 and 1.0 milliliter of the acid and neutral fractions from extracts of Redhaven peach seeds collected June 2 and 7 almost completely inhibited any growth of coleoptile. The only exception was the 0.5 millimeter of the neutral fraction from the June 7 collection which reduced the growth of the coleoptile in comparison to the control of phosphate buffer solution alone.

Coinciding closely with the observed high hormone accumulation in the peach seeds on June 15, was the gradual change of the endosperm from a free-nuclear state to a cellular condition which took place in 1958 for the Redhaven variety from approximately June 14 to June 18. Luckwill (57) also found a close correlation between high hormone content of the seeds of the Beauty of Bath apple and the time of cellular formation of the endosperm. He believed the location of the natural hormone may be in the endosperm since the nucellus was almost completely absorbed by the endosperm at the time he made these observations. By contrast, the Redhaven peach endosperm did not appear to be any larger than one-tenth the size of the nucellus at the time of the last sampling, June 28.

High daily rate of fruit drop of Redhaven, commonly referred to as "June" drop, between June 21 and June 28, 1958 appeared after the high concentration and content of natural hormones of the peach seeds observed June 15 (Figures 4, 16 and 17). This did not conform with the findings of Luckwill (58) working with the apple. He reported that high hormone content of apple seeds corresponded with the termination of the "June" drop.

As the avena straight growth tests revealed the presence of natural occurring hormones in the seed, studies were made in hopes of identification. Dried chromatographed papers which had been spotted with 0.05 milliliter of the neutral fraction and of the acid fraction of the seed extracts of the June 15

sample were scanned with a 2537 "Mineralight" for ultraviolet absorption. A light blue spot was found at Rf[1] 0.89 on the chromatograph paper of the neutral fraction, but no noticeable spot was observed on the chromatograph paper of the acid fraction.

Chromatograph papers were spotted with EtIA and with IAA in the solvent 8:1:1 volume ratio of 2-propanol, ammonia and distilled water, dried, and scanned with the "Mineralight" as for the neutral and acid fractions of the peach seed extracts. A blue spot was found for the chromatographed EtIA at Rf 0.91, and a white spot was evident for the chromatographed IAA at Rf 0.50.

For further evaluation these chromatograms of the acid and neutral fractions containing the natural extracted hormones were cut into strips and biologically assyed with the avena straight growth test. Growth responses were observed at the Rf ranges of 0.5 to 0.6, 0.6 to 0.7, and 0.7 to 0.8 for the acid fraction and of 0.8 to 0.9 and 0.9 to 1.0 for the neutral fraction (Table 14 and Figures 18 and 19). The Rf of these growth response areas would be about 0.63 for the acid fraction, and 0.93 for the neutral fraction the mid-points of each Rf spot.

Sen and Leapold (86) have reported the use of Erlicher's reagent to determine the presence of indole compounds. Accordingly, Erlicher's reagent

---

[1] Rf is the distance the substance migrated, divided by the distance the solvent front migrated.

Table 14

The Summary Data on the Chromatographed Paper of Diethyl Ether Extracts of Redhaven Peach Seeds Using 2-Propanol, Ammonia, Water (8:1:1 v/v) Solvent and Determined by Avena Straight Growth Test

	Rf range[2]										Control
	.0-.1	.1-.2	.2-.3	.3-.4	.4-.5	.5-.6	.6-.7	.7-.8	.8-.9	.9-.10	
Acid Fraction											
Average final coleoptile length (mm)	7.80	7.80	7.70	7.85	7.80	8.75**	8.20*	8.60**	8.00	8.05	7.80
$S_{\bar{x}}$	0.41	0.33	0.33	0.41	0.60	0.40	0.40	0.66	0.22	0.54	0.41
"t" test between strips	0	0.7	0.9	0.2	4.2[1]	3.1[1]	1.6	2.7[1]	0.1		
Final length of coleoptile/5 mm (its initial length)	1.56	1.56	1.54	1.57	1.56	1.75	1.64	1.72	1.60	1.61	1.56
Neutral Fraction											
Average final coleoptile length (mm)	7.45	7.20**	7.50	7.60	7.55	7.55	7.60	7.80	8.35**	11.05**	7.60
$S_{\bar{x}}$	0.52	0.25	0.45	0.66	0.47	0.42	0.44	0.42	0.45	0.73	0.24
"t" test between strips	1.4	1.9	0.4	0.2	0	0.3	1.1	2.8[1]	9.9[1]		
Final length of coleoptile/5 mm (its initial length)	1.49	1.44	1.50	1.52	1.51	1.51	1.52	1.56	1.67	2.21	1.52

*Significantly different than control at 5 percent level.

**Significantly different than control at 1 percent level.

[1]Significantly different between strips at 1 percent level.

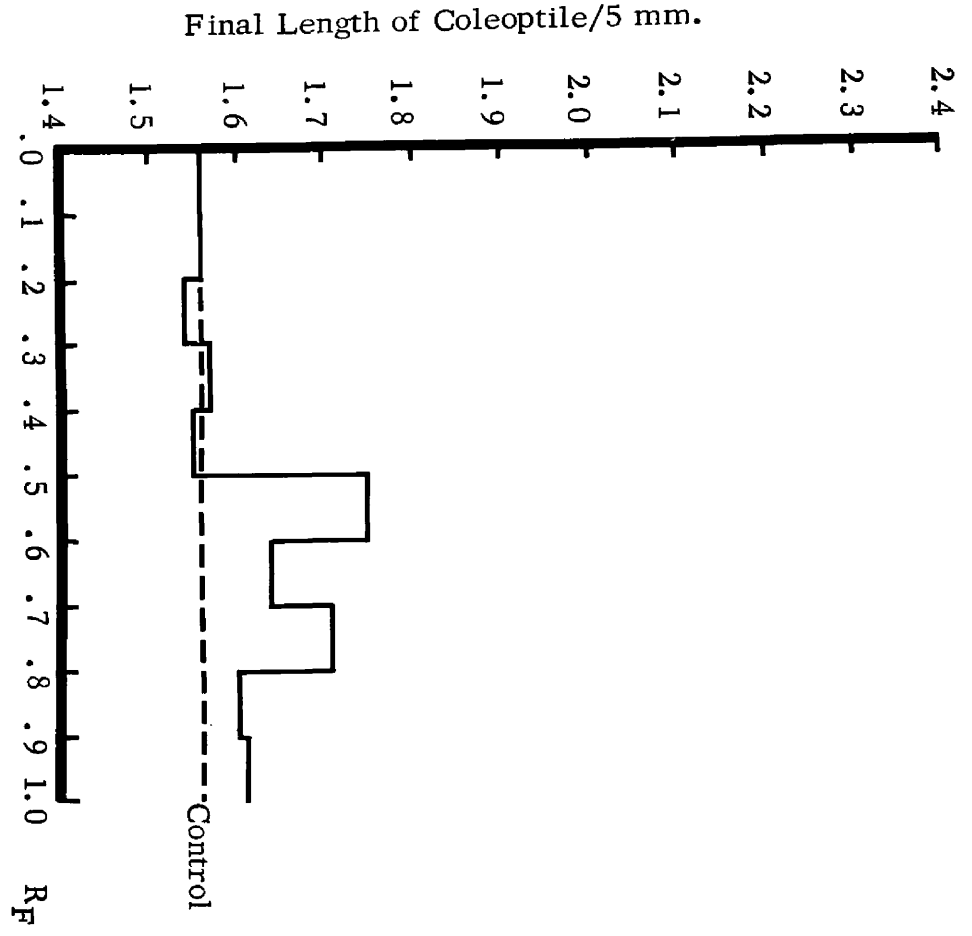
[2]Rf range is the distance the solvent front migrated divided into tenths.

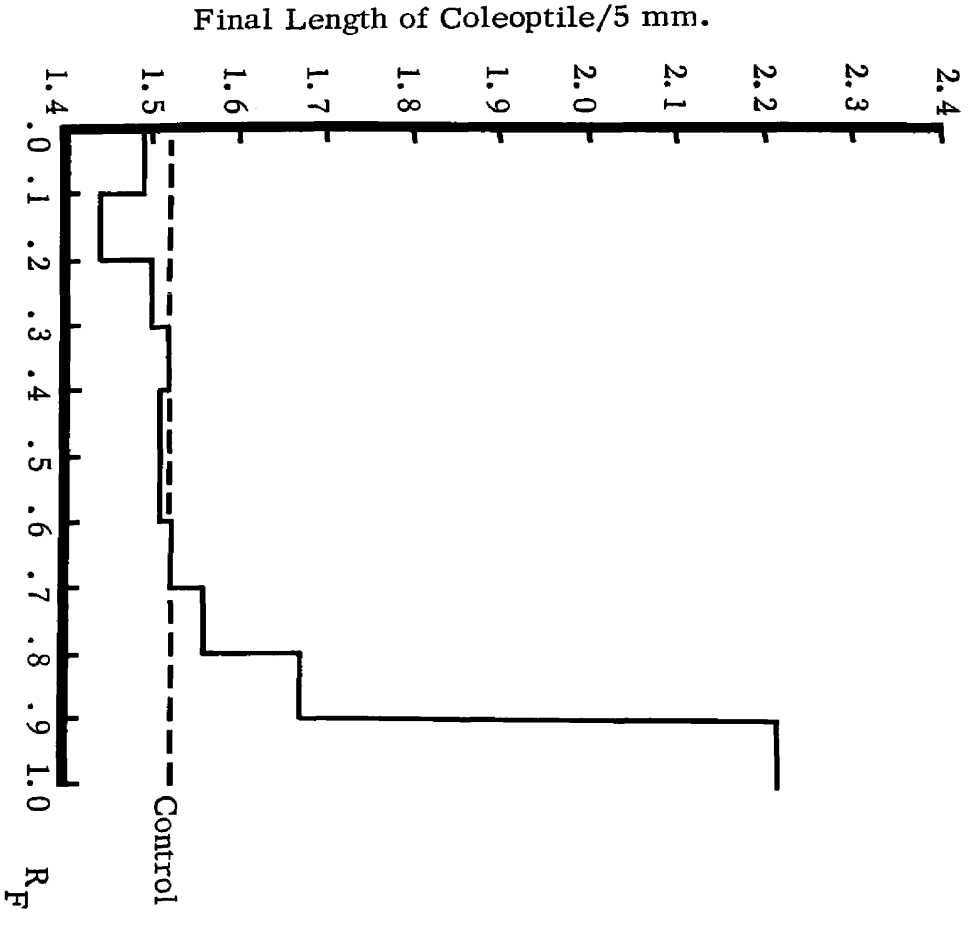
Figure 18

Histogram of the chromatographed paper of the acid fraction of the ether extracts of Redhaven seeds collected on June 15, 1958, as determined by the avena straight growth test. Acid fraction chromatographed in 2-propanol:NH_3:H_2O (8:1:1 v/v).

Figure 19

Histogram of the chromatographed paper of the neutral fraction of the ether extracts of Redhaven seeds collected on June 15, 1958, as determined by the avena straight growth test. Neutral fraction chromatographed in 2-propanol:NH_3:H_2O (8:1:1 v/v).


Final Length of Coleoptile/5 mm.
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
.0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0
R_F
Control


Final Length of Coleoptile/5 mm.
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
.0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0
R_F
Control

was sprayed on chromatograph papers of the neutral and acid fraction of the extracted hormones. No noticeable spot was formed on the chromatograph paper of the acid fraction treated with Erlicher's reagent. However, a light blue spot was located on chromatograph paper of the neutral fraction. Chromatograph papers of EtIA and IAA were also treated with Erlicher's reagent. A blue spot was found at Rf 0.95 on the EtIA paper, while an orange spot was evident at Rf 0.50 on the IAA paper.

On the basis of these evaluations, it appears that the natural hormone present in the acid fraction of the extraction from the Redhaven peach seeds is not IAA. There is a strong indication, however, that the natural hormone present in the neutral fraction of the extraction is EtIA.

DISCUSSION

Two plant regulators, N-1-naphthylphthalamic acid (NPA) and naphthaleneacetic acid (NAA), when applied to peach trees three and 42 days after bloom, respectively, at the correct concentration have significantly reduced the fruit set as was found in this study. But it has been observed that NPA applications to peach trees later than seven days after full bloom do not reduce the fruit set (2). Whereas, Hibbard and Murneek (33) and Kelley (44) have found that an application of NAA at low concentrations does not result in effective fruit thinning when applied to peach trees during a period of approximately 30 days following bloom. Therefore, it is apparent that the application of the two materials are effective in reducing the set of blossoms and fruits from peach trees at two different developmental stages of the reproductive organ. A histological study of the developing flower and fruit was therefore necessary to establish the stage of development during which the application of these materials to peach trees would result in effective thinning.

Ovules of Redhaven peaches were examined anatomically at full bloom, at four, and at seven days after bloom in 1958. From these examinations, it was observed that megagametogenesis was in progress in the peach ovule during the seven day period after bloom (Table 10). Syngamy did not take place in the peaches of the Redhaven trees used in this study in 1958

until during a seven-day period between seven and 14 days after bloom (Table 10). This conformed with the findings of Harrold (32) who reported that syngamy took place about nine days after bloom in the peach of the Carman variety.

The NPA applications made three days after bloom on young Redhaven trees significantly reduced the number of blossoms in 1958 (Table 10). This is a strong indication, therefore, that the process by which peach flowers are removed by the result of an NPA application may involve the prevention of syngamy. Similarly, Luckwill (62) has observed that NAA sprayed on open apple flowers before pollination induced incompatibility between pollen tubes and stylar tissue, and therefore has suggested that this may account for thinning action of NAA when applied at this stage.

Further evidence to support this hypothesis of peach thinning with NPA was found by observing the number and type of blossoms which dropped from the NPA treated trees. A much greater number of blossoms dropped from the NPA treated trees than from the control trees during the first heavy daily drop of blossoms of the control trees.

On the basis of the suggestion by Murneek (70) that apple blossoms of the first heavy drop may be divided into unpollinated flowers and pollinated flowers where syngamy has failed, the pistils of the peach flowers which dropped from the control and NPA treated trees during the first heavy drop were examined for growth and development. A higher portion of the abscissed

flowers from the NPA treated trees contained a "developed" pistil than those flowers which dropped from the control trees. On the basis of this finding and assuming that pollination had stimulated pistil development (77), the increase drop from the NPA treated trees over the controls apparently was due to a heavier drop of pollinated flowers.

Microscopic studies of abscissed flowers with a "developed" pistil from the NPA treated trees revealed no evidence that fertilization had taken place. Thus, it appears that NPA may hinder, in some manner, the act of syngamy either indirectly or directly, and subsequently causing abscission of the flower. NPA evidently does not interfere with the pollination, assuming that the high number of abscissed flowers with "developed" pistils indicate pollination.

Treatments of NAA at 30 ppm made on June 7 and on June 14, 1958 resulted in a significant reduction of fruit set on Redhaven peach trees (Table 3). However, a treatment of NAA at the same concentration made on June 21 did not result in any significant fruit reduction (Table 3). This would indicate that the Redhaven variety was sensitive to NAA used at 30 ppm for thinning purposes 35 and 42 days after bloom, but not 49 days. For the treatments of NAA which reduced fruit set significantly this would be seven and 14 days after "shuck-off", a means of timing used by Kelley (44) in Illinois.

Hibbard and Murneek (33) found the most favorable time to thin the

Elberta peach with NAA was 34 days after bloom, but Kelley (44) found the most favorable time to this this variety with NAA was 38 days after bloom one year and 48 days after bloom another year. However, it is interesting that in each case the effective applications of NAA occurred approximately 14 days after "shuck-off". These investigators found no significant thinning when NAA was applied at "shuck-off", 21 days and 28 days after "shuck-off", but thinning occurred when the treatment was made seven and 14 days after "shuck-off". From these experiences it appears that timing the applications of NAA resulting in effective thinning could be based more advantageously on days after "shuck-off" rather than days after bloom.

Relating the time of NAA application with "June" drop, Kelley (44) observed that applications of NAA made during or after "June" drop, 21 to 28 days after "shuck-off", were not effective in thinning peach trees. These observations corroborate the findings of this study as the NAA application made on Redhaven peach trees June 21, 1958 at the beginning of the second period of "June" drop did not result in significant thinning. However, the two earlier treatments made on June 7 and on June 14 gave significant thinning (Table 3, and Figure 4). The two treatments of June 7 and June 14 were made during the first period of "June" drop occurring June 7 to June 21. From the results of this study it would seem that Kelley's (44) reference to "June" drop would conform to the second period of "June" drop shown in Figure 4. This second period of "June" drop was typified by a heavier daily fruit drop than the first period.

The NAA application on Redhaven trees June 14 appeared to delay the second period of "June" drop three or four days (Figure 4). Nevertheless, this period was not extended over any longer time by the NAA applications contrary to the findings of Kelley (44) for the Elberta peach and of Teubner and Murneek (96) for the Jonathan apple. The delay in fruit abscission by the use of NAA on the Redhaven peach trees as found in this study may perhaps be explained by the temporary positive effect of the NAA on the abscission zone of the fruit as described by Murneek and Teubner (76) for the pedicels of apples when NAA is used for thinning.

An attempt was made to relate the three stages of development of the peach fruits as reported by Tukey (98) with the effectiveness of NAA treatments in thinning. The two treatments of NAA on the Redhaven variety in 1958, which significantly thinned the fruits, were made during Stage I, the time of rapid increase in size of the pericarp when the development of the embryo was in an "arrested" state (Figure 7). The late NAA treatment which did not significantly reduce fruit set, was made at the beginning of Stage II, June 21, when the rate of embryo growth was rapid and the rate of growth of the pericarp had declined (Figure 7). Thus, it would appear that after the embryo enters the period of rapid growth stage, thinning can no longer be accomplished because of the increasing resistance of the embryo to abortion. Rietsema, Satina and Blakeslee (82) found evidence also that young Datura

embryos are more sensitive to the effects of indoleacetic acid than advanced embryos.

Assuming that embryo abortion is involved in the thinning mechanism of NAA, it appears that the embryo is susceptible to NAA applications when it is in the filamentous and the spherical stages and that it is resistant in the transitional and later stages (Tables 3 and 10). Teubner and Murneek (96) believed that the apple embryo was most susceptible in the 8- to 16-celled stage which would place it in the filamentous stage, as classified by Meyer (67). It is doubtful that the use of NAA on peaches at the non-injurious rate of 30 ppm before June 7, one week after "shuck-off", would effectively reduce the fruit set, since Hibbard and Murneek (33) and Kelley (44) found that an application at "shuck-off" or earlier was not effective. The Redhaven peach embryos were about 46-celled one week after "shuck-off" and, therefore, were more advanced than the 8- to 16-celled embryos of the apple (Table 8). Embryos of the Halehaven variety were similar to Redhaven in development throughout 1957 and 1958 (Tables 6 and 7). It may be conjectured from this that the peach embryo is not as sensitive to NAA during the early part of the filamentous stage as the apple embryo. However, failure of NAA to reduce the fruit set when the embryos are in the early part of the filamentous stage may be attributed to the limited leaf surface on the peach trees during this early stage, resulting in reduced absorption of the thinning agent.

It is believed that a close study of the early development of the peach embryo would contribute to the subject of fruit thinning by NAA. The type of embryo found in the Halehaven and the Redhaven peach appeared to be similar to that of the Geum urbanum which was classified by Johansen (39) as the Asterad type. This type is typified by a single transverse division in the zygote which is followed by an oblique division in the terminal cell and a second transverse division in the basal cell. Teubner and Murneek (96) observed the same type of development in apple embryos of the Jonathan variety. Apparently, the embryo type for the apple differs with the variety as Meyer (67) reported an embryo development similar to the Solanad type for McIntosh.

The primary difference in the early embryo development between the apple and the peach appears to be the length of time the embryo remains in the filamentous stage. The peach embryo as found in this study remained in the filamentous stage 28 days (Tables 8, 9 and 10) as compared to 10 days for the apple embryo reported by Meyer (67). The length of time in which the peach embryo remained in the spherical and transitional stage (Tables 8, 9 and 10) was about the same as found in the apple by Meyer (67). Therefore, the so-called "arrested" stage composed of the filamentous and spherical stages of the peach embryo would be about two or three weeks longer than that of the apple with the longer time of the filamentous stage creating the difference.

The length of time of the "arrested" stage of the peach appears to coincide closely with that of the pear (68). Mitchell (68) observed that embryos of the Bartlett pear remained microscopic for eight weeks after bloom after which time they increased in length rapidly. This compares to about eight weeks after bloom for the peach embryo as observed in this study.

Luckwill (62) found that the greatest thinning effect obtained from NAA on the Crawley Beauty variety of apple was during cellular formation of the endosperm. Based on Luckwill's report, the thinning action of NAA on peach trees was studied to determine if it was related to endosperm development. The two NAA treatments made in 1958 on Redhaven peaches, which resulted in significant thinning, were applied about three days and 11 days before cellular formation of the endosperm (Figure 7). Whereas, the NAA treatment which did not result in significant thinning of Redhaven peaches was made three days after cellular formation (Figure 7). The NAA applications 42 days after bloom on Halehaven peach trees in 1957 and 1958 were just before cellular formation of the endosperm and also resulted in significant thinning (Table 3). Therefore, it appears that the application made when the endosperm is in the free-nuclear state just before cellular development, may be vital for the thinning action of NAA.

Observations from this study indicate that cellular formation of the endosperm occurred about two weeks after "shuck-off". Assuming that the time of "shuck-off" reflects seed and fruit development more so than the

time of full bloom, it becomes evident that the NAA treatments made by Hibbard and Murneek (33) and Kelley (44) which gave effective thinning must have been applied just before cellular formation in the endosperm.

It would be difficult to state that the thinning action of NAA is more dependent on the development of the endosperm than the development of the embryo, since they are inseparable. But, it would be even more difficult to believe that the thinning action of NAA on peaches is independent of the spectacular cellular change of the endosperm.

If the time of "shuck-off" can be used for determining the time of the cellular formation of the peach endosperm for the purpose of applying a thinning agent, as NAA, it would seem that a more exact method could be used. The process of "shuck-off" occurs slowly over a period of about a week, the rate of which can be altered easily by rain and wind. Linear size of the young fruit increased very rapidly before and during the cellular formation of the endosperm, increasing at the rate of about two millimeters for every three days (Figures 5, 6 and 7). The size of the fruit of the Halehaven and Redhaven peach was approximately 30 millimeters, or one and three-sixteenth inches during the endosperm change (Figures 5, 6 and 7). The time of NAA application for effective thinning could be determined possibly by measuring the linear size of pericarp at three- or four-day intervals beginning about one week after "shuck-off". When the linear dimension of the fruit

reaches about 29 millimeters, or one and one-eighth inches, a NAA application at this time and at the right concentration should give effective thinning.

From the suggestion by Luckwill (57, 59) that the accumulation of natural hormone in the apple endosperm could control abscission of the young fruit, a study was made in 1958 to relate the natural hormone content and concentration in Redhaven peach seeds with the natural "June" drop. It was found from this study that the natural hormone content of the peach seeds was greatest on June 15, which was during the first period of "June" drop, about one week before the second period of "June" drop (Figures 4 and 17). In contrast, Luckwill (59) found a low accumulation of hormone in apple seeds during "June" drop.

The concentration of the natural hormone in the neutral fraction of the peach seed extracts was lower during the second period of "June" drop than at the other sampling dates (Figures 4 and 16). Even the concentration of this hormone did not appear to be related to natural fruit drop.

Since the endosperm of the peach seeds did not appear to be any larger than the nucellus during the sampling period, most of the natural hormone biologically assyed could have come from the nucellus. Luckwill (57, 58) was working with apple seeds in which the endosperm made up most of the volume. But since the greatest natural hormone content was found at the time of cellular formation of the peach endosperm, it would seem that the largest portion of the hormone was seated in or near the embryo sac.

The natural hormone accumulation in peach seeds decreased as the rate of growth of the endosperm and embryo increased from June 21 to June 28, 1958 (Figures 7, 16 and 17). After finding the same relationship between the hormone content and embryo growth in apple seeds, Luckwill (57) suggested that the hormone plays a part in embryo development during the period of its rapid growth. Teubner and Murneek (96) found that the activity of the indoleacetic acid oxidase system of apple embryos was greatest in the rapidly developing embryos. The activity of this system may explain partially the decrease of hormone accumulation during the increased growth of the embryo, assuming that the hormones which were isolated could be oxidized by the same system.

It is difficult to relate the thinning effectiveness of NAA on peach trees with natural hormone accumulation in the peach seeds. The two treatments of NAA on Redhaven peach trees that resulted in significant thinning were made one day and eight days before the time of the greatest natural hormone content in the peach seed (Table 3, and Figure 17). The NAA application made six days after the time of the greatest hormone content resulted in no significant thinning of peach trees (Table 3 and Figure 17). It is possible that the addition of an exogenous supply of NAA to the seed would result in the inhibition of the embryo through competition with the natural hormone of a high content, as Teubner and Murneek (96) suggested in thinning apples.

More work is necessary to substantiate or disprove this suggestion.

Of the two apparent hormones extracted from the Redhaven peach seeds, there is a strong indication that one of the natural hormones was ethyl indoleacetate. There is no positive evidence that the other hormone was a indole derivative compound. Teubner (94) identified ethyl indoleacetate as the natural hormone present in apple seeds. Of three natural hormones isolated from seeds, fruits, and leaves of apples, Luckwill (63) found one hormone with properties similar to ethyl indoleacetate. The other two hormones did not appear to be indole derivative compounds. The two unidentified natural hormones were found to be present in apple seeds only before and during the time of cellular formation of the endosperm. The indole derivative hormone was present before, during and after the time of the endosperm change from free-nuclear to cellular form.

A natural inhibitor to the growth of coleoptile sections of oats was found in the neutral fraction of the June 15 extract from peach seeds (Table 14 and Figure 19). The inhibitor was located on chromatograms at Rf 0.1 to 0.2, using a 2-propanol, ammonia, water solvent of a 8:1:1 volume ratio. It was not possible to identify it. Luckwill (63) isolated two inhibitors from apple seeds of which one was present only before and the other present only after cellular formation of the endosperm. He could not positively identify them.

The results of these studies indicate quite definitely that two plant

regulators, NPA and NAA, are promising blossom and fruit thinning agents of peach trees. The use of NPA has the advantage of decreasing blossom set early in the season, for increased fruit size, and for an increased number of flower buds the following spring. At the present time NAA appears to be the more favorable of the two as a thinning agent because of the possibility of thinning after the danger of spring frost.

SUMMARY

1. A series of studies were conducted during the period of 1955 through 1958 to determine the time of application, and the value of naphthaleneacetic acid (NAA), N-1-naphthylphthalamic acid (NPA), and 3-chloro-isopropyl-N-phenyl carbamate (3-chloro IPC) for blossom and fruit thinning of peaches.

a. Redhaven trees were over thinned by NPA at 300 ppm applied three days after full bloom in 1956 and 1958. Trees treated with NPA at 200 and 400 ppm in 1955; with NPA at 200, 250 and 300 ppm in 1957; and with NPA at 250 ppm in 1958, had to be hand-thinned. In 1956, Halehaven trees were thinned effectively with 300 ppm NPA applied three days after full bloom. Halehaven trees treated with NPA at 200 and 400 ppm in 1955; with NPA at 200, 250 and 300 ppm in 1957; and with NPA at 250 and 300 ppm in 1958, required hand-thinning.

b. The use of 3-chloro IPC at 400 ppm on Redhaven and Halehaven four weeks after bloom in 1956 and 1957 did not thin sufficiently to eliminate hand-thinning. It's use did hasten ripening of the fruit as measured by firmness of flesh, and did result in undesirable "beaked" peaches (Figure 1). Because of the undesirable shape of the peaches resulting from it's use, 3-chloro IPC was eliminated from further testing.

c. NAA at 300 ppm applied 42 days after bloom, two weeks after "shuck-off", in 1957 and 1958, gave significant fruit thinning on Redhaven

and Halehaven trees. An application of NAA on Redhaven trees 35 days after bloom, one week after "shuck-off", at 30 ppm resulted in significant thinning in 1958; but an application of NAA 49 days after bloom resulted in no apparent thinning.

d. The size of Redhaven and Halehaven peaches was increased in 1956 by the use of NPA at 300 ppm. However, the use of 3-chloro IPC at 400 ppm increased the size of the fruit of only the Redhaven variety.

e. An application of NAA at 30 ppm 42 days after bloom on Redhaven and Halehaven trees in 1957 significantly reduced the number of flower buds in the spring of 1958. There was a significant increase in the number of flower buds on Redhaven trees treated with NPA at 300 ppm three days after bloom in 1957 when compared to those trees receiving no treatment.

2. In order to evaluate the pattern of fruit drop, a study was initiated in 1958 to compare the natural drop of blossoms and young fruits from Redhaven peach trees with the drop resulting from the use of NAA and NPA as thinning agents.

a. The daily blossom drop of the control trees was heaviest during a period of seven days after bloom. Most of the drop from the Redhaven peach trees, which received an application of NPA at 300 ppm three days after bloom took place during this same period. Sixty-five percent of the blossoms

which dropped seven days after bloom from the NPA treated trees were pollinated, whereas only approximately 20 percent of the blossoms which dropped from the control trees were pollinated. There was no indication that syngamy had taken place in the pollinated pistils of the abscissed flowers.

b. The so-called "June" drop of the control trees was divided into a first and second period of heavy fruit drop. The first period of heavy fruit drop occurred approximately 35 days after bloom and remained constant for 14 days. The second period of drop occurred about 49 days after bloom and lasted for 11 days. An application of NAA at 30 ppm 42 days after bloom appeared to delay the second period of "June" drop by three days, but it did not extend this period for any longer time.

3. Anatomical investigations were made in 1957 and 1958 on Halehaven and Redhaven peaches so as to relate the development of the embryo, endosperm, and pericarp with the time of application of the plant regulators, NPA and NAA, for more effective thinning.

a. Microscopic examinations of the Redhaven peach revealed that syngamy took place between seven and 14 days after bloom in 1958. The peach embryo of both the Halehaven and Redhaven varieties grew very slowly in length, width and cell number for about 35 days after syngamy during the so-called "arrested" stage. The embryo continued in the filamentous stage, linear in shape, for a 28-day period, a form which developed immediately

after the first division of the zygote, 14 days after bloom. The embryo became bulbous in shape, the spherical stage, approximately 42 days after bloom which continued for seven to ten days. Rapid growth of peach embryo was observed about 49 days after bloom during the transitional stage, when the embryo changed to a triangle shape. The rapid rate continued throughout the early cotyledonary stage which followed seven days later.

b. The endosperm of the peach continued in a free-nuclear state for approximately 32 days after syngamy and changed to a cellular condition about 42 days after full bloom, two weeks after "shuck-off". The endosperm grew rapidly in width and length approximately seven days after cellular formation.

c. The seed and pericarp of the peach continued to grow at a rapid rate for a period of 49 days after bloom when a sharp decline occurred. The endocarp of the peach began to harden about 35 days after bloom. "Shuck-off" was found to occur approximately 30 days after full bloom in 1957 and 1958 for both the Redhaven and Halehaven varieties.

4. Fruit collections from Redhaven trees were made June 2, 1958, four weeks after bloom, and at weekly intervals thereafter for a period of four weeks in order to determine the relative concentration and content of natural hormones occurring in peach seeds.

a. The natural hormone content of both acid and neutral fractions

from peach seed extracts reached a maximum in quantity on June 15, six weeks after bloom. The concentration of the natural hormone in the seeds was highest on June 15 for the acid fraction of the extract. The hormone concentration of the neutral fraction was relatively the same for the first three dates of sampling, June 2, 7 and 15, after which time it decreased markedly.

b. Chromatograph papers were spotted with the acid and neutral fractions of extracts from peach seeds and were migrated with a 8:1:1 volume ratio solvent of 2-propanol, ammonia, and water. From various qualitative tests, it appeared that the neutral hormone present in the acid fraction of the extraction from the Redhaven peach seeds was not IAA. There was a strong indication, however, that the natural hormone present in the neutral fraction of the extraction was EtIA.

The results of these studies indicate quite definitely that two plant regulators, NPA and NAA, are promising blossom and fruit thinning agents of peach trees. Of these two, NAA appears to be more favorable as a thinning agent because of the possibility of thinning after the danger of spring frost.

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