# EMBRYO AND ENDOSPERM DEVELOPMENT IN THE PEACH (PRUNUS PERSICA, (L.) BATSCH.) IN RELATION TO THE INDUCTION OF FRUIT ABSCISSION WITH NAPHTHALENEACETIC ACID

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# EMBRYO AND ENDOSPERM DEVELOPMENT IN THE PEACH (PRUNUS PERSICA, (L.) IN RELATION TO THE INDUCTION OF FRUIT ABSCISSION WITH NAPHTHALENEACETIC ACID

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# ABSTRACT

# EMBRYO AND ENDOSPERM DEVELOPMENT IN THE PEACH (*PRUNUS PERSICA*, (L.) BATSCH.) IN RELATION TO THE INDUCTION OF FRUIT ABSCISSION WITH NAPHTHALENEACETIC ACID

By Stanley Josiah Leuty

Naphthaleneacetic acid (NAA-30ppm) was applied to Redhaven and Halehaven peach trees approximately six days prior to, during and six days after the onset of cell wall formation in the endosperm. The NAA spray applied just as cell walls began to form in the endosperm of the Redhaven fruit samples induced significant (70 percent) abscission, whereas, the earlier spray applied when the endosperm was completely free nuclear was ineffective. The final NAA spray, applied to Redhaven when the endosperm of samples was cellular, also induced significant (65 percent) abscission. No significant abscission was induced in Halehaven irrespective of time of application. Subsequent histological investigations conducted on samples harvested at times corresponding to the three NAA applications, revealed that the endosperm of the Halehaven fruits was either completely free nuclear or completely cellular at the times of application.

An examination of several parameters for the purpose of finding an index of the onset of cell wall formation, revealed that fruit and seed measurements were more reliable as indices than heat units, "days after full bloom" or "days after petal fall." Pericarp length was found to be a particularly reliable index of this stage of endosperm

development. Pericarp length at the time of cytokinesis in the endosperm varied between cultivars but was remarkably consistent within a cultivar from season to season.

The variations in pericarp length and the variations in endosperm development of Redhaven peaches in a population sample of fruits harvested at the time of cytokinesis in the endosperm of a ten fruit sample, were used to provide a basis for a study of the site of action of NAA. Approximately 38 percent of the fruits (the largest fruits in the sample) contained endosperm in which cell walls were beginning to form. This figure represents the fruits that would have been expected to survive an NAA spray applied at the time the sample was harvested. The theoretical value of 38 percent survival was remarkably consistent with actual thinning results, in which 30 percent fruit survival followed an NAA spray applied when cell walls were just beginning to form in the endosperm of a ten fruit sample.

A histological study of the embryos and endosperms of naturally abscising Redhaven peach fruits in comparison to those of persisting fruits, revealed that abscising fruits contained either abnormal embryos and endosperms, or smaller embryos and endosperms, than were found in the persisting fruits.

The effects and site of action of NAA were studied by applying the material at 60 ppm, and then collecting treated and non-treated fruit samples at 14 hours, 1 through 7 days and 14 days after treatment, and again at harvest. NAA reduced the rate and amount of fleshy pericarp growth but did not markedly affect overall growth of the seed. The rate of embryo enlargement and development was markedly enhanced by the

NAA; the endosperm of sprayed fruits formed cell walls more rapidly than that of non-sprayed fruits.

Ring-labeled NAA-$C^{14}$ was found to accumulate in the seeds (dry weight basis) of peach fruits following foliar application. This supports the premise that the seed is the site of NAA action in inducing fruit abscission.

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# INTRODUCTION

The selective removal of a portion of the peach fruits early in the growing season insures maximum size and quality of the remaining fruits, and promotes continued productivity of the tree. Currently, hand and pole thinning are the methods most commonly employed but these methods are expensive and often difficult to perform sufficiently early in the growing season. By the time that hand and pole thinning operations have been completed, much of the trees' carbohydrate reserves have been utilized by those developing fruits which are removed in the thinning operation. Consequently, reserve food is wasted, and the late thinning has had little promotional effect on flower bud initiation. In peach growing regions where the risk of a late spring frost is severe, however, the earlier blossom thinning methods (utilizing hand-operated "switches," or caustic sprays) are of limited value. In such regions <u>fruit</u> thinning methods are preferred.

Investigations into the use of growth regulators as a more timely, efficient, and economical means of thinning peaches began in the early 1940's. Despite years of research no growth regulator is available that will consistently thin peaches satisfactorily.

Naphthaleneacetic acid (NAA) has proven to be fairly satisfactory as a thinning agent for apples, and it will induce fruit abscission in peaches, but its performance with the latter fruit has been erratic and inconsistent. A survey of the literature reveals that NAA has induced satisfactory abscission in peaches when the material was applied four to six weeks after full bloom, but applications during this interval have not always resulted in satisfactory thinning. Since NAA occasionally induces fruit abscission, it was felt that peaches may reach a particular stage of development (during the interval four to six weeks after full bloom) at which they are sensitive to the material.

The thinning action of NAA has been attributed to inhibition of embryo growth (91, 107), the reduction of natural auxin production (44), a nutritional competitive mechanism (105), and various other mechanisms (26, 44, 73, 74, 88, 99, 117). Determining the distribution within the fruit, of foliar applied NAA, would help to identify the site of action, contribute information concerning the mechanism of action, and provide a basis for the more consistently effective use of NAA in peach thinning.

Because of the inconsistent performance of NAA as a peach thinning agent, and the dearth of specific information concerning the manner in which NAA induces peach abscission this study was instigated to determine: (1) if the NAA-sensitive period of peach can be related to a specific stage of fruit development; (2) a convenient parameter which can be employed to identify the NAA-sensitive period; (3) whether or not the same parameter can be employed as a general timing index for certain thinning sprays for various peach cultivars; (4) the development of the embryo and endosperm in naturally abscising fruits as a basis for a comparative study of embryo and endosperm development in NAA treated fruits; and (5) the site of action of foliar applied NAA.

# LITERATURE REVIEW

## Development of Chemical Thinning

In 1941 Burkholder and McCown (13) reported upon their attempt to increase the fruit set of apples using bloom sprays of various chemicals including naphthaleneacetic acid (NAA); instead of increasing the fruit set, the NAA caused a marked reduction--a reduction which increased with an increase in concentration of the NAA applied. In 1943 Greene (41) endeavored to increase the fruit set on notoriously light setting apple cultivars with early sprays of NAA and various other growth regulators. His results with NAA were similar to those achieved by Burkholder and McCown.

Schneider and Enzie (97, 98) were the first investigators to apply NAA for the specific purpose of removing excess apple fruits. Their experiments demonstrated that the material, particularly in the amide form, was promising as a chemical thinning agent for apples. Davidson et al. (24) confirmed the findings of Schneider and Enzie and further found that either full bloom or petal fall applications of NAA resulted in satisfactory thinning of certain apple cultivars. Other investigators (48, 104) compared the thinning effects of NAA with those of the caustic blossom removal sprays (DN-type compounds) and were favorably impressed with the potential of the growth regulating material to induce fruit abscission in the apple.

Chemical thinning investigations did not progress as rapidly with the peach as with the apple. Experiments with caustic spray materials were conducted by many investigators including Batjer and Moon (2), Hoffman and VanDoren (47), Kenworthy (60), Murneek and Hibbard (90), and Havis (45). Their results, while somewhat variable, demonstrated that caustic materials applied to peach trees during the bloom period reduced the set of fruit. However, in northern peach growing regions where the risk of a late spring frost is severe, blossom thinning methods (utilizing either hand-operated "switches" (7, 89, 118) or caustic sprays) are of limited value. In such regions fruit thinning, either by pole or by hand is preferable (7, 118).

Growth regulating type chemicals were first tried as thinning agents on peaches in the mid-1940's. In 1947 two groups, Southwick, et al. (100) and Murneek and Hibbard (90), published results of experiments comparing the thinning effects achieved with caustic versus NAA sprays on peaches. The caustic materials resulted in a significant reduction in fruit set when applied at full bloom, but NAA did not induce significant thinning. The growth regulator was applied at various times from full bloom up to 20 days after by Southwick and his group but in no instance did thinning occur. Murneek and Hibbard on the other hand, found that applications of NAA to various peach cultivars at full bloom actually increased the fruit set over that of the controls. Hibbard and Murneek (46, 87) later reported successful thinning of Elberta peaches in two consecutive seasons, when NAA sprays were applied between one and two weeks after shuck-off.

Since Hibbard and Murneek's report of successful thinning with NAA, this material has proven to be inconsistent in the induction of abscission of young peach fruits. However, intermittent reports of successful thinning have been published by several investigators, including Langer

(62), Kelley (55, 56, 57, 58, 59), Lombard (65) and Donoho (29). These sporadic reports of success indicate that NAA may induce fruit abscission at certain stages of fruit development.

## Indices Employed in the Timing of NAA Thinning Sprays

After Schneider and Enzie (97, 98) pointed out the potential of NAA as a chemical thinning agent for the apple, many scientists attempted to identify optimal timing of spray applications for the most effective thinning (1, 25, 49, 50, 76, 86, 101, 102, 109). Thompson (109) observed that NAA can be used successfully for the spray thinning of apples if the material is applied during the interval between full bloom and about three weeks after full bloom. In general, the earlier a given concentration is applied during this interval the greater the thinning effect. Luckwill (76) suggested that cell wall formation in the endosperm of apple seeds may define the limits beyond which NAA would be ineffective in inducing abscission.

Since biennial bearing in peaches is not as serious as it can be with apples, very early thinning is not as essential. The timing of hand thinning of peaches is relatively non-critical (32, 33, 34, 35, 38, 61), although Tukey and Einset (116) reported that very early annual thinning promoted fruit bud initiation, and resulted in somewhat larger fruits at harvest. Hand thinning will increase fruit size at harvest, when it is carried out between the end of June drop and the beginning of the second period of rapid growth of the fleshy pericarp, but will result in an unnecessary depletion of carbohydrate material produced by the tree (5, 6, 17, 32, 63, 111).

Timing of NAA sprays for peach thinning appears to be extremely critical. Attempts to thin peaches with NAA sprays applied during the interval between full bloom and 20 days after, met with complete failure (90, 100). However, successful thinning was accomplished during two consecutive seasons when the application was delayed until one to two weeks after shuck-off (46). Batjer and Rogers (4) stated that, while NAA spray applications of 20 to 40 ppm at four to five weeks after full bloom sometimes resulted in satisfactory thinning of peaches, the results were usually quite variable. Thompson (108), reported that, because of inconsistent results NAA could not be recommended as a thinning material for peaches.

Kelley (55, 56, 57, 58, 59) considered "days after full bloom" (the timing index some research workers were using) to be too variable a basis for timing NAA thinning sprays for peaches. During three consecutive seasons he found that NAA sprays applied at two weeks after shuck-off satisfactorily thinned several peach cultivars. The timing of Kelleys' spray applications in relation to full bloom varied considerably during the three years; that is, the "two weeks after shuck-off" stage of development occurred at 38, 48 and 37 days after full bloom in 1952, 1953, and 1954 respectively.

Despite the fact that NAA sprays gave inconsistent results, as was again pointed out by Horsfall and Moore (52), this material was not abandoned entirely as a possible peach thinning agent. Lombard (65) and Lombard and Mitchell (66) investigated the changing levels of naturally occurring auxin(s) present in the seed, during the interval between four weeks after full bloom through June drop, in relation to the fruit abscission induced by NAA applications. Significant thinning resulted from NAA sprays applied at 35 and 42 days after full bloom; the natural auxin level of the peach seed reached its peak 45 days after full bloom.

## Growth and Development of the Peach

No attempt has been made to completely characterize the early embryogeny of the peach, as has been done for the apple by Meyer (84). Such embryological classifications have also been reported for numerous other genera (54, 82). However, investigations have been conducted which reveal considerable information about the early growth of the embryo (65), and other portions of both the seed and fruit. Connors (17) reported that the growth of the fleshy pericarp proceeds in three stages; during the first stage, the fleshy pericarp enlarges rapdily; this is followed by a period in which pericarp enlargement occurs very slowly, and then by a second period of rapid expansion. The existence of three distinct periods of growth in the peach was confirmed by Lilleland (63), Tukey (111), and Lott and Ashley (67). Tukey (111) carried this work several steps farther by inter-relating the growth of the fleshy pericarp, the endocarp, the nucellus and integuments, and the embryo. He was able to show that the duration of the first period of growth of the peach fruit is fairly constant in all cultivars, whereas, that of the second growth period is exceedingly variable. With early ripening cultivars, the duration of the second stage is shorter than with late maturing cultivars. Further, the third growth period varies somewhat in duration (longer in late- than in early-maturing cultivars) but the variation is not as marked as that of the second growth period. Tukey (111), and Harrold (43) reported that the growth of the integuments and nucellus of the anatropous peach ovule parallels the development of the fleshy pericarp during its initial period of rapid growth. The developing ovule reaches its full size by about eight weeks after full bloom (just prior to pit hardening).

Ragland (96) and Harrold (43) discovered that the embryo sac of a developing peach fruit begins to elongate shortly before fertilization. It continues to elongate rapidly so that by two weeks after fertilization the embryo sac has almost reached the chalazal end of the ovule. According to Harrold (43), the endosperm remains uninucleate for a period of nine to ten days after fertilization. By this time the primary endosperm nucleus has migrated to a point in the embryo sac half-way between the micropylar and chalazal ends. The first nuclear division is followed by a rapid succession of numerous divisions of the endosperm nuclei. The embryo sac becomes lined with a peripheral layer of free-nuclear endosperm in which cell walls begin to form about the sixth week after full bloom.

The zygote nucleus of some peach cultivars undergoes its first division about twelve days after fertilization (43, 65, 101). Development of the embryo as observed by Tukey (111), Harrold (43) and Lombard (65), proceeds very slowly until a considerable amount of cellular endosperm has formed. The embryo then enters a period of rapid growth (beginning just prior to the second stage of fleshy pericarp development) and completes its development by about the twelfth week after full bloom. This coincides closely with the beginning of stage three of fleshy pericarp development.

## Natural Abscission of Immature Fruits

Blake (6) reported on an investigation in which he compared the development of small versus large, and early versus late blooming peach flower buds. He discovered that the largest fruits developed from the largest flower buds, and that the fruits which were destined to drop could be detected by their size (or lack of it) by a given date. In addition, late blooming buds, despite hand pollination, showed a very high percentage of drop about two weeks after bloom. Blake recognized three distinct waves of natural abscission.

To better understand some of the causes of fruit abscission Detjen (27) investigated this phenomenon in relation to pollination, fertilization and embryo abortion. He divided the natural abscission pattern of peach fruitlets into two major waves. The first major wave of drops (abscising fruits), consisted for the most part of non-pollinated flowers and he postulated "external factor(s)" as the causal agent. The fruitlets in the second wave developed from fertilized flowers; however, embryo abortion which was caused by some internal factor, had occurred in these fruitlets. In his investigation of apple fruit abscission, Detjen (27) noted that with the exception of the very early drops most of the abscised fruitlets contained fertilized ovules. He again cited embryo abortion as the major causal factor of abscission. Detjen concluded from these investigations that, since lack of fertilization of the ovules of the abscised fruitlets made up only a small percentage of the total number of abscised fruitlets, physiological drop could not be attributed to a lack of pollination or fertilization. Further, the factor causing embryo abortion appeared to be the chief cause of abscission of immature fruits.

In a later study on the physiological dropping of fruits from a genetic point of view, Detjen and Gray (28) investigated and compared the shedding performances of trees of different kinds of fruits, of different species of fruits, of different cultivars and of trees of the same cultivar. No correlation was found in fruit abscission between different kinds or species of fruits. However, different cultivars shed fruits at a comparable time and rate, although variations were noted among unlike cultivars. They concluded that fruitlet shedding of each cultivar seems to be governed by separate factors, the manifestations of which are reasonably constant from year to year. In addition, they concluded that the genetic individuality of the trees is the overall factor governing the abscission of immature fruitlets.

Howlett (53) examined several factors affecting fruit setting of the Stayman Winesap apple, to gain some indication of the reasons for the notoriously heavy fruit drop of this cultivar. After the first wave of abscission the fruit set was usually reduced to 15 percent or less. The first drops consisted of non-fertilized flowers and fruitlets in which only one to three eggs had been fertilized. Howlett observed that irregularities occurred frequently during the meiotic divisions of megasporogenesis, resulting in non-functional gametes as well as weak and unstable embryos. Such irregularities were not as common in the heavier setting cultivar, Jonathan.

Natural drop patterns of the apple were investigated by Murneek (85) wherein four waves of abscission were observed, the peaks of which occurred at intervals of approximately twelve to fourteen days. Of the four waves only the first consisted largely of non-fertilized flowers. He also discovered that the variety of pollen used did not appear to have any characteristic influence on the nature of shedding of immature apples. Embryo abortion was considered to be the chief cause of abscission of enlarging fruits.

Bradbury (8) reported that the natural abscission pattern of the sour cherry was almost identical to that reported for the peach by Blake (6). There were three distinct periods of natural abscission; the first period attained its maximum rate about two weeks after full bloom; the second about one week later and the third, about three weeks after the second. Bradbury concluded that neither lack of pollination, nor failure of pollen tubes to reach the ovarian cavities was an important factor in the abortion of sour cherries. Her results suggested that fruit abortion was a nutritional problem.

Harrold (43) also found three naturally occurring waves of abscission of immature peach fruitlets similar to those described above for sour cherry. He suggested that the fruits in the second and third abscission waves, abscise because of an upset of the vascular system in the chalazal region of the developing ovule.

Tukey (112, 113) observed that embryo abortion occurred in early ripening sweet and sour cherries as a varietal characteristic. Embryo abortion was also a characteristic of abscising fruitlets of later drop waves. Tukey suggested that embryo abortion may be a varietal character which is responsible for the earliness with which some cultivars ripen their fruit. To test this hypothesis, Tukey (114, 115) induced embryo abortion early in the second phase of pericarp development; this resulted in a marked inhibition of fruit development followed in turn by shrivelling and abscission. However, destruction of the embryo at the time of the transition between the second and third stages of pericarp growth resulted in normal growth for a short time, followed by earlier ripening and failure of the fruit to reach full size. Destruction of the embryo during the third stage of pericarp development resulted in an increased rate of fruit growth, earlier ripening and occasionally increased size at maturity over that attained by the control fruits. Tukey concluded that early ripening of early cultivars of peaches and cherries was due in part at least, to embryo abortion, rather than embryo abortion being caused by the rapid increase in pericarp development.

Dorsey (36) examined developing peach fruits for the purpose of studying the causes of "Buttons" in the J. H. Hale peach. He concluded that the most probable cause of "Buttons" is single fertilization; that is, during the course of fertilization either the egg or the fusion nucleus (but usually not both) becomes fertilized in potential "Buttons." But, as Dorsey pointed out, the "Buttons" survive all three normal drop waves, ripen later than the other portion of the crop, and sometimes reach a surprisingly large size, despite the fact that embryo and/or endosperm development is suppressed or greatly retarded.

Brink and Cooper (9, 10, 11), Cooper, Brink and Albrecht (18), and Cooper and Brink (19, 20, 21, 22) devoted many years to the study of embryo-endosperm interrelationships to determine the various causes of ovule mortality. They examined ovule development in self-pollinated versus cross-pollinated plants, in interspecific and in intergeneric crosses, and concluded that the primary cause of hybrid seed collapse is poor growth of the endosperm. There was no indication that the embryo itself was a factor contributing to seed failure. Brink and Cooper suggested that suboptimal endosperm development induced modifications in associated maternal tissues, which in turn reacted unfavorably on the endosperm. When the endosperm became starved and broke down, the embryo continued to grow for a time, although it was greatly retarded. Brink and Cooper demonstrated that an interspecific hybrid embryo could be reared into a plant, if it was freed from the degenerating endosperm and reared on a suitable medium.

## The Distribution of Naturally Occurring Growth Substances in Fruits in Relation to Abscission

In 1939 Gustafson (42) reported on the distribution of auxin in various tissues of several fruits. The highest concentration occurred in the ovules. Gustafson therefore suggested that developing seeds are the centers of auxin production.

Luckwill (68) extracted an unknown, active growth substance from developing apple seeds. The relative quantity of this growth substance varied with the stages of seed development (69). Luckwill and Woodcock (80) conducted a preliminary investigation into the nature of the unknown hormone but did not identify it. Teubner (106) isolated an active growth factor from apple endosperm and chromatographically identified it as the ethyl ester of indoleacetic acid.

During the next several years Luckwill (70, 71, 72, 75, 77, 79) related the auxin content of apple seeds to their morphological development, to better understand the mechanisms involved in immature fruit abscission. The auxin content of the endosperm was considerably higher than that of other seed components (70). He suggested that the small amounts of auxin present in the embryo diffused there from the endosperm, the latter being the apparent production site of auxin. Fluctuations in auxin content of the seed were found to be associated with definite stages in endosperm development. Luckwill postulated that apple fruits are prevented from abscising by hormonal stimuli diffusing from the developing seeds and that the endosperm is the probable source. Since apples with the fewest seeds tend to abscise most readily, Luckwill suggested that this abscission was due to a reduction in auxin production below a critical level.

To support his contention that apple fruitlet abscission is under hormonal control, Luckwill (71, 76) demonstrated that the first peak of hormone production coincided with the end of the first wave of dropping fruitlets (ignoring the initial wave of non-pollinated flower drops). Similarly, the second major hormone peak marked the end of the final wave of abscission of immature fruits. Luckwill postulated the existence of a third stimulus which reaches its peak at fertilization; this stimulus is either of a different nature than the later two or, it has its origin outside of the seed. Three acidic growth promoting, and two acidic growth inhibiting substances have been isolated from apple leaves and fruits, but no chemical identification has been made (78).

Nitsch (92, 93) in reviewing the role of plant hormones in developing fruits stated that seeds, in addition to controlling fruit abscission by releasing a hormone, also control fruit growth by releasing a chemical stimulus of the auxin type. Numberous observations on the relationship of fruit size and seed number support this view. In the strawberry (and perhaps in certain other fruits as well) the effect of the ovule is continuous, since enlargement of the receptacle is inhibited by ovule removal as late as 21 days after pollination (92). The major auxin peak coincides with the occurrence of cytokinesis in the endosperm of the achenes of strawberry (92), and seeds of the apple (71, 76).

Fruit development, in relation to growth factors in black currant fruits, was investigated by Wright (119) in an attempt to relate naturally occurring auxins, fruit morphogenesis, and fruit abscission. Three auxins, designated auxins 1, 2 and 3, and one growth inhibitor, were isolated but were not chemically identified. A close correlation was found between the two periods of rapid growth exhibited by the black currant berry, and the two major peaks in the amounts of auxins 1 and 3. In addition, whenever auxin 2 was present in relatively large amounts the berry abscission rate was very low. No evidence was found that linked the inhibitor with any essential role in the control of growth processes within the fruit.

The natural auxin content of the peach seed has also been investigated in relation to morphological development, in an attempt to understand the mechanism of NAA induced abscission. Lombard (65) compared the natural auxin content of peach seeds before, during, and after cytokinesis in the endosperm. Employing chromatographic techniques, he detected, but did not identify, two active areas, the activities of which reached peaks just as cell walls began to form in the endosperm.

Stahly and Thompson (103) investigated the auxin levels of developing Halehaven peach ovules and identified three different auxins, one of which reached a maximum level at the time that the endosperm became cellular. The second attained its maximum level just as the endosperm began to develop most rapidly, while the third auxin peak seemed to be associated with the commencement of rapid embryo growth.

Powell and Pratt (95) identified only two periods during Halehaven seed development when growth substances were present in relatively large amounts. The first major peak was attained just as rapid cell division and growth was occurring in the nucellus and endosperm (late stage I of fleshy pericarp development). The second peak was correlated with rapid embryo development.

## Effects, Mode of Action, and Distribution of Applied Growth Regulators

### (1) Effects of Applied Growth Regulators

In addition to inducing the abscission of certain developing peach fruitlets, NAA has been reported to cause foliar injury (56, 62, 65). Langer (62) reported that a NAA spray applied when a sample of fruits averaged three-quarters of an inch in length, caused severe terminal dieback of the lateral branches of Redhaven peach. Kelley (56) observed that both the acid and amide forms of NAA caused terminal dieback. A reduction in the number of fruit buds following NAA application was noted, although the reduction was not sufficient to alter the following season's crop--except where higher-than-necessary concentrations were employed (56). Injury to terminal growth of Redhaven but not Halehaven was also reported by Lombard (65).

Thompson and Rogers (110) noted that NAA was not suitable as a chemical thinning agent for peaches because of its inconsistent thinning action, and its tendency to cause injury to the foliage. Tafuro (16) stated that while NAA is generally effective as a thinning agent for peaches, if applied four to five weeks after petal fall, it frequently causes some terminal shoot killing and yellowing of the foliage, and the fruits remaining on the trees tend to be in clusters.

Donoho and Mitchell (30) applied a NAA spray to Redhaven peaches 28 days after full bloom which induced satisfactory thinning, but the fruits from the thinned trees were somewhat smaller at maturity than controls.

## (2) Mode of Action of Applied Growth Regulators

To gain an insight into the mode of action of NAA Britten (12) studied its effect on the developing maize caryopsis. For the first eight or nine days the ovaries treated with NAA were longer and larger than those non-treated, but from that time on growth of the treated ovaries lagged, such that at maturity they were smaller than the controls. The early increase in size resulted from a greater elongation of the cells of the nucellus and pericarp of the treated ovaries. The embryos appeared normal until eight days after the NAA was applied; from this time until maturity the embryos of the treated ovaries were smaller and lagged behind the controls with respect to differentiation. The endosperm did not appear to be as sensitive to NAA as the embryo and the treated kernels were viable at maturity.

Crane and Punsri (23) investigated the effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on apricots, by making a comparative study of the ovules of treated and non-treated fruits. The 2,4,5-T was applied as a spray at the beginning of pit hardening. At harvest, the seed and endosperm lengths of the treated fruits (Royal cultivar), were about 5 percent longer than those of the controls, whereas, with the Tilton cultivar, these measurements were about the same in both treated and control samples. For a short time after the 2,4,5-T application the treated Royal embryos were shorter than the controls, but gradually the embryos of the sprayed fruits became longer and remained longer than those of the non-treated fruits. The embryos of the sprayed and non-sprayed Tilton apricots were similar in size throughout the season.

### (3) Distribution of Applied Growth Regulators.

Maxie et al. (83) followed the distribution of $C^{14}$ from carboxyl-labeled 2,4,5-T in the fruit tissues of the Tilton apricot. They found that the $C^{14}$ accumulated in the integuments. Chromatograms of extracts of the integuments suggested that the material was present in its original molecular form, but since the amounts involved were small, the authors could not be sure of their identification. Since maximum accumulation of $C^{14}$ occurred in the integuments when the tissue was dead and drying out, the authors concluded that the accumulation was non-metabolic.

Donoho (29) and Donoho et al. (31), investigated the translocation of $C^{14}$ labeled NAA in the peach and apple. They demonstrated that the $C^{14}$ of ring-labeled NAA is translocated through the conductive system into the developing fruit and seed.

## Suggested Mechanisms of Action of NAA

Struckmeyer and Roberts (105) suggested that the induction of fruit abscission by NAA is probably accomplished through increased nutritional competition, the latter being a result of a heavier initial fruit set. That is, the initial effect of NAA (that of temporarily delaying abscission) causes additional competition for nutrients. More of the "weaker" young fruits cannot compete successfully and thus abscise. Nitsch (92) supported this contention.

Batjer and Hoffman (3) postulated that since NAA increased the respiration rate of fruits when it was applied just prior to harvest (26, 99), perhaps when applied as a thinning agent NAA similarly increases the respiration rate thus effecting a reduction in the food supply. Abscission of the "weaker" fruits could thus be induced.

Luckwill (73, 74) observed that apples are sensitive to NAA only as long as the endosperm is in the free nuclear condition. He suggested that abscission of immature apple fruits is controlled by hormones diffusing from the endosperm of the seed. Luckwill postulated that applied NAA induces seed abortion thereby reducing the flow of hormones down the pedicel of the developing fruit. When the auxin flow across the abscission zone drops below a critical level, abscission results.

Van Overbeek (117) offered still another theory as to the mechanism of the thinning action of NAA on apples. He suggested that the NAA stimulates excessive elongation of the cells at the base of the pedicel. The rapidly developing apple fruit invokes such a strain on the existing carbohydrate supply that the elongating cell walls at the base of the fruit stalk cannot acquire strengthening cellulose deposits quickly enough. Van Overbeek did not consider the resulting drop to be true abscission.

Murneek (88) suggested that abscission of apple fruits may be a result of an unfavorable auxin-ethylene balance. He hypothesized that, upon reaching the ovule, NAA may uncouple oxidative phosphorylation. The hormone content of the endosperm may then drop, thus reducing fruit growth. The embryo may then grow more slowly and sub-normal metabolism could ensue. The consequence of this sub-normal metabolism could possibly be an increase in ethylene production leading to fruit abscission.

Teubner and Murneek (91, 107) examined the possibility that embryo abortion is the mechanism involved in hormone thinning of fruits. They stated that, since the first visible effect of the NAA thinning spray on fruit development was "an immediate and complete cessation of growth," a nutritional competitive mechanism, as suggested by Struckmeyer and Roberts (105), is probably not the causal factor of induced abscission. Teubner and Murneek examined the abscising fruits of NAA treated, versus those of non-treated apples, at various intervals from the time of application. In addition to the aforementioned retardation of fruit development, which was apparent in a few fruits three days after treatment, the NAA spray caused a marked inhibition of embryo growth.

Teubner and Murneek were not satisfied that Van Overbeek's proposed mechanical fracture of elongated, weakened cells at the base of the pedicel, was responsible for NAA induced abscission. They pointed out that this elongation of cells had not been demonstrated. In addition, they were able to show that NAA applied to defruited apple pedicels delayed their abscission.

Hartmann and Howlett (44) in their investigations into the effects of NAA on fruit setting and development in the apple, discovered that on a given date an increased concentration of applied NAA caused more embryo degeneration. In addition, NAA sprays reduced the total seed number per fruit, and the total weight of seeds per fruit. Therefore, Hartmann and Howlett suggested that NAA, by suppressing seed development, effects a reduction in the amount of natural auxin produced, thus causing fruit abscission.

Although a number of theories have been advanced, the thinning action of the growth regulating material NAA has not been satisfactorily explained.

# MATERIALS AND METHODS

## The Relative Effectiveness of Naphthaleneacetic Acid Thinning Sprays Applied at Different Stages of Endosperm Development

To test the hypothesis that fruit abscission can be more readily induced with NAA near to, or during, cell wall formation in the endosperm, NAA (30 ppm) was applied approximately one week prior to, during, and six days after the onset of cytokinesis in the endosperm of Redhaven and Halehaven peach fruits. Spray dates were estimated to coincide with the desired developmental stage by comparing length and width measurements of the fruits and seeds to those taken by Lombard (65). Both cultivars received the NAA sprays on the same days. Comparable non-treated trees were used as a control.

Each treatment was replicated six times, employing a single mature tree of each cultivar for each replication. A randomized block design was used. Approximately two and one-half to three gallons of spray solution (sodium salt of NAA--30 ppm) was applied per tree with a hand gun (350 psi). Environmental conditions at the times of the applications of NAA are summarized in Table 1.

To establish the degree of fruit abscission induced by the NAA applications, fruit counts were made on all trees under test prior to the first application. Six representative branches, with approximately 100 developing fruits per branch, were tagged on each tree. The number of persisting fruits was determined during the latter part of July. Following this count the selected branches were hand thinned and the remaining fruits were counted. The number of fruits thinned chemically

Table 1.--Environmental conditions at the times of application of NAA to Redhaven and Halehaven peach trees.

| Date NAA Applied | Temperature | | Weather Conditions | Time Required for Trees to Dry (Minutes) | Hour of Application |
|---|---|---|---|---|---|
| | Minimum | Maximum | | | |
| June 15 | 44 | 70 | Slight breeze, sunny | 20 | 5:00 P.M. |
| June 24 | 44 | 70 | Fairly strong breeze, sunny, then showers | 15 | 12:00 Noon |
| June 30 | 68 | 96 | Cloudy, calm | 35 | 7:00 A.M. |

and naturally, and those removed by hand, were then compared statistically by analysis of variance. A comparison among treatment means was made using the procedure outlined by Duncan (37).

At the time of each NAA application a random sample of ten non-treated fruits of each cultivar was collected. Length and width measurements were taken of both fruits and excised seeds, and the seeds were immediately killed and fixed in FAA[1] for subsequent anatomical observations. The development of the embryo and endosperm was followed in random samples of ten fruits (non-treated), collected at 12, 24, 48, 72 and 96 hours after the first two NAA applications. Length and width measurements were also taken of these fruits and their seeds. The seeds were killed and fixed in FAA immediately after measuring.

Length and width measurements of fruits and seeds were made with vernier calipers. Pericarp length consisted of the distance between the point of attachment of the peduncle to the fruit and the base of the style--the point at which the style and ovary are fused--whereas, width measurements were made across the suture, at the point of greatest width. Seed length measurements were of the greatest longitudinal distance, and seed width measurements were of the greatest transverse diameter.

All seed samples were left in FAA for at least 24 hours (8 to 12 hours of which they were under vacuum) prior to dehydration and embedding in paraffin. Both the ethyl alcohol--chloroform, and the tertiary butyl

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[1] 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.

alcohol (54) series were employed for dehydration. The integuments were either pierced several times with a needle, or a small portion was removed from the side of the seed in order to avoid seed and embryo sac collapse during dehydration and infiltration. The concentration of the solutions employed in both the dehydrating and the infiltrating processes, was increased very gradually, for the same reason.

The seeds were embedded in Fisher Tissuemat, (melting range 56°-58°C) and were sectioned longitudinally at 12 u with a rotary microtome. The sections were affixed to glass slides with Haupts adhesive (54), and were stained by one of the following procedures: (a) safranin: aniline blue, (b) safranin: fast green, or (c) safranin: haematoxylin (54). Upon completion of staining the sections were taken to xylol then mounted in either Canada Balsam or Piccolyte.

## The Comparative Reliability of Various Parameters as Indices of the Onset of Cytokinesis in the Endosperm of the Redhaven Peach

If peach fruits can be induced to abscise by NAA applied near to, or during cell wall formation in the endosperm, a convenient, reliable, parameter indicative of this stage of endosperm development, must be found. Temperature and crop growth are closely related (51); therefore, it was felt that heat units, accumulated from full bloom until the beginning of cell wall formation in the endosperm, might serve as an index of the onset of cytokinesis. A Hygro-Thermograph was placed in the University peach orchard in a standard weather box, situated four feet above the ground; the temperature data were collected during the 1962 and 1963 growing seasons, and accumulated heat units for the interval between full bloom and the beginning of cytokinesis in the Redhaven peach were determined. Heat units were calculated from daily mean temperatures employing 40° and 50°F as bases. Thus, daily heat units equals (mean temperature minus base temperature) x 24.

Fruit measurements are indicative of certain developmental changes within the seed (43, 111). Therefore, length and width measurements of fruit and seed samples of Redhaven peaches were made at various intervals in 1961, 1962 and 1963, to determine whether or not a consistent relationship existed between one or more of these parameters and the date of onset of cytokinesis. The seeds were examined microscopically to determine the onset of cell wall formation.

The dates of full bloom, petal fall and shuck split were determined for the Redhaven peach in 1961, 1962 and 1963. The number of days elapsing between each of these dates and the date of the onset of cytokinesis were determined. The number of days elapsed was compared over three seasons for reliability as an index of the beginning of cell wall formation.

### A Comparison of Pericarp Length and the Occurrence of Cell Wall Formation in the Endosperm of Several Cultivars of Peach

Preliminary results indicated that pericarp length was a good index of the onset of cytokinesis in the Redhaven peach. To determine the uniformity of this index among several peach cultivars, fruit samples were harvested and measured, in 1962 and 1963, to determine their average size, particularly pericarp length, at the time of the onset of cytokinesis. The cultivars examined included, Halehaven, Richhaven, Rochester, Redskin (1962 only), Ambergem (1963 only) and Elberta (1963 only), in addition to Redhaven. Samples were harvested daily in 1962, and every other day in 1963: the collection of samples commenced when the endosperm was considered to be in a free nuclear condition, and continued until the endosperm was considered to be completely cellular. At each sampling date ten fruits (if available) were collected from each cultivar. Length and width measurements were taken on fruits and seeds as described earlier, and the seeds were prepared for histological examination. Longitudinal sections of the seeds were examined to identify initiation of cytokinesis. With cultivars that could be harvested during more than one growing season, pericarp lengths at the times of the onset of cell wall formation, were compared. The consistency of pericarp length from year to year, as an index of the beginning of cytokinesis in the endosperm of several cultivars, was determined.

### Size Distribution and Variations in Endosperm Development at the Time of Cytokinesis in the Endosperm of a Total Population of Redhaven Peach Fruits

If NAA did induce abscission in fruits at a specific stage of development, it was important to know what percent of the total fruits were in a given stage at a given time. Accordingly, the distribution of fruits on a tree was determined on the basis of pericarp length and endosperm development. All fruits from two representative branches of Redhaven peach were harvested at the time of the onset of cytokinesis in a standard ten fruit sample. Each fruit in the sample (190 fruits) was measured and placed in one of five size ranges, based on pericarp length. Seeds were excised, measured, and a random sample of ten seeds from each of the five sized groups was prepared for histological observation, and development of the endosperm was recorded.

## Comparison of Embryo and Endosperm Development in Naturally Abscising and Persisting Redhaven Peaches

To gain a better understanding of the causes of natural abscission of peach fruits, and to establish a possible relationship between embryo and endosperm development and fruit abscission, three groups of fruit samples of the Redhaven peach, separated on the basis of pericarp length, were harvested on June 13, 1962. The three groups were:

(1) Ten small fruits which had obviously stopped growing and which were ready to abscise.

(2) Ten fruits of medium size which were considered to be potential drops.

(3) Ten large fruits which would have persisted until harvest under normal conditions.

Length and width measurements of the fruits and seeds were taken, the seeds were killed and fixed in FAA, embedded in paraffin, sectioned longitudinally, and slides prepared. The mounted sections were then examined and compared. Special attention was given to a comparison of the relative development of the embryo and endosperm of the three classes of fruits, so as to identify a possible role of these tissues in natural abscission.

## The Effects and Site of Action of Naphthaleneacetic Acid When Applied as a Foliar Spray to Redhaven Peach Trees

### (1) Foliar Injury Induced by Naphthaleneacetic Acid

An objective evaluation was made of the degrees of lateral branch killing induced on Redhaven terminals by NAA (30 ppm) applied on three different dates in 1961. Each tree, in each of the four treatments, was examined and assigned an injury rating factor, Table 2. Injury ratings for the six whole tree replications in each treatment, were averaged in order to estimate the variation in lateral branch killing between treatments. Environmental conditions at the times of the three spray applications are summarized in Table 1.

### (2) Site of Action of Naphthaleneacetic Acid

To identify the site of action, NAA was applied at a supra-optimal concentration (60 ppm) to one Redhaven peach tree, just as cytokinesis was beginning in the endosperm (1962). The application was made with a three gallon hand sprayer, and the tree was sprayed to the drip point using approximately two gallons of solution. An adjacent tree was used as a control. Treated and non-treated fruit samples (ten of each) were collected at 14 hours, 1 through 7 days inclusive, 14 days after treatment, and at harvest. Each sample consisted of ten fruits from <u>one</u> branchlet. Fruits were measured and the seeds were prepared for subsequent anatomical examination. Sections of seeds from treated and non-treated fruits were compared to identify any modification in embryo and/or endosperm development. Embryo measurements were taken from median sections. With embryos in the pre-cotyledonary developmental stages, length and width consisted of the greatest longitudinal and transverse distances, respectively. With embryos in the cotyledonary stage, length measurements were still of the greatest longitudinal distance, but widths were measured at a point immediately below the

Table 2.--The injury rating factors used to evaluate the variation in degree of lateral branch killing on terminals of the Redhaven peach.

| Percent of Lateral Branches Killed per Terminal | Injury Rating |
|---|---|
| 0 | 0 |
| 1-10 | 1 |
| 11-30 | 2 |
| > 30 | 3 |

epicotyl region. Average cell length and width measurements were determined from the cells in these regions.

Samples of fruit from NAA- and non-treated trees were collected at harvest. The seeds were removed, after-ripened (14), and their viability was determined.

## The Distribution of Foliar Applied Ring-labeled Naphthaleneacetic Acid in Leaf, Fruit and Seed Tissues of the Redhaven Peach

The distribution of foliar absorbed NAA within leaf, fruit, and seed tissues was followed to localize the site of action. Approximately 0.05 uc of ring-labeled NAA-$C^{14}$ was applied (dropwise with a Tuberculin syringe) to each of four or five leaves (upper surface) adjacent to a selected fruit. Each treatment consisted of five fruits, and the treatments were replicated three times. The NAA-$C^{14}$ was applied just as cell walls began to form in the endosperm. Samples, each consisting of five fruits, and all adjacent treated leaves, were harvested at 2, 5, 10, 15, and 20 days after treatment. One fruit from each replication, at each sampling date, was hand sectioned and dried. The dried sections were then exposed for a period of five weeks to Kodak X-ray film (Blue Brand), in order to determine the gross distribution of the ring-labeled NAA-$C^{14}$ in the pericarp and seed tissues.

Each sample of leaves was washed with 95 percent ethyl alcohol, prior to maceration in a Virtis Model 45 homogenizer. Seeds were excised from the four fruits remaining in each replication; the resulting pericarp and seed samples were separately macerated in the homogenizer. Leaf, pericarp, and seed tissues were then extracted for 12 hours with 95 percent ethyl alcohol. The homogenate was filtered, washed with

95 percent ethyl alcohol, dried, and finally weighed. Filtrates and washings were evaporated to dryness on a steam bath. The residues were then taken up in five milliliters of 95 percent ethyl alcohol; a one milliliter aliquot of each of the resulting solutions was placed in a stainless steel planchet and dried slowly under an infra red heat lamp. The plated samples were counted with a Tracerlab Versa-Matic II Scaler and a TGC-14 gas flow carbon counter, and the distribution of the $C^{14}$ (presumably NAA-$C^{14}$ since the material was ring-labeled) was then calculated.

## RESULTS

### The Relative Effectiveness of Naphthaleneacetic Acid Thinning Sprays Applied at Different Stages of Endosperm Development

To test the hypothesis that peaches can be thinned with NAA applied just as cell walls begin to form in the endosperm, the chemical was applied approximately one week prior to, during, and six days after the onset of cytokinesis in the endosperm of Redhaven and Halehaven peaches. Microscopic examination of longitudinal sections of seeds from fruits harvested at the times of NAA applications, revealed that the first spray was applied when the endosperm of both cultivars was completely free nuclear. Nine days later when the second NAA spray was applied, cell walls had just begun to form in the endosperm of the Redhaven peach whereas, that of Halehaven was completely cellular. At the time of the third spray the endosperm in the samples of both cultivars was completely cellular. The stages of endosperm development in the Redhaven peach when sprayed are illustrated diagrammatically in Figure 1, B.

The mean pericarp length of both Redhaven and Halehaven were similar at the times of the three NAA applications, yet endosperm development differed markedly, Table 3. These results suggest that different peach cultivars may undergo cytokinesis at different times, in relation to the development of the pericarp. Average pericarp length of the Redhaven samples increased 6.8 mm (or approximately 0.8 mm per day) in the nine days that elapsed between the first two NAA applications. However, pericarp length increased only 2.2 mm (or approximately 0.4 mm per day) during the six day period between the

Figure 1.--Diagrammatic representation of the stages of development of the seed, (A) embryo sac, (B) and embryo, (C) of Redhaven peach at the times of application of NAA at 30 ppm.

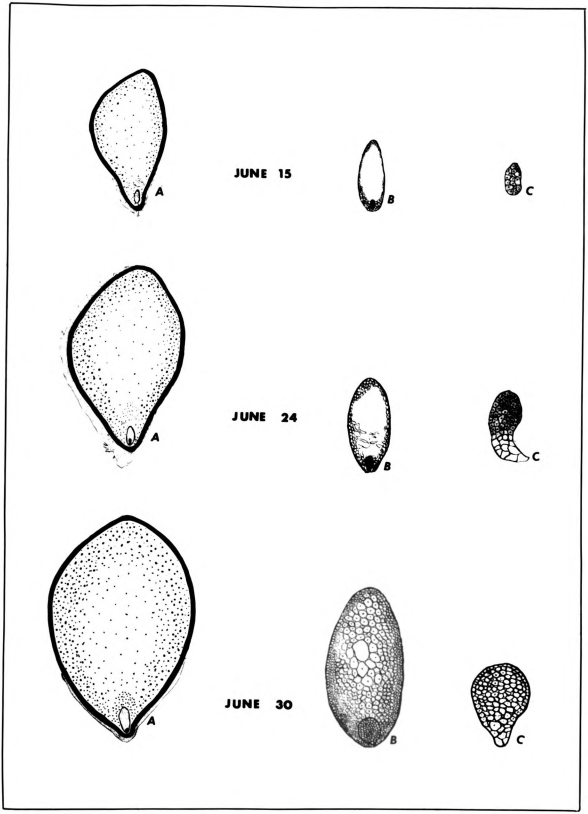

A
JUNE 15
B
C
A
JUNE 24
B
C
A
JUNE 30
B
C

Table 3.--Pericarp length and stage of endosperm development of peach fruits at the times of application of NAA at 30 ppm.

| Date NAA Applied | Days After Full Bloom | Stage of[1] Endosperm Development | | Pericarp Length[2] | |
|---|---|---|---|---|---|
| | | Redhaven | Halehaven | Redhaven | Halehaven |
| June 15 | 33 | FN | FN | 24.0 | 24.2 |
| June 24 | 42 | Cy | C | 30.8 | 30.2 |
| June 30 | 48 | C | C | 33.0 | 34.4 |

[1] FN = free nuclear
Cy = beginning of cell wall formation
C = completely cellular

[2] Average of 10 fruits and measured in millimeters

second and third sprays. Pericarp enlargement in Halehaven differed similarly during the two intervals.

The effects of NAA on fruit abscission when applied at different stages of endosperm development are recorded in Table 4. The spray applied to Redhaven just as cell walls were beginning to form in the endosperm induced significant abscission whereas, NAA applied nine days earlier, when the endosperm was completely free nuclear, failed to induce significant abscission. NAA applied when the endosperm of the Redhaven peach was completely cellular also induced significant abscission but to a lesser degree than that induced by the second NAA application. The endosperm of the Halehaven peach at the time of the first NAA application (33 days after full bloom), was completely free nuclear, and at the second and third applications, completely cellular. None of the three sprays induced significant thinning of Halehaven peaches.

The degrees of thinning achieved with Redhaven peaches by the three applications of NAA are illustrated in Figure 2. Variations in the amount of thinning achieved are presented as percent thinning induced by NAA above natural abscission of the non-treated control.

These results indicate that NAA may induce fruit abscission when applied at the beginning of cytokinesis in the endosperm or immediately thereafter. Further, cytokinesis may represent a critical time when the fruit is more sensitive to exogenous auxin, and variability between calendar date and this stage of development could explain the erratic results reported for NAA.

Table 4.--Effect of NAA on thinning of **Redhaven and Halehaven peaches when applied at** 33, 42, and 48 **days after full bloom.**

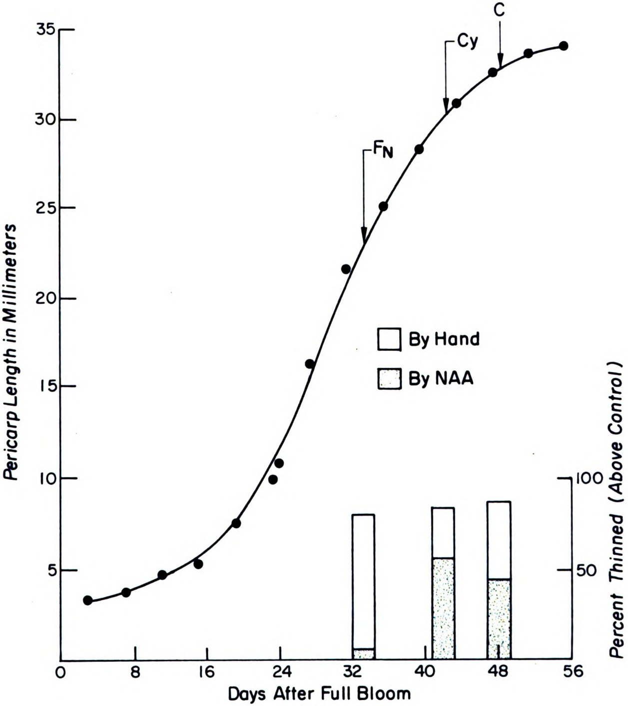
| NAA (30 ppm) at | Endosperm[1] Development |  | Percent Thinned[2] |  | Percent Thinning Necessary |  |
|---|---|---|---|---|---|---|
|  | Redhaven | Halehaven | Redhaven | Halehaven | Redhaven | Halehaven |
| 33 AFB[3] | FN | FN | $48^a$ | $55^a$ | 79 | 81 |
| 42 " | Cy | C | $70^b$ | $62^a$ | 86 | 83 |
| 48 " | C | C | $65^b$ | $62^a$ | 85 | 84 |
| No treatment | - | - | $45^a$ | $58^a$ | 81 | 83 |

[1] FN = free nuclear, Cy = cytokinesis, C = completely cellular.

[2] Means with different superscripts differ at P = 0.01.

[3] AFB = days after full bloom.

Figure 2.--Pericarp length, endosperm development, and days after full bloom in relation to the degree of thinning of Redhaven peach induced by NAA applied on three different dates. FN = free nuclear, Cy = cytokinesis, C = completely cellular.


Pericarp Length in Millimeters
35
30
25
20
15
10
5
0
8
16
24
32
40
48
56
Days After Full Bloom
Percent Thinned (Above Control)
100
50
C
Cy
F_N
By Hand
By NAA

## The Comparative Reliability of Various Parameters as Indices of the Onset of Cytokinesis in the Endosperm of the Redhaven Peach

If peach fruits are more sensitive to exogenous auxins during cell wall formation in the endosperm, then a convenient, reliable index is essential for the effective timing of a thinning spray. Since environmental conditions, which influence fruit growth, vary so markedly from year to year the number of days between full bloom and the beginning of cell wall formation in the endosperm would be expected to be quite variable. An assessment of the temperature patterns above a selected base would minimize variation due to differences in temperature between different growing seasons. Accumulated heat units have been employed as a fairly accurate index of the total environmental conditions required by certain crops from the time of planting until maturity (51). Therefore, employing as bases 40°F and 50°F, the number of heat units accumulated from the time of full bloom until the onset of cytokinesis in Redhaven peach was determined in 1962 and 1963. These determinations are summarized in Tables I, II, III and IV of the Appendix. Employing 40°F as the base temperature, accumulated heat units (Table 5) from the time of full bloom until the onset of cell wall formation, during 1962 and 1963, were 22,812 and 20,736 units respectively. Using 50°F as the base temperature, 14,052 units were accumulated in 1962 and 11,280 in 1963 (Table 5). With either base, considerable variation was apparent in number of heat units accumulated and rate of fruit development. Further, there must be other factors not related to temperature responsible for this variation.

Fruit and seed measurements were next examined in relation to cytokinesis in the endosperm, with the belief that factors modifying fruit development would be collectively manifested by a tissue dependent on endosperm development. The relationship between the beginning of cell wall formation in the endosperm, "days after full bloom," and length and width of the fruit and seed of Redhaven peach over several seasons, are recorded in Table 6. There was considerable variation in calendar date (13 days) and "days after full bloom" (6 days) as indices of cytokinesis. By contrast, all parameters of fruit and seed were extremely uniform. Pericarp length was particularly reliable (0.5 mm variation over four years) and perhaps most readily obtained.

The relationship between date of cytokinesis, days after full bloom, days after petal fall, and days after shuck split, and pericarp length of the Redhaven peach is recorded in Table 7. The indices "days after full bloom" and "days after petal fall" were both excessively variable (six and seven days variation, respectively) whereas, "days after shuck split" was somewhat more reliable (four days variation) as an index of cell wall formation in the endosperm. However, of the indices compared, average pericarp length was the most reliable.

### A Comparison of Pericarp Length and the Occurrence of Cell Wall Formation in the Endosperm of Several Cultivars of Peach

That variations occur in pericarp length of different cultivars at the time of the onset of cell wall formation in the endosperm, was observed in Redhaven and Halehaven peaches in the 1961 thinning experiments. Evidence was presented in the previous section demonstrating that pericarp length may serve as a reliable index of cytokinesis. For proper timing and optimal thinning we should be able to group or

Table 5.--Summary of accumulated heat units.

| Year | Base of 40°F | Base of 50°F |
|---|---|---|
| 1962 | 22,812 | 14,052 |
| 1963 | 20,736 | 11,280 |

Table 6.--Relationship between the onset of cytokinesis in the endosperm, days after full bloom, and fruit and seed measurements of Redhaven peach.

| Date of Cytokinesis | Days AFB[1] | Pericarp[2] Length | Pericarp[2] Width | Seed[2] Length | Seed[2] Width |
|---|---|---|---|---|---|
| June 14, 1958[3] | 42 | 30.8 | 25.5 | 12.9 | 7.8 |
| June 24, 1961 | 42 | 30.8 | 27.5 | 11.7 | 7.4 |
| June 11, 1962 | 36 | 30.5 | 25.5 | 11.9 | 7.3 |
| June 16, 1963 | 40 | 30.3 | 26.0 | 11.0 | 6.5 |

[1] Days AFB = days after full bloom.

[2] Average of 10 fruits and measured in millimeters.

[3] After Lombard (65).

Table 7.--Relationship between date of cytokinesis in the endosperm and days after full bloom, petal fall, and shuck split, and pericarp length in the Redhaven peach.

| Approximate Date of Cytokinesis | Days After Full Bloom | Days After Petal Fall | Days After Shuck Split | Pericarp Length |
|---|---|---|---|---|
| 6-14-58[1] | 42 | - | - | 30.8 |
| 6-24-61 | 42 | 34 | 23 | 30.8 |
| 6-11-62 | 36 | 29 | 25 | 30.5 |
| 6-16-63 | 40 | 36 | 27 | 30.3 |

[1] After Lombard (65).

identify the appropriate time for commercially important cultivars. Therefore, at various intervals during the 1962 and 1963 seasons, length and width measurements of fruits and seeds were taken of several commercially important peach cultivars. Immediately after measuring, the seeds were placed in FAA for subsequent histological processing and microscopic examination. The results are presented in Table 8 and Figure 3. Where data are available for more than one year, comparisons can be made between the indices cited, from one year to the next. The dates of the onset of cytokinesis in a given cultivar were again found to vary considerably from year to year, as were the number of days between full bloom and the beginning of cytokinesis. However, the pericarp lengths of the individual cultivars at the times of cytokinesis were remarkably consistent from one year to the next. This consistency in average pericarp length at a given stage of development, is illustrated more clearly in Figure 3.

These results demonstrate that average pericarp length of a representative fruit sample is a very reliable index of the beginning of cytokinesis in a given cultivar of peach. Further, peach cultivars differ as to the time of cytokinesis in relation to pericarp growth. Consequently, a given NAA application on a given date, irrespective of cultivar, cannot be the basis of a sound thinning program.

### Size Distribution and Variations in Endosperm Development at the Time of Cytokinesis in the Endosperm of a Total Population of Redhaven Peach Fruits

Information concerning the variation in pericarp length and the variation in endosperm development of a given cultivar in a peach

Table 8.--Relationship between the date of the beginning of cytokinesis, days after full bloom, and average pericarp length in several cultivars of peach.

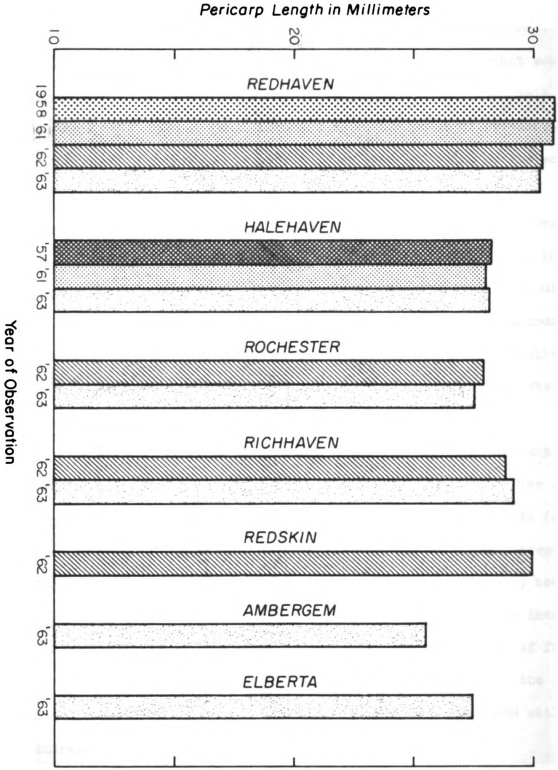
| Cultivar | Date of the Onset of Cytokinesis | Days after Full Bloom | Average Pericarp Length at Cytokinesis[1] |
|---|---|---|---|
| Redhaven | 6-14-58[2] | 42 | 30.8[2] |
| | 6-14-61 | 42 | 30.8 |
| | 6-11-62 | 36 | 30.5 |
| | 6-16-63 | 40 | 30.3 |
| Halehaven | 6-14-57[2] | 43 | 28.3[2] |
| | 6-19-61 | 37 | 28.0 |
| | 6-12-63 | 36 | 28.2 |
| Rochester | 6-10-62 | - | 27.9 |
| | 6-12-63 | - | 27.6 |
| Richhaven | 6-9-62 | 34[3] | 28.8 |
| | 6-14-63 | 38 | 29.1 |
| Redskin | 6-10-62 | - | 29.9 |
| Ambergem | 6-13-63 | - | 25.5 |
| Elberta | 6-16-63 | - | 27.4 |

[1] Average of 10 fruits and measured in millimeters.

[2] After Lombard (65). These measurements were made when the endosperm was in the "early cellular" stage of development.

[3] Approximate.

Figure 3.--Pericarp length at the time of the onset of cytokinesis in the endosperm of several peach cultivars.


Pericarp Length in Millimeters
10
20
30
REDHAVEN
1958
'61
'62
'63
HALEHAVEN
'57
'61
'63
ROCHESTER
'62
'63
RICHHAVEN
'62
'63
REDSKIN
'62
AMBERGEM
'63
ELBERTA
'63
Year of Observation

orchard, at the time when cell walls are beginning to form in the endosperm of the fruits in a representative sample, would contribute to an understanding of the mode of action of NAA. Such information could enable the approximation of the degree of thinning that would be expected to result from an NAA spray applied just as cytokinesis was beginning. Thus, one could theorize as to the stage of development at which peach fruits would be sensitive to NAA, and which fruits would survive a NAA application.

Therefore, the complete population of two representative branches of Redhaven, was harvested just as cell walls began to form in the endosperm. The size distribution (pericarp length) of this population is presented in Figure 4. The distribution curve obtained approximates a normal distribution, particularly if the extremely small fruits, which were obviously about to abscise at the time the sample was harvested, are not included.

To relate variations in endosperm development with pericarp length, the total population fruit sample was divided into five sized groups, based on pericarp length, and ten representative seeds from each group were killed and fixed in FAA. Permanent slides, prepared from these 50 seeds, were studied to determine a relationship between pericarp length and endosperm development, Table 9. With an increase in length, there was a corresponding increase in the number of fruits in which the endosperm was becoming cellular. In addition, the estimated amount of cell wall formation per embryo sac increased with an increase in pericarp length.

Photomicrographs of typical embryo sacs in permanently mounted

Figure 4.--Range in pericarp length of 190 Redhaven peaches--the entire population from two representative branches--harvested just as cell wall formation began in the endosperm of a representative ten fruit sample.

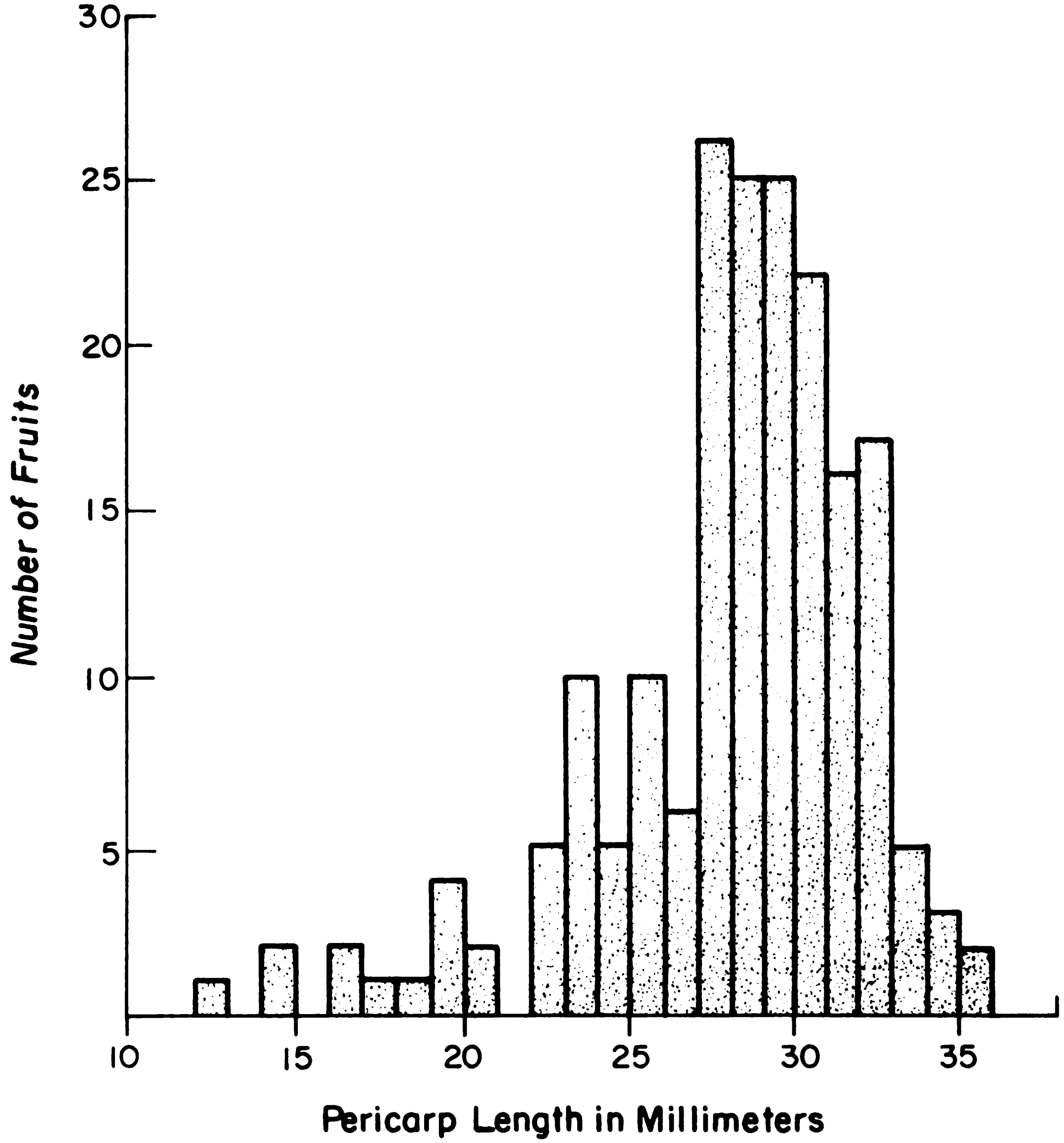

30
25
20
15
10
5
Number of Fruits
10
15
20
25
30
35
Pericarp Length in Millimeters

Table 9.--Relationship between pericarp length and endosperm development of Redhaven peach fruits.

| Pericarp[1] Length | No. of Fruits with Endosperm Becoming Cellular | Percent of Fruits with Endosperm Becoming Cellular | Estimated Amt. of Cell Wall Formation per Embryo Sac[2] |
|---|---|---|---|
| 22.6 | 0 | 0 | 0 |
| 26.4 | 1 | 10 | 10 |
| 29.2 | 4 | 40 | 17 |
| 31.2 | 4 | 40 | 6.5 |
| 33.2 | 10 | 100 | 61 |

[1] Average of 10 samples and measured in millimeters.

[2] Percent

longitudinal sections of seeds prepared from fruits of the smallest (designated A), of the medium (designated B), and of the largest pericarp lengths (designated C), are presented in Figure 5. These photomicrographs illustrate the extremes in endosperm development found in the population of Redhaven peach fruits. The completely free nuclear condition of the endosperm of the smallest fruits is illustrated in A, whereas B reveals the early cellular condition of the endosperm found in 40 percent of the fruits in the middle size range. In addition, the fruits in the second largest size group (not pictured) contained endosperms in a similar stage of development to that pictured in Figure 5B. The completely cellular condition of the endosperm of the largest fruits is illustrated in C. All fruits in the largest classification contained endosperm in varying stages of cell wall formation.

Combining some of the data in Table 9 with that in Figure 4, presents a clearer picture of the relationship between pericarp length and cell wall formation in the endosperm of the Redhaven peach Figure 6. The stippled portion of Figure 6 represents the percent of the fruits examined (of a given size classification) in which cell wall formation had begun in the endosperm. The curve is the distribution, based on pericarp length, of the total fruit population. Cellular endosperm was present in approximately 38 percent of the total population (represented by the histogram in Figure 6).

### Comparison of Embryo and Endosperm Development in Naturally Abscising and Persisting Redhaven Peaches

A study of the embryo and endosperm in naturally abscising fruits, in comparison to that in persisting fruits was initiated to better

Figure 5.--Photomicrographs illustrating the extremes in endosperm development of Redhaven peaches harvested just as cell walls began to form in the endosperm of representative fruits. (A) Embryo sac illustrating the free nuclear condition of the endosperm as found in the smallest fruits; (B) Embryo sac illustrating the early cellular condition of the endosperm as found in representative medium sized fruits; (C) Embryo sac illustrating the completely cellular condition of the endosperm as found in the largest fruits. Approximately 135X.

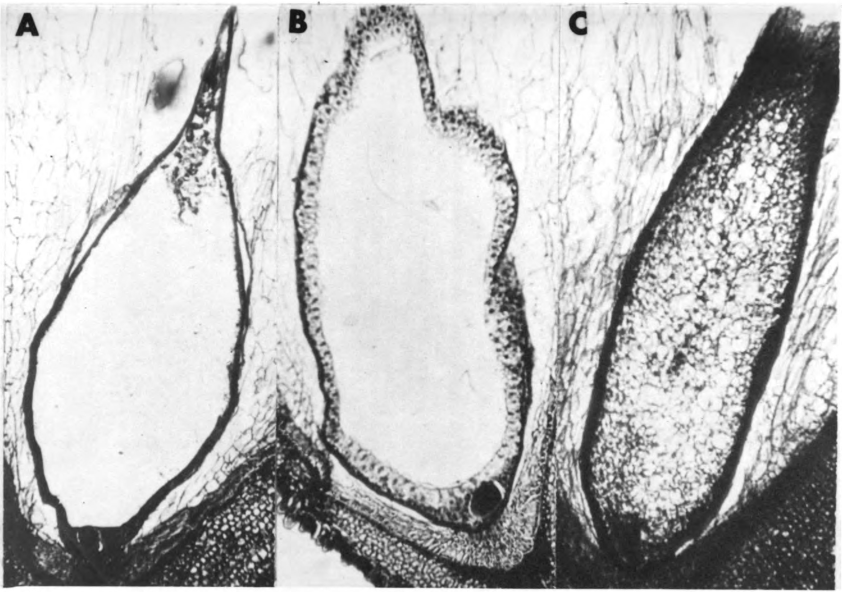
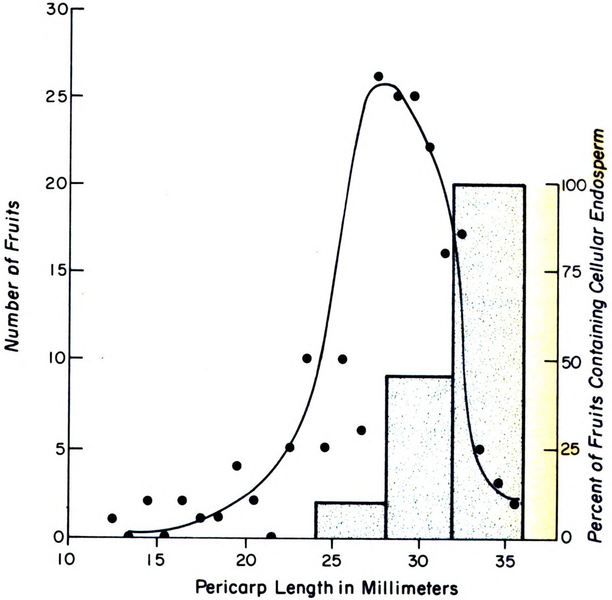

A
B
C

Figure 6.--Relationship between pericarp length and cell wall formation in the endosperm of the Redhaven peach--June 16, 1963. The stippled portion represents the fruits that would be expected to persist following a NAA application. The clear portion represents the fruits containing only free nuclear endosperm (62 percent of the total population).


Number of Fruits
Percent of Fruits Containing Cellular Endosperm
Pericarp Length in Millimeters
0
5
10
15
20
25
30
10
15
20
25
30
35
0
25
50
75
100

understand natural abscission, and to provide a basis for investigating the mode of action of NAA. Three classes of Redhaven fruits were harvested for this study--ten fruits about to abscise, ten medium sized fruits considered potential drops, and ten large, persisting fruits. All were harvested on the same date.

The relationship between physiological condition, length and width of pericarp and embryo, and endosperm development in naturally abscising and persisting Redhaven peaches, is summarized in Table 10. The seeds taken from the smallest fruits were in varying stages of abortion. For example 70 percent of these seeds contained no embryos and 20 percent contained gigantic embryos. Ten percent of the seeds from abscising fruits contained an embryo that appeared normal. None of these seeds contained normal endosperm. In fact, endosperm could be found in only 40 percent of the seeds from the abscising fruits, and in each instance this endosperm was degenerating. Embryos were absent in the embryo sacs without endosperm, and these embryo sacs were small and appeared to be degenerating.

An examination of the mounted sections of seeds taken from fruits considered potential drops, revealed normal appearing embryos in 90 percent of the fruits. In each case the endosperm also appeared normal and was completely free nuclear. There was no indication that cell wall formation was about to start, despite the fact that these fruits were harvested two days after the onset of cytokinesis in the endosperm of the standard ten fruit sample. The fruits in this potential drop classification averaged approximately six millimeters less in pericarp length than those in the standard sample harvested two days earlier.

Table 10.--Relationship between physiological condition, length and width of pericarp and embryo, and endosperm development in naturally abscising and persisting Redhaven peaches--June 13, 1962.

| Physiological Condition | Pericarp[1] Length | Width | Embryo[2] Length | Width | Endosperm Development | Number Aborting |
|---|---|---|---|---|---|---|
| Abscising | 18.9 | 14.3 | 75.8[3] | 42.3 | Degenerating, if present[4] | 10 |
| Potential drops | 24.8 | 19.8 | 82.1 | 46.8 | All samples free nuclear | 1 |
| Persisting | 32.2 | 27.7 | 93.3 | 54.6 | Forming cell walls in half of samples | 0 |

[1] Average of 10 samples, measured in millimeters.

[2] Average of 10 samples measured in microns.

[3] Only three of the 10 samples contained embryos and two of these were gigantic, relative to the size of the embryo sac.

[4] Six of the 10 samples contained no endosperm.

Ten percent of the seeds in this group contained an aborting, gigantic embryo, no endosperm, and a degenerating embryo sac.

In the case of the third classification, composed of seeds from large, obviously persisting fruits, microscopic examination of longitudinal sections of the ten seeds revealed that cell wall formation in the endosperm was progressing in 50 percent of the samples. The remaining samples contained abundant free nuclear endosperm. All of the embryos appeared to be developing normally.

The fruits in the three groups thus appeared to differ in overall size, in embryo size and development (if present), and in endosperm development. In most instances, obviously aborting seeds contained either abortive embryos or no embryos, and degenerating or missing endosperms. It was not possible to determine whether the endosperm degenerated first causing the embryo to abort, or whether the reverse was the case. However, in one seed the endosperm was degenerating while the embryo remained normal in appearance.

## The Effects and Site of Action of Naphthaleneacetic Acid When Applied as a Foliar Spray to Redhaven Peach Trees

### (1) Foliar Injury Induced by Naphthaleneacetic Acid

The variations in amount of lateral branch killing per Redhaven peach terminal, caused by NAA sprays applied on three different dates in 1961, were evaluated and are summarized in Table 11. The injury usually took the form of browning and senescence of those leaf clusters nearest the apex of the terminal. Since the portion of the tree injured by the NAA produces the crop for the following year this type of injury could markedly reduce the crop potential.

Table 11.--Degree of lateral branch killing to Redhaven peach tree terminals caused by applications of NAA (at 30 ppm) on three different dates.

| Date Applied | Days from Full Bloom | Endosperm[1] Development | Degree of Injury |
|---|---|---|---|
| June 15, 1961 | 33 | FN | 1.30 |
| June 24, 1961 | 42 | Cy | 0.83 |
| June 30, 1961 | 48 | C | 2.67 |
| No treatment | - | - | 0 |

[1] FN = free nuclear, Cy= cytokinesis, C = completely cellular.

NAA applications on different dates resulted in marked differences in degree of injury, Table 11. A comparison of the degrees of lateral branch killing with the times required for the sprays to dry (Table 1), reveals that as the drying times increased, the degree of injury also increased. Varietal sensitivity also plays a role in the injurious effects of NAA on peach foliage. Halehaven trees sprayed with the same concentration of NAA (30 ppm), under identical conditions, and at the same times as the Redhaven trees, exhibited no sign of injury.

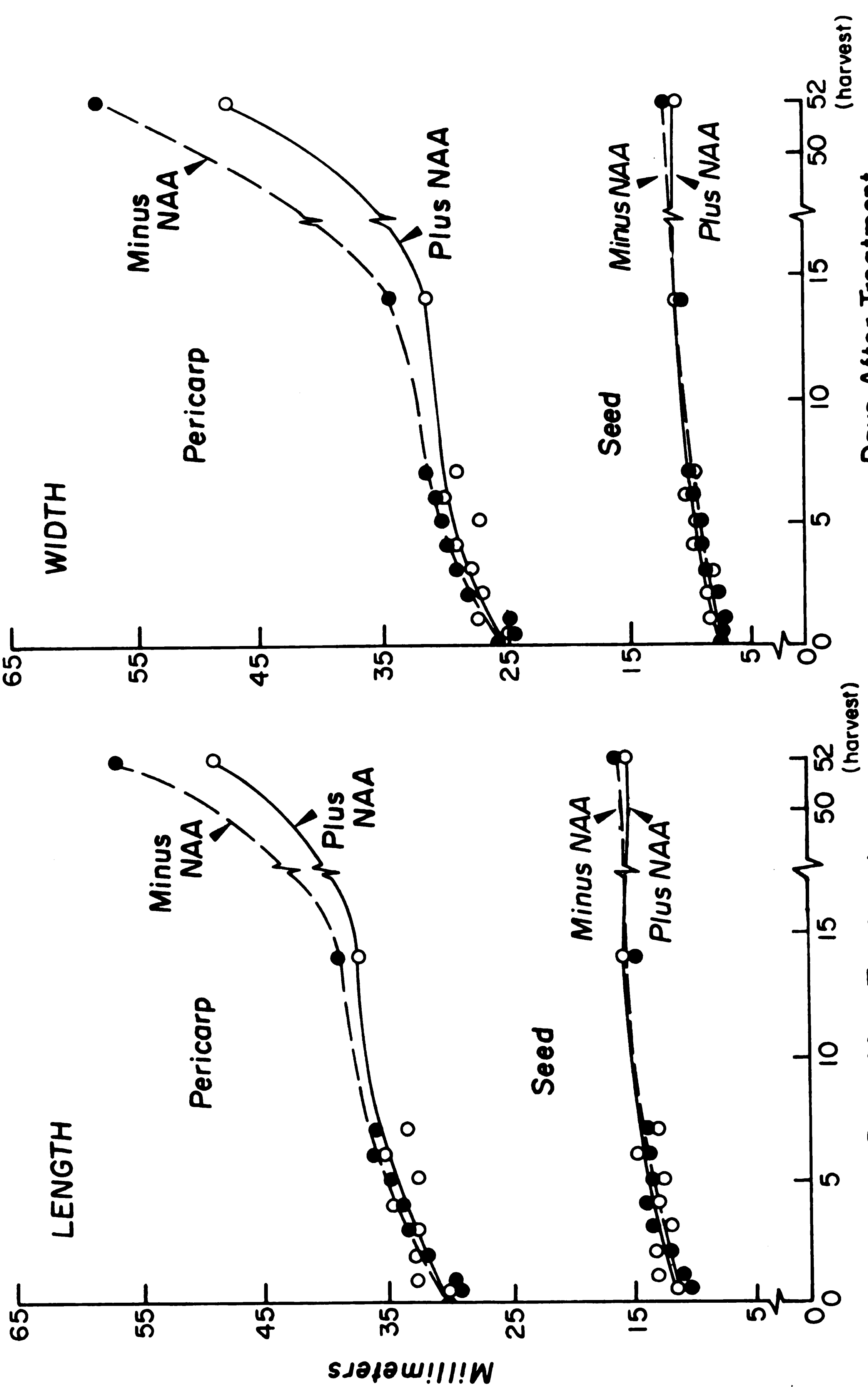
The response of Redhaven peach trees to a NAA spray in 1962, differed markedly from the 1961 response. In 1962 NAA was applied to one Redhaven tree at 60 ppm--double the concentration employed in 1961--in order to study its site of action. Virtually no lateral branch killing resulted. Instead, the foliage became yellow and some abscission occurred.

### (2) Site of Action of Naphthaleneacetic Acid

Knowledge of the site of action of NAA would contribute to an understanding of its mode of action. In addition, such information is essential for the efficient and consistently effective use of NAA as a thinning agent. Thus, the effects of NAA on pericarp and seed enlargement were examined; further, permanent microscope slides of longitudinal sections of seeds from treated and non-treated fruits, were critically studied.

The differences in pericarp and seed size induced by the supra-optimal concentration of NAA are illustrated in Figure 7 and summarized in Tables V and VI in the Appendix. The NAA did not materially affect

Figure 7.--Relationship between fruit and seed measurements of treated (NAA - 60 ppm) and non-treated Redhaven peaches.


LENGTH
WIDTH
Millimeters
Pericarp
Seed
Minus NAA
Plus NAA
Days After Treatment
0
5
10
15
50
52
(harvest)
5
15
25
35
45
55
65

size of the seed, as determined by length and width measurements. In addition, after-ripened seeds from treated and non-treated fruits harvested at maturity, showed no differences in viability. However, development of the pericarp was considerably inhibited by the NAA and this reduction became more pronounced as the season progressed. At maturity the treated fruits were approximately 15 percent shorter and 19 percent narrower than the non-treated fruits.

Cell wall formation in the endosperm occurred more rapidly in seeds from treated Redhaven peach fruits than in those from non-treated fruits. In addition, at the times of the first four sample collections (14 hours, two and three days after treatment) cell wall formation occurred in a much higher percentage of the seeds from fruit samples treated with NAA than in the non-treated samples, Table 12.

The average length and width of the embryos from NAA treated and non-treated Redhaven peaches are illustrated in Figure 8, and recorded in Table VII (Appendix). NAA induced a considerable increase in embryo size. Embryos in samples collected 14 days after the NAA was applied averaged approximately 25 percent longer and 18 percent wider, compared to non-treated controls harvested at the same time.

At maturity however, the embryos from treated and non-treated Redhaven peaches were similar in size, those from the non-treated fruits being slightly larger. The size relationship of the embryos of treated and non-treated fruits at each time of sampling, during the 14 days after application of NAA, is illustrated in Figure 9. These photomicrographs were taken of representative longitudinal sections of seeds, removed from treated and non-treated fruit samples harvested at six,

Table 12.--Comparison of endosperm development in treated (NAA - 60 ppm) and non-treated Redhaven peaches.

| Time from Treatment | Estimated Amt. of Cell Wall Formation per Embryo Sac (Percent) | | Percent of Samples in Which Cytokinesis was Occurring | |
|---|---|---|---|---|
| | No Treatment | NAA | No Treatment | NAA |
| 14 hrs. | 0 | 28 | 0 | 60 |
| 1 day | 0 | 41 | 40 | 86 |
| 2 days | 47 | 49 | 78 | 90 |
| 3 " | 31 | 34 | 89 | 100 |
| 4 " | 80 | 87 | 100 | 100 |
| 5 " | 78 | 85 | 90 | 100 |
| 6 " | 94 | 98 | 100 | 100 |
| 7 " | 100 | 98 | 100 | 100 |
| 14 " | 100 | 100 | 100 | 100 |

Figure 8.--Comparison of length and width of embryos from treated (NAA - 60 ppm) and non-treated Redhaven peaches collected and measured at various intervals after treatment.

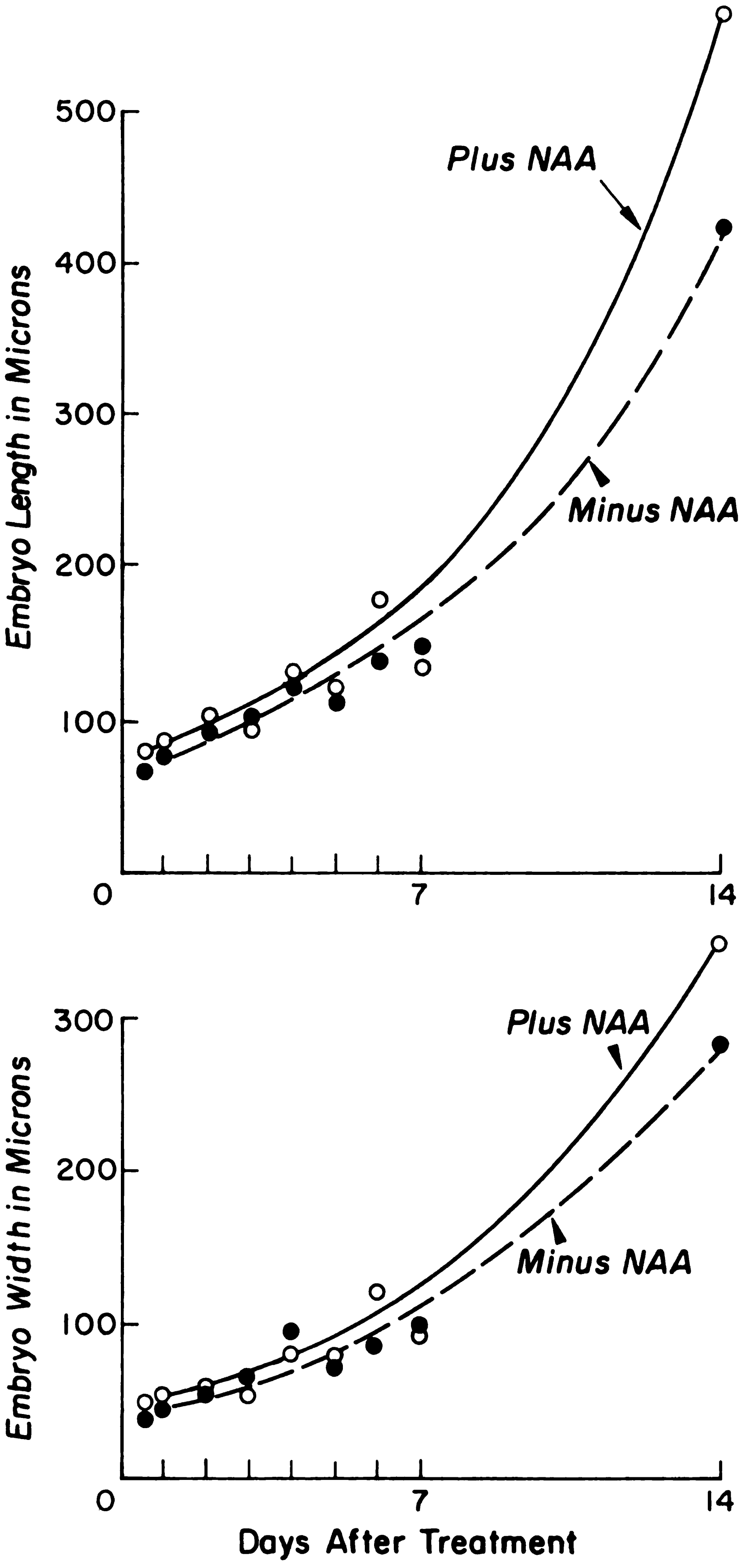
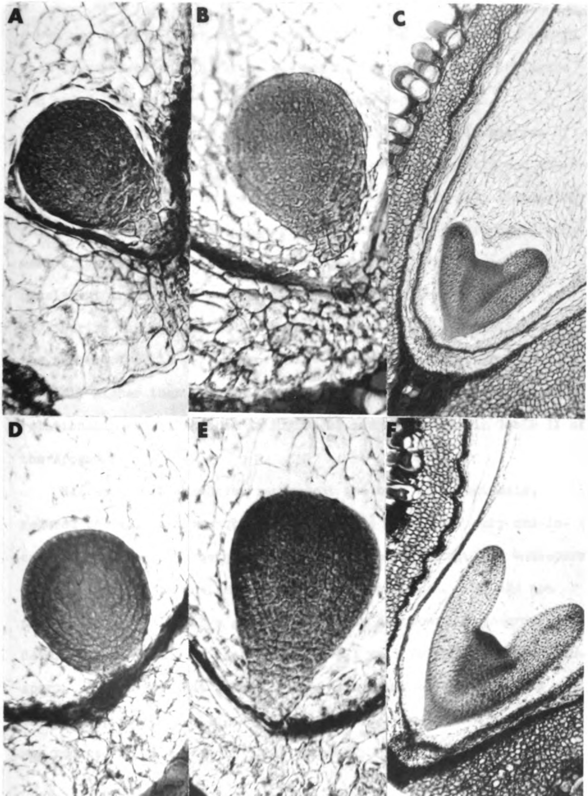

Embryo Length in Microns
500
400
300
200
100
0
7
14
Plus NAA
Minus NAA
Embryo Width in Microns
300
200
100
0
7
14
Plus NAA
Minus NAA
Days After Treatment

Figure 9.--Photomicrographs illustrating embryo enlargement in seeds from non-treated (top row) and NAA treated (bottom row) Redhaven peaches. (A) and (D), (B) and (E), and (C) and (F) illustrate embryo development at 6, 7 and 14 days after treatment, respectively. Approximate magnification is 375X for (A), (B), (D) and (E), and 90X for (C) and (F).


A
B
C
D
E
F

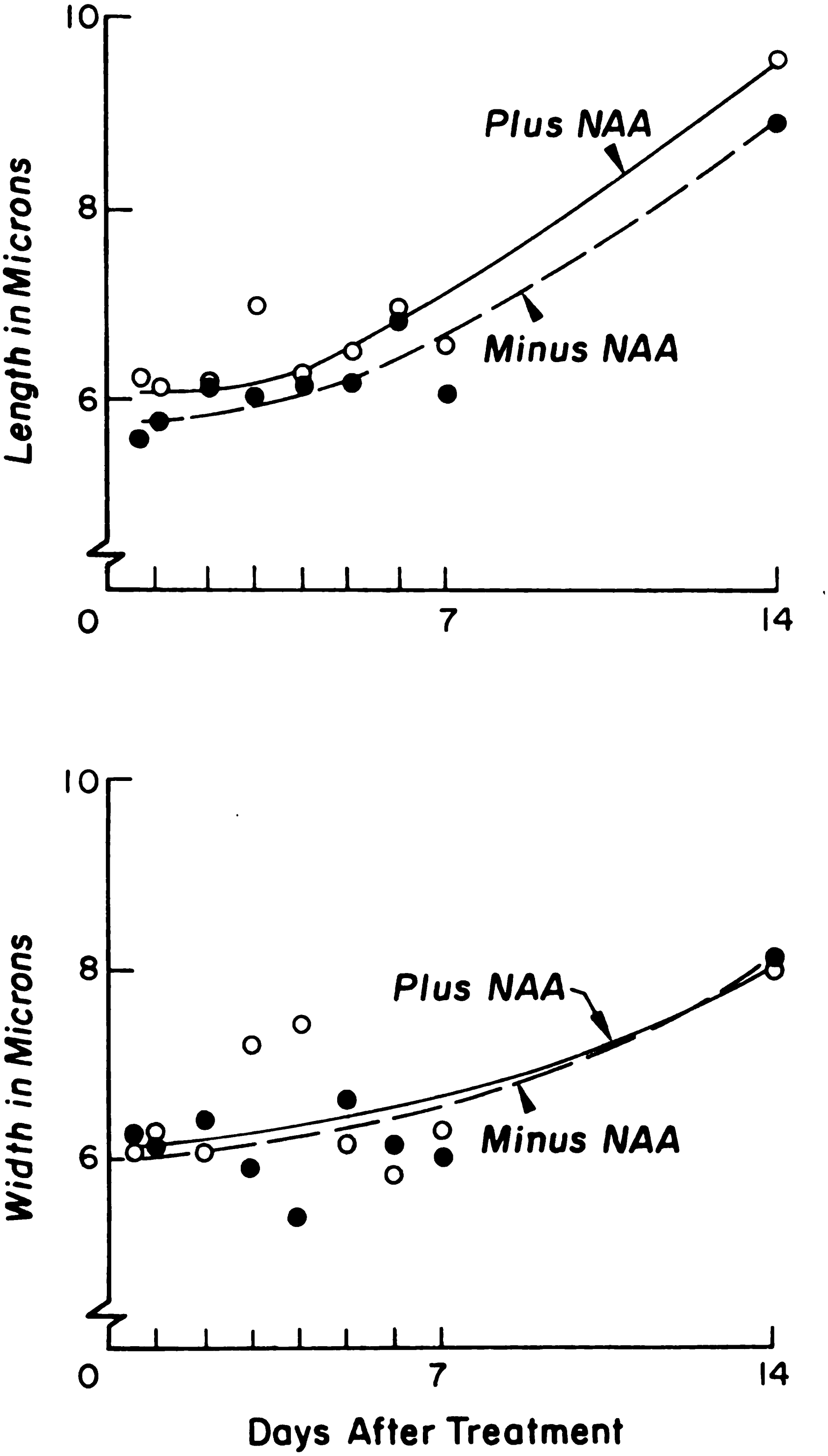
seven and 14 days after application of NAA. The difference in embryo size is not apparent in Figure 9-A and D, becomes apparent in Figure 9-B and E, and is quite pronounced in Figure 9-C and F.

Measurements of the length and width of the cells in embryos of treated and non-treated fruits are illustrated in Figure 10 and summarized in Table VIII in the Appendix. During the 14 days immediately following the NAA application, cells in the embryos of treated fruits were slightly longer than those from non-treated fruits but no such consistent trend could be seen in the width of embryo cells.

During the initial 14 day period following the NAA application, the number of cells per embryo in the seeds from treated fruits was markedly higher than that in the embryos of non-treated fruits. This relationship is illustrated in Figure 11 and summarized in Table IX of the Appendix.

NAA applied to Redhaven peaches at the time of cytokinesis, reduced the rate and amount of growth in the fleshy pericarp and increased the rate of growth and development in the embryo and endosperm. There was only a slight, negative effect on the seed. NAA did not promote more total embryo growth; it merely hastened embryo growth and development. Similarly, NAA hastened cell wall formation in the endosperm. It appears that growth in the meristematic regions of the fruit was promoted by NAA, whereas, growth in the expanding regions was reduced in rate, and in total amount.

Figure 10.--Comparison of average length and width of embryo cells from treated (NAA - 60 ppm) and non-treated Redhaven peach fruits.


10
8
6
Length in Microns
Plus NAA
Minus NAA
0
7
14
10
8
6
Width in Microns
Plus NAA
Minus NAA
0
7
14
Days After Treatment

## The Distribution of Foliar Applied Ring-labeled Naphthaleneacetic Acid in Leaf, Fruit and Seed Tissues of the Redhaven Peach

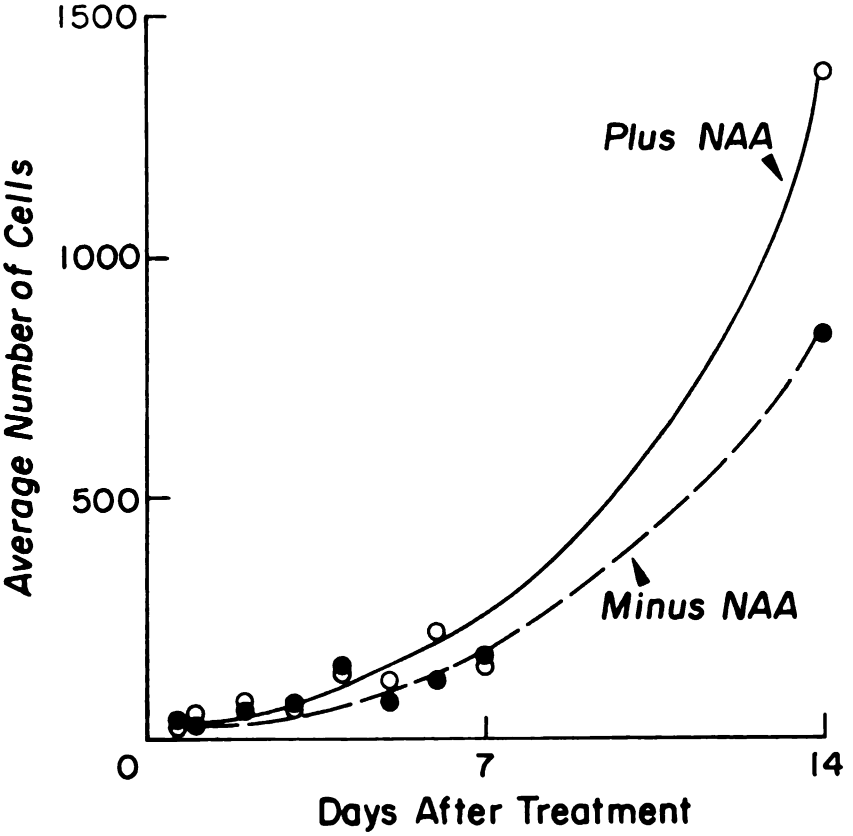
Determining the relative amounts of NAA present in the various peach tissues at intervals following the application of a known quantity of the material, and determining the site of action of NAA--even in a gross manner--would contribute to a better understanding of the mode of action of NAA. Thus, in an experiment designed to determine the distribution and the approximate site of action of NAA, a known amount of ring-labeled NAA-$C^{14}$ was applied to each of four or five peach leaves subtending the fruits in each of three replications.

The ethanol soluble $C^{14}$ in leaf, pericarp and seed tissues, for each sampling date, are illustrated graphically in Figure 12 and are summarized in Table X of the Appendix. The total amounts of $C^{14}$ extracted from the leaves and from the pericarp were higher (at each sampling date) than those found in the seed extracts. However, on a dry weight basis the amount extracted from the seeds was considerably higher than that extracted from the pericarp and leaf tissues. The amount of $C^{14}$ per gram dry weight that could be removed from the seeds was highest with seeds from fruits harvested five days after treatment. With the leaf extracts the samples at 15 days yielded the greatest amount of $C^{14}$.

The relative amounts of $C^{14}$ extracted from the pericarp and seed tissues at various intervals after treatment, are summarized in Table 13 on the basis of percent of the total amount extracted from the whole fruit. The fruit extracts contained approximately two to five times as much total $C^{14}$ as the seed extracts. The extraction data indicate

Figure 11.--Relationship between the number of cells in median longitudinal sections of embryos from treated (NAA - 60 ppm) and non-treated Redhaven peaches, harvested at various intervals after treatment.

Figure 12.--Amount of $C^{14}$ (expressed as cpm/gm dry weight) extracted from samples of leaves, pericarp and seeds of Redhaven peach at various intervals following the foliar application of ring-labeled NAA-$C^{14}$ on June 16, 1963.


1500
1000
500
0
Average Number of Cells
Plus NAA
Minus NAA
7
14
Days After Treatment

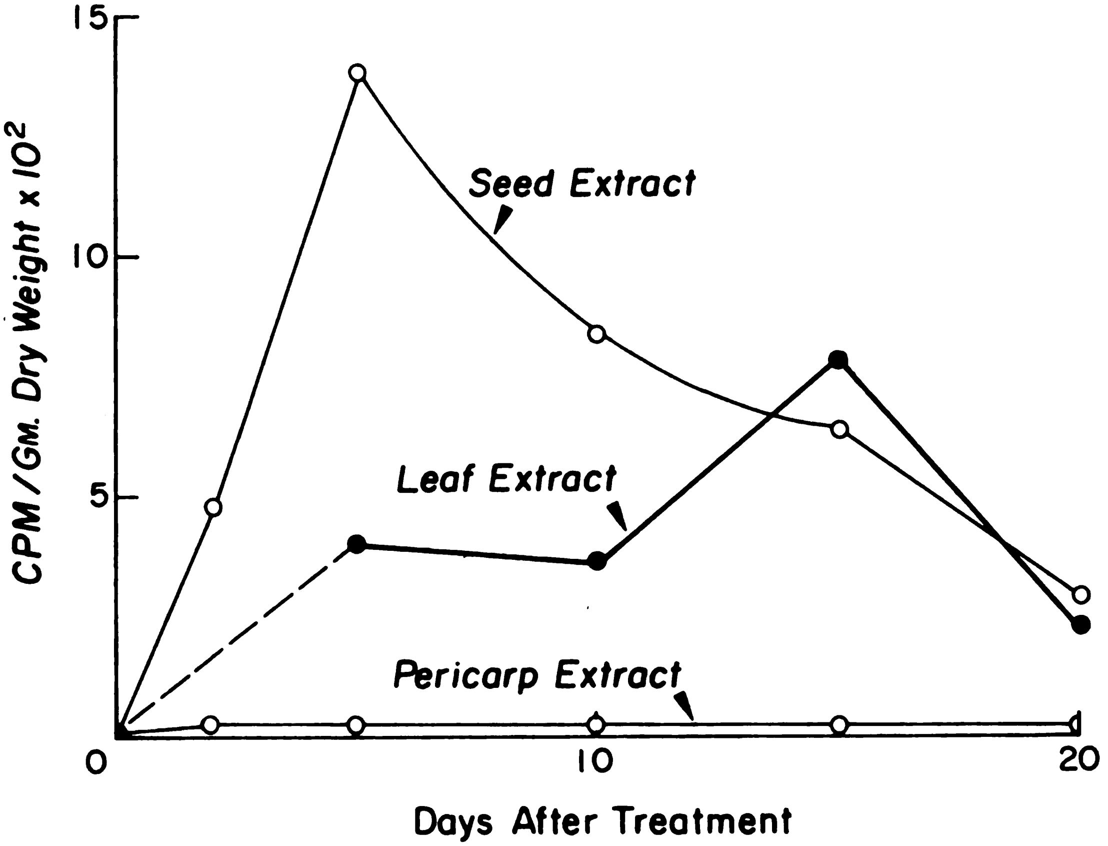

15
10
5
0
CPM / GM. Dry Weight x $10^2$
Seed Extract
Leaf Extract
Pericarp Extract
10
20
Days After Treatment

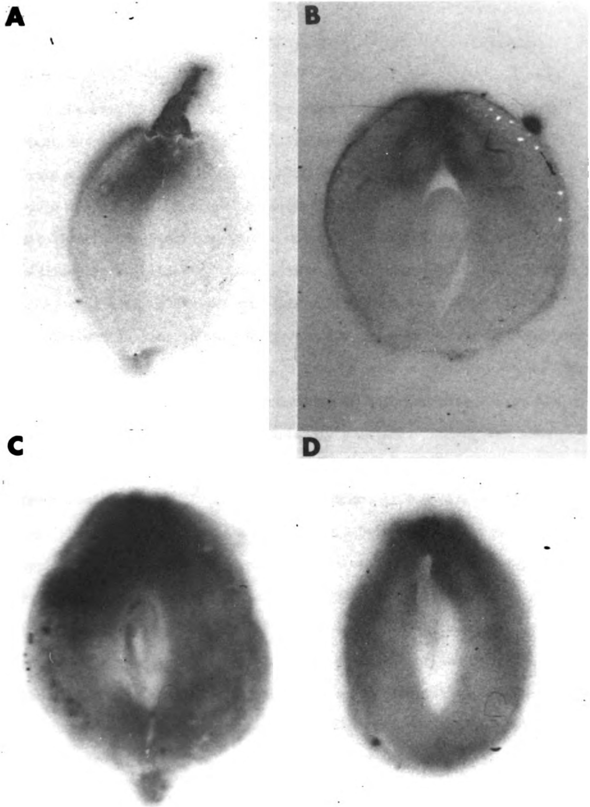
Table 13.--Percent of total $C^{14}$ found in fruit, extracted from pericarp and seed tissues at various intervals from the time of foliar application of NAA-$C^{14}$ to Redhaven peaches.

| Days from Treatment | Percent of Total $C^{14}$ Found in Fruits, Extracted from | |
|---|---|---|
| | Pericarp | Seeds |
| 2 | 85 | 15 |
| 5 | 60 | 40 |
| 10 | 65 | 35 |
| 15 | 69 | 31 |
| 20 | 82 | 18 |

that less $C^{14}$ was present in the samples at 15 and 20 days than at five and 10 days.

At each sampling date, several treated fruits were hand sectioned (longitudinally) with a razor blade, and the sections were dried prior to making autoradiograms. Four of these autoradiograms are presented in Figure 13-A, B, C, and D, representing the harvest dates occurring 2, 5, 10, and 15 days, respectively, after the application of NAA-$C^{14}$. (The seed in Figure 13-D shrank considerably during the drying process so that it appears very small in the autoradiogram.) Differences in geometry and in contact of sections with the film, permit only a qualitative comparison between the autoradiograms. It is apparent that the $C^{14}$ from foliar applied ring-labeled NAA-$C^{14}$ moved into the fruit tissues of the peach in less than two days after application, and it was fairly uniformly distributed. Since the NAA-$C^{14}$ was ring-labeled, the $C^{14}$ found in the fruit and seed tissues was presumably NAA-$C^{14}$ in its original molecular form.

Figure 13.--Autoradiograms of longitudinal sections of Redhaven peach fruits harvested at 2 days (A), 5 days (B), 10 days (C), and 15 days (D) after foliar treatment with ring-labeled NAA-$C^{14}$. The seed in (D) shrank in size during drying procedures. Approximately 3X.


A
B
C
D

# DISCUSSION

The original hypothesis, that auxin induced abscission in the peach may be related to stage of fruit development, was supported by field experimentation in 1961. From the studies reported herein, one would expect maximum response to exogenous auxin, of fruits in which cytokinesis was occurring in the endosperm. Significant abscission was induced in Redhaven, when NAA was applied during cell wall formation, or shortly after the cellular condition was attained. No significant abscission was noted when NAA was applied during the free nuclear stage.

Significant abscission was induced in Redhaven even though the endosperm of the samples was completely cellular. During the nine days that elapsed between the first two NAA applications the peach fruits enlarged rapidly averaging approximately 0.8 millimeters per day, whereas, during the six day interval between the second and third applications the rate of enlargement was only 0.4 millimeters per day. Perhaps the Redhaven fruits were sensitive to the NAA for a longer than "normal" period because of a reduced rate of growth and development during the second interval. That NAA does not induce significant abscission when applied after the occurrence of cytokinesis, has been adequately demonstrated by the work of Donoho (29), Kelley (55, 56), and Lombard (65). Undoubtedly, if the third NAA application had been delayed a few days, or if a fourth application had been made a few days after the third, little, if any significant abscission would have been induced.

Irrespective of the time of application, NAA did not induce significant fruit abscission with the Halehaven cultivar. This was consistent with the original hypothesis, because NAA was applied when the endosperm was either completely free nuclear or completely cellular, and this may have been before and after the sensitive stage. Cell wall formation actually began in the endosperm of Halehaven samples four days after the first NAA application and five days prior to the second. The fact that NAA induced significant abscission when applied to Redhaven peaches six days after the onset of cytokinesis in the endosperm of representative samples, and yet did not induce significant abscission when applied to Halehaven five days after the onset of cytokinesis, suggests that the Halehaven peach is either less sensitive or sensitive over a shorter period of time.

These results suggest that the onset of cytokinesis forms a line of demarcation between the fruits which are sensitive to NAA and those which have passed the sensitive stage. However, not all peach fruits containing only free nuclear endosperm are sensitive to NAA; if they were, the sprays applied to Redhaven and Halehaven peaches when the endosperm was completely free nuclear (1961) would have induced abscission, and such was not the case. There exists an upper and a lower threshold, above and below which peach fruits are not sensitive to NAA thinning sprays. Presumably the sensitive fruits are those which are approaching the point of forming cell walls in the endosperm. In all probability the smaller fruits which have not reached the suggested NAA-sensitive stage of development are those which would abscise in the June drop.

The range in average pericarp length at which the peach is susceptible to NAA is apparently very narrow. Halehaven peaches (in 1961) were not thinned significantly by sprays applied when representative fruit samples averaged approximately 24 and 30 millimeters in length. Halehaven would be expected to be sensitive to NAA applied when the average pericarp length in a representative sample was 28 millimeters. Theoretically, a margin of two millimeters average pericarp length was the difference between significant thinning and no thinning with the Halehaven peach in 1961.

Similarly, with Redhaven in 1961, NAA applied when ten representative fruits averaged 30.8 millimeters resulted in 70 percent fruit abscission. Sprays applied when fruit samples averaged approximately six millimeters smaller or two millimeters larger resulted in non-significant, and 65 percent abscission, respectively. These results suggest that the range in average pericarp length at which NAA would be expected to induce satisfactory thinning of a given cultivar, is less than plus or minus two millimeters of the length at which cytokinesis has been shown to occur. The individual fruits in a representative sample would be more variable in length and the desired results could still be achieved. For example, in 1961 the NAA spray applied to Redhaven that resulted in the greatest degree of thinning, was applied when the average pericarp length was 30.8 millimeters, but the range in pericarp length was 29 to 33 millimeters.

The work reported herein has established that Redhaven fruit abscission can be induced by an application of exogenous auxin during cytokinesis in the endosperm. To fully utilize this phenomenon a convenient parameter by which this stage could be identified in the field, is needed. Several such parameters (accumulated heat units, days after full bloom, after petal fall and after shuck split, and, fruit and seed measurements) were examined and compared.

The use of accumulated heat units as an index of the occurrence of cytokinesis in the peach is not feasible--at least not when full bloom is taken as the starting date for the accumulation. Heat units--especially when calculated from mean temperatures--are, at best, an approximation. Environmental factors other than temperature are involved in the growth and development of the peach. Therefore, an approximate index of the environment based on a single environmental factor cannot be expected to be very accurate, or reliable, as an index of an exact morphological stage of fruit development.

Calendar date, number of days after full bloom, after petal fall and after shuck split, (indices currently used to time thinning sprays in commercial practice) were inconsistent as indices of the beginning of cytokinesis in various peach cultivars. The use of one of these indices--particularly one of the first three--as a basis for the timing of NAA thinning sprays on peaches could understandably lead to inconsistent thinning results.

On the other hand, it would be logical to assume that such parameters as pericarp and seed measurements, which are inter-related with development of the endosperm and embryo, would provide a more reliable basis for determining the onset of cytokinesis. Pericarp length was shown to be a particularly reliable index of this stage of endosperm development in a given peach cultivar. Each cultivar studied began cell wall formation at a relatively constant pericarp length; however, cytokinesis in different cultivars may occur at different pericarp lengths. The reliability of average pericarp length and the ease by which this parameter can be determined, indicated that it is a suitable "in-the-orchard" index of the onset of cytokinesis.

It has been established that Redhaven peaches are most sensitive to NAA at the time of the onset of cytokinesis. In addition, average pericarp length has been shown to be a reliable index for this stage of development. Of interest would be the determination of the percentage of fruits of a given population (for a tree or for an entire orchard), which would be expected to persist to maturity, that might be induced to abscise following a NAA thinning spray. Thus, the population study was instigated and the information gained from it added considerable support to the original hypothesis. That is, the number of Redhaven peaches of the population sample that contained varying amounts of cellular endosperm amounted to 38 percent of the total. If NAA had been applied at the time the population sample was harvested, most of the fruits with cell walls forming in the endosperm (that is, 38 percent of the total) would have been expected to persist following the spray. This theoretical value of 38 percent was remarkably consistent with the 1961 thinning response of Redhaven, where 30 percent of the fruits survived a NAA spray applied just as cell walls were beginning to form.

The remaining 62 percent of the fruits (those in which cell wall formation had not begun), would have been expected to abscise following a NAA spray applied at the time the population sample was harvested.

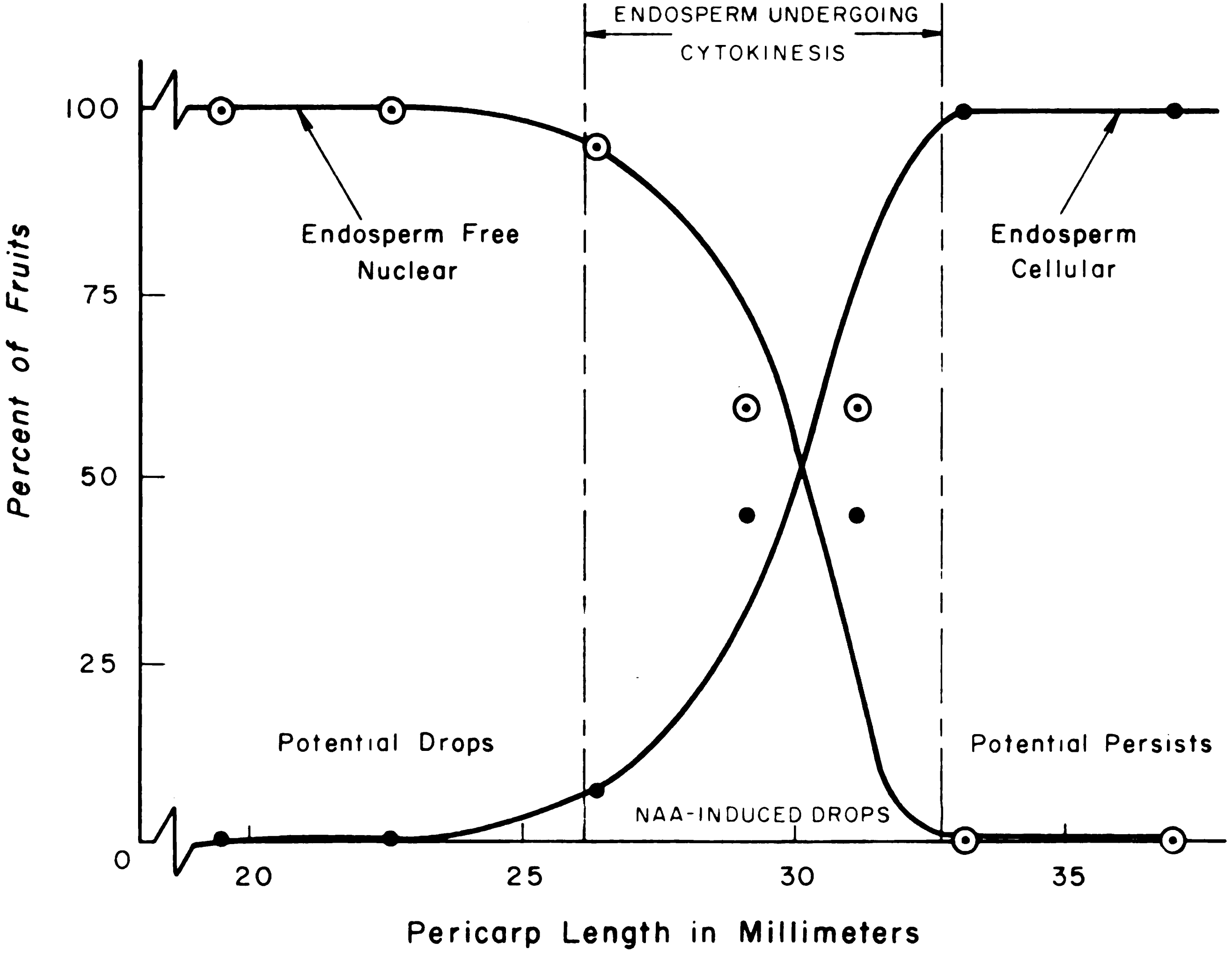
Undoubtedly, most of this postulated fruit abscission would have occurred without the NAA (since 45 percent abscission occurred in the control trees in 1961), but some of it would have been induced by NAA. Figure 14 is the result of an attempt to explain natural abscission, NAA-induced abscission, and non-abscission, based on size distribution of the fruit and associated variations in endosperm development present in a total population of Redhaven peach fruits, at the time of cytokinesis.

The two extreme areas on the chart, representing fruits which contained completely free nuclear endosperm and those which contained completely cellular endosperm, can be logically assumed to represent potentially abscising, and persisting fruits, respectively. The central area of the chart, representing fruits in which the endosperm was undergoing cytokinesis, undoubtedly includes areas representing fruits that would have normally abscised and those that would have normally persisted. In addition, the central area probably includes the area representing those fruits which would have been sensitive to an NAA spray applied at the time the population sample was harvested. It must be emphasized that not all of the fruits represented by the "undergoing cytokinesis" area would have been sensitive to the NAA; if they were, excessive abscission would have resulted.

Information concerning the causes of natural abscission could, perhaps, aid in determining those fruits which would be sensitive to an appropriately timed NAA spray. Naturally abscising Redhaven peach fruits were observed to contain either no embryo or a gigantic embryo, and either no endosperm or degenerating endosperm. One of the

Figure 14.--A projected scheme to explain natural abscission, NAA-induced abscission, and non-abscission, based on size distribution of the fruit and associated variation in endosperm development present in a total population of Redhaven peach fruits, at the suggested time of NAA application.

## A PROJECTED SCHEME FOR NAA-INDUCED ABSCISSION IN A TOTAL FRUIT POPULATION IN RELATION TO ENDOSPERM DEVELOPMENT



abscising fruits contained an apparently normal (but small) embryo and degenerating endosperm. It seems unlikely that those fruits containing no embryo or endosperm, had reached this stage of development without having been fertilized. Normally, non-fertilized flowers abscise very soon after petal fall (8, 27, 28, 43). Probably fertilization occurred and the product of this fertilization degenerated at a later date. The remnants of the embryos and endosperms could have been lost during the histological procedures.

Luckwill (70) has suggested that fruit abscission in apples is controlled by hormonal stimuli diffusing from the endosperm. If this is also true for peaches then the lack of normal endosperm in the seeds of the aborting fruits was the sole cause of the abortion. The causal factor of endosperm degeneration may have been a genetic problem in that the primary endosperm nucleus may have been weak and slow growing. Such slow growth could have reduced the growth rate of the entire fruit (due to a reduction in hormone production (93)). The slow growing fruit may have been less able to compete successfully with fast growing fruits for nutrients, thus aggravating the condition of a reduced growth rate.

Insufficient endosperm could also result in embryo starvation and eventual abortion. However, two of the naturally abscising fruits contained relatively gigantic embryos (which almost filled their embryo sacs) and very little endosperm. In all probability, genetic factors were responsible for the giant embryos. Normal endosperm would be unable to grow quickly enough, sufficiently early in the growing season, to supply such embryos with nutrients. Despite the fact that the embryos appeared to be very large, they were probably lacking nutrients. A lack of adequate endosperm could again have induced the fruit abortion.

The reason for abortion of the potentially dropping Redhaven fruits is less clear. Besides the expected size differences between the components of these fruits and the components of the persisting fruits, only one difference was apparent; the endosperm in the persisting fruits was much more abundant and more advanced. On the other hand, the less abundant endosperm of the potential drops was completely free nuclear in all samples. Such slower developing endosperm could again account for the reduced growth and possible eventual abscission as theorized above for the definitely abscising fruits.

The effects on peach fruits of an appropriately timed NAA application bear some resemblance to those on apples as discussed by Teubner and Murneek (107), and some resemblance to those on maize caryopses, as discussed by Britten (12). For example, Teubner and Murneek reported that an application of NAA to apples retarded fruit growth very markedly; the reduction in size of NAA treated apples became apparent in a few of the treated fruits three days after application, and in all of the treated fruits eight days after application. Britten reported that NAA promoted the growth of maize ovaries for eight to nine days after application, but from that time until maturity ovary growth lagged behind that of non-treated controls. Peach fruits too, were inhibited markedly in their growth following the application of NAA, but this inhibition did not become apparent quite as early as Teubner and Murneek reported for the apple. This difference could be explained on the basis of the different types of growth exhibited by the two fruits. The growth of the apple fruit is characterized by a sigmoid curve, whereas, the peach (fleshy pericarp) exhibits two periods of rapid growth separated by a period of retarded pericarp enlargement. Shortly after NAA application the Redhaven peach fruits entered the period of retarded pericarp growth, perhaps masking any early size difference between NAA treated and non-treated fruits.

NAA reportedly inhibits catalase activity (40), and may promote the activity of the IAA oxidase system (39). The net effect of such actions could be a reduced rate of fruit growth resulting from the destruction of some of the natural growth promoting substances diffusing from the seed (92). Such an explanation could account for the observed reduction in growth of the fleshy pericarp of the Redhaven peach.

Britten (12), and Teubner and Murneek (107), reported that the endosperm of maize caryopses and of apple fruits, respectively, was not noticeably altered by the applications of NAA. As reported herein, the only marked change in the endosperm of NAA treated peaches was earlier cell wall formation as compared to non-treated fruits.

Teubner and Murneek (107) examined embryo development mainly in abscising fruits, although the embryo development in some persisting fruits was also examined. Nevertheless some valid comparisons can be made between their work and that reported herein. They reported that NAA markedly inhibited embryo growth in the apple. When embryo growth was inhibited before the embryo reached the 60-celled stage, it aborted and the fruit abscised. In most of those apples in which embryo growth had reached the 60-celled stage the embryo also aborted but the fruits persisted with a full complement of aborted seeds.

Britten (12) reported that the embryos from NAA treated caryopses were apparently normal for eight days following treatment and then lagged in growth and differentiation until maturity. Viability was not impaired.

In contrast, the embryos of NAA treated Redhaven peach fruits showed a marked increase in average length, width and cell number during the first 14 days after treatment, in comparison to the controls. At harvest however, the embryos of treated and non-treated fruits differed little, if any, in size and both were viable.

The IAA destruction potential of the apple embryo is high during the early period of its rapid growth (107). If, as this suggests, IAA destruction is essential for rapid growth of the apple embryo, perhaps, the same condition exists in the peach embryo. Thus, if NAA enhances the activity of the IAA oxidase system this could account for the increased <u>rate</u> of embryo growth observed with the Redhaven peach.

It is evident from a morphological point of view, that the mode of action of NAA in the pericarp tissues of the fruits cited, is remarkably consistent. It is equally evident (again, from a morphological point of view) that the mode of action of NAA on the embryos is remarkably <u>inconsistent</u>. One thing is apparent; an application of NAA induces its most profound, morphologically visible effects on the embryo and pericarp tissues. Whether these effects are direct, as a result of the NAA itself, or indirect, as a result of NAA upsetting the delicate hormone balance (or other chemical effects) within the seed and fruit, remains to be seen.

That the seed is the site of NAA action is further suggested by NAA distribution studies employing ring-labeled NAA-$C^{14}$. These studies revealed the presence of $C^{14}$ in the pericarp and seed tissues within two days after application of the material to the foliage. After a five-day absorption period approximately one and one-half times as much $C^{14}$ was extracted from the pericarp as from the seed. However, the concentration of $C^{14}$ (on a cpm/gm. dry weight basis) was approximately 70 times higher in the seed than in the pericarp. At 15 and 20 days after treatment, the concentration of $C^{14}$ (presumably NAA-$C^{14}$ since the material was ring-labeled) was lower, suggesting that the seed has a capacity to metabolize the NAA. Preliminary studies designed to demonstrate that peach seeds can decarboxylate NAA-$C^{14}$ (employing carboxyl-labeled material) have proven inconclusive.

There is evidence however, to indicate that Redhaven peach seeds (and apple seeds) can decarboxylate indoleacetic acid (IAA) (29). IAA-oxidase activity of peach seeds (measured by determining the rate of decarboxylation of carboxyl-labeled IAA-$C^{14}$ by whole seeds) was found to be highest in seeds from fruits 18 to 25 millimeters long. Further, the IAA-oxidase activity reached its lowest level when average pericarp length was approximately 30 millimeters, but immediately increased to a second peak when the fruits were in excess of 36 millimeters long.

Lombard (65) reported the natural growth substance level of peach seeds to be low when pericarp length was 18 to 27 millimeters, high when the fruits reached approximately 30 millimeters, and low again at 37 millimeters pericarp length.

It was established herein, that cytokinesis begins in the endosperm of Redhaven, when the fruits average 30 to 31 millimeters in length. Perhaps, at this stage of endosperm development, when the natural auxin level is high and the destruction factor is low, any additional exogenous auxin transported to the seed could augment the high auxin level, perhaps to the point of upsetting the metabolism of the embryo and endosperm such that abscission may occur. Before and after cytokinesis, the destruction factor levels are high, thus maintaining the natural auxin levels low; additional exogenous auxin would not raise natural growth substance levels sufficiently high to trigger fruit abscission.

In essentially all fruit growth regulator work, investigators have more or less accepted the premise that the seed produces growth factors which diffuse through the peduncle and prevent an abscission zone from forming. The possibility exists that an abscission factor is produced (when certain conditions exist in the seed) which upon diffusion through the peduncle would accelerate the formation of an abscission zone. Liu et al. (64) and Ohkuma et al. (94) have recently isolated abscission factors from cotton burs and young cotton fruit, respectively, (designated Abscisin I and Abscisin II, respectively) which accelerate the formation of an abscission zone on young, excised, debladed petioles. Perhaps a similar factor can be induced to form in the peach at a specific time, by an exogenous auxin application.

The mechanism of action of NAA has been studied by many investigators (4, 44, 73, 74, 88, 91, 105, 107, 117) but no completely satisfactory explanation accounting for its diverse actions has yet been advanced. Nevertheless, it would seem that the various actions of NAA on the component parts of peach fruits are manifestations of some form of control NAA exerts over the natural growth or abscission factors produced by the seed.

# SUMMARY

1. In an attempt to identify a sensitive period (to exogenous auxin) in the development of the peach fruit, NAA (at 30 ppm) was applied to Redhaven and Halehaven peach trees when the fruits were at three stages of development. The sprays were applied approximately six days prior to, during, and six days after the onset of cytokinesis in the endosperm.

a. The NAA spray applied just as cell walls began to form in the endosperm of Redhaven peach induced significant (70 percent) abscission whereas the earlier spray applied when the endosperm was completely free nuclear was ineffective. NAA applied six days after the onset of cytokinesis also induced significant (65 percent) fruit abscission.

b. None of the three sprays induced significant abscission of Halehaven peaches. Subsequent histological examination of fruit samples revealed that the endosperm of the Halehaven fruits was either completely free nuclear or completely cellular at the times of application.

2. Several parameters were examined during two or more seasons, for the purpose of identifying a reliable index of the beginning of cell wall formation.

a. Heat units (accumulated from full bloom until the onset of cell wall formation) were unsatisfactory as an index of the beginning of this stage of endosperm development in the Redhaven peach.

b. Fruit and seed measurements were found to be reliable as indices of cell wall formation. Pericarp length was a particularly reliable index.

c. "Days after full bloom" and "days after petal fall" were inconsistent indices of cytokinesis in the Redhaven peach. "Days after shuck split" was somewhat more reliable but was not as accurate as pericarp length as an index of the onset of cytokinesis.

3. If growth regulators (having NAA-like action) are to be used successfully as peach thinning agents an index of the beginning of cell wall formation in the endosperm must be established for commercially important cultivars. Since pericarp length was a reliable index of this stage of endosperm development in the Redhaven peach, its use was examined in relation to other cultivars. Pericarp length varied between cultivars at the time of the beginning of cell wall formation, but within a cultivar it was a very consistent index from year to year.

4. To provide a partial basis for a study of the site of action of NAA, the variations in pericarp length and the variations in endosperm development of a total population sample of Redhaven fruits, were studied. The sample (consisting of 190 fruits) was harvested just as cell wall formation began in a ten fruit sample.

a. The size distribution "curve" of the fruits (based on pericarp length) approached a normal distribution.

b. Cell wall formation in the endosperm had advanced farthest in the largest fruits.

c. Approximately 38 percent of the fruits contained endosperm in which cell walls were forming. These fruits would have been expected to survive an NAA spray applied at the time the population sample was harvested.

5. A study of the embryo and endosperm of naturally abscising fruits in comparison to those of persisting fruits was initiated.

a. Most of the abscising fruits contained no embryos, a few contained gigantic embryos and one contained a small, but apparently normal embryo. Whenever endosperm was found in the embryo sacs, it was degenerating.

b. Fruits which were considered as potential drops, contained completely free nuclear endosperm and apparently normal (but small) embryos, in most instances.

c. Fruits which were classified as persisting, contained abundant endosperm. In 50 percent of the samples cell walls were beginning to form in the endosperm. The embryos were normal and larger than those in the other two groups.

6. The effects induced by NAA when applied as foliar sprays to peach trees were also examined.

a. NAA induced either lateral branch killing on terminals, or an overall yellowing of the foliage of the Redhaven peach, the form of injury differing from one year to the next. The Halehaven peach was not injured by NAA regardless of the time of application.

b. NAA (60 ppm) reduced the rate and amount of fleshy pericarp growth, but did not markedly affect the growth of the seed. The endosperm of sprayed fruits formed cell walls somewhat more rapidly than that of non-sprayed fruits. In addition, the supra-optimal concentration of NAA enhanced the <u>rate</u> of embryo growth but had little, if any, effect on final embryo size.

7. In order to determine the distribution of NAA in Redhaven peach fruits, ring-labeled NAA-$C^{14}$ was applied to leaves subtending selected fruits. At various intervals thereafter fruit and leaf samples were harvested.

a. Autoradiograms of longitudinal, hand sections of whole fruits prepared at each harvest date, revealed that $C^{14}$ was present in the fruits within two days after treatment.

b. Extracts of leaf, pericarp and seed tissues revealed that the relative concentration of $C^{14}$ was considerably higher in seed than in pericarp or leaf tissues.

c. The greatest amount of extractable $C^{14}$ was taken from fruit samples harvested five days after the application of the ring-labeled NAA-$C^{14}$.

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# APPENDIX

Table I.—Accumulated heat units (base temperature 40°F) from the time of full bloom until the onset of cell wall formation in the endosperm of Redhaven peach--1962.

| Date | Mean Temp. °F | Heat Units (°F-50) x 24 | Accumulated heat units |
|---|---|---|---|
| May 6 | 67.5 | 660 | 660 |
| 7 | 47.5 | 180 | 840 |
| 8 | 47.5 | 180 | 1020 |
| 9 | 50.5 | 252 | 1272 |
| 10 | 55 | 360 | 1632 |
| 11 | 61 | 504 | 2136 |
| 12 | 55 | 360 | 2496 |
| 13 | 68 | 672 | 3168 |
| 14 | 77 | 888 | 4056 |
| 15 | 82 | 1008 | 5064 |
| 16 | 77 | 888 | 5952 |
| 17 | 75.5 | 852 | 6804 |
| 18 | 77.5 | 900 | 7704 |
| 19 | 73.5 | 804 | 8508 |
| 20 | 74.5 | 828 | 9336 |
| 21 | 61 | 504 | 9840 |
| 22 | 62 | 528 | 10368 |
| 23 | 75.5 | 852 | 11220 |
| 24 | 59 | 456 | 11676 |
| 25 | 60.5 | 492 | 12168 |
| 26 | 55 | 360 | 12528 |
| 27 | 54.5 | 348 | 12876 |
| 28 | 64 | 576 | 13452 |
| 29 | 76.5 | 876 | 14328 |
| 30 | 75 | 840 | 15168 |
| 31 | 75 | 840 | 16008 |
| June 1 | 56.5 | 396 | 16404 |
| 2 | 55.5 | 372 | 16776 |
| 3 | 58 | 432 | 17208 |
| 4 | 70 | 720 | 17928 |
| 5 | 72 | 768 | 18696 |
| 6 | 68 | 672 | 19368 |
| 7 | 63.5 | 564 | 19932 |
| 8 | 64.5 | 588 | 20520 |
| 9 | 71 | 744 | 21264 |
| 10 | 71 | 744 | 22008 |
| 11 | 73.5 | 804 | 22812 |

Table II.--Accumulated heat units (base temperature 50°F) from the time of full bloom until the onset of cell wall formation in the endosperm of Redhaven peach--1962.

| Date | | Mean Temp. °F | Heat Units (°F-50) x 24 | Accumulated heat units |
|---|---|---|---|---|
| May | 6 | 67.5 | 420 | 420 |
| | 7 | 47.5 | 0 | 420 |
| | 8 | 47.5 | 0 | 420 |
| | 9 | 50.5 | 12 | 432 |
| | 10 | 55 | 120 | 552 |
| | 11 | 61 | 264 | 816 |
| | 12 | 55 | 120 | 936 |
| | 13 | 68 | 432 | 1368 |
| | 14 | 77 | 648 | 2016 |
| | 15 | 82 | 768 | 2784 |
| | 16 | 77 | 648 | 3432 |
| | 17 | 75.5 | 612 | 4044 |
| | 18 | 77.5 | 660 | 4704 |
| | 19 | 73.5 | 564 | 5268 |
| | 20 | 74.5 | 588 | 5856 |
| | 21 | 61 | 264 | 6120 |
| | 22 | 62 | 288 | 6408 |
| | 23 | 75.5 | 612 | 7020 |
| | 24 | 59 | 216 | 7236 |
| | 25 | 60.5 | 252 | 7488 |
| | 26 | 55 | 120 | 7608 |
| | 27 | 54.5 | 108 | 7716 |
| | 28 | 64 | 336 | 8052 |
| | 29 | 76.5 | 636 | 8688 |
| | 30 | 75 | 600 | 9288 |
| | 31 | 75 | 600 | 9888 |
| June | 1 | 56.5 | 156 | 10044 |
| | 2 | 55.5 | 132 | 10176 |
| | 3 | 58 | 192 | 10368 |
| | 4 | 70 | 480 | 10848 |
| | 5 | 72 | 528 | 11376 |
| | 6 | 68 | 432 | 11808 |
| | 7 | 63.5 | 324 | 12132 |
| | 8 | 64.5 | 348 | 12480 |
| | 9 | 71 | 504 | 12984 |
| | 10 | 71 | 504 | 13488 |
| | 11 | 73.5 | 564 | 14052 |

Table III.--Accumulated heat units (base temperature 40°F) from the time of full bloom until the onset of cell wall formation in the endosperm of Redhaven peach--1963.

| Date | Mean Temp. °F | Heat Units (°F-50) x 24 | Accumulated Heat Units |
|---|---|---|---|
| May 7 | 57 | 408 | 408 |
| 8 | 76 | 864 | 1272 |
| 9 | 71 | 744 | 2016 |
| 10 | 58 | 432 | 2448 |
| 11 | 46.5 | 156 | 2604 |
| 12 | 51.5 | 276 | 2880 |
| 13 | 56.5 | 396 | 3276 |
| 14 | 57 | 408 | 3684 |
| 15 | 61 | 504 | 4188 |
| 16 | 60.5 | 492 | 4680 |
| 17 | 61.5 | 516 | 5196 |
| 18 | 60.5 | 492 | 5688 |
| 19 | 60 | 480 | 6168 |
| 20 | 57 | 408 | 6576 |
| 21 | 55.5 | 372 | 6948 |
| 22 | 46 | 144 | 7092 |
| 23 | 45 | 120 | 7212 |
| 24 | 47 | 168 | 7380 |
| 25 | 52.5 | 300 | 7680 |
| 26 | 54.5 | 348 | 8028 |
| 27 | 60 | 480 | 8508 |
| 28 | 62.5 | 540 | 9048 |
| 29 | 54.5 | 348 | 9396 |
| 30 | 59.5 | 468 | 9864 |
| 31 | 62 | 528 | 10392 |
| June 1 | 64 | 576 | 10968 |
| 2 | 71.5 | 756 | 11724 |
| 3 | 72.5 | 780 | 12504 |
| 4 | 74 | 816 | 13320 |
| 5 | 75 | 840 | 14160 |
| 6 | 77 | 888 | 15048 |
| 7 | 72.5 | 780 | 15828 |
| 8 | 73.5 | 804 | 16632 |
| 9 | 73 | 792 | 17424 |
| 10 | 75.5 | 852 | 18276 |
| 11 | 49.5 | 228 | 18504 |
| 12 | 57 | 408 | 18912 |
| 13 | 56.5 | 396 | 19308 |
| 14 | 57.5 | 420 | 19728 |
| 15 | 60.5 | 492 | 20220 |
| 16 | 61.5 | 516 | 20736 |

Table IV.--Accumulated heat units (base temperature 50°F) from the time of full bloom until the onset of cell wall formation in the endosperm of Redhaven peach--1963.

| Date | | Mean Temp. °F | Heat Units (°F-50) x 24 | Accumulated Heat Units |
|---|---|---|---|---|
| May | 7 | 57 | 168 | 168 |
| | 8 | 76 | 624 | 792 |
| | 9 | 71 | 504 | 1296 |
| | 10 | 58 | 192 | 1488 |
| | 11 | 46.5 | 0 | 1488 |
| | 12 | 51.5 | 36 | 1524 |
| | 13 | 56.5 | 156 | 1680 |
| | 14 | 57 | 168 | 1848 |
| | 15 | 61 | 264 | 2112 |
| | 16 | 60.5 | 252 | 2364 |
| | 17 | 61.5 | 276 | 2640 |
| | 18 | 60.5 | 252 | 2892 |
| | 19 | 60 | 240 | 3132 |
| | 20 | 57 | 168 | 3300 |
| | 21 | 55.5 | 132 | 3432 |
| | 22 | 46 | 0 | 3432 |
| | 23 | 45 | 0 | 3432 |
| | 24 | 47 | 0 | 3432 |
| | 25 | 52.5 | 60 | 3492 |
| | 26 | 54.5 | 108 | 3600 |
| | 27 | 60 | 240 | 3840 |
| | 28 | 62.5 | 300 | 4140 |
| | 29 | 54.5 | 108 | 4248 |
| | 30 | 59.5 | 228 | 4476 |
| | 31 | 62 | 288 | 4764 |
| June | 1 | 64 | 336 | 5100 |
| | 2 | 71.5 | 516 | 5616 |
| | 3 | 72.5 | 540 | 6156 |
| | 4 | 74 | 576 | 6732 |
| | 5 | 75 | 600 | 7332 |
| | 6 | 77 | 648 | 7980 |
| | 7 | 72.5 | 540 | 8520 |
| | 8 | 73.5 | 564 | 9084 |
| | 9 | 73 | 552 | 9636 |
| | 10 | 75.5 | 612 | 10248 |
| | 11 | 49.5 | 0 | 10248 |
| | 12 | 57 | 168 | 10416 |
| | 13 | 56.5 | 156 | 10572 |
| | 14 | 57.5 | 180 | 10752 |
| | 15 | 60.5 | 252 | 11004 |
| | 16 | 61.5 | 276 | 11280 |

Table V.--Comparison of the length and width of fruits from treated and non-treated Redhaven peach trees, collected and measured at various intervals--1962.

| Time from Treatment | Fruits Receiving | | | |
|---|---|---|---|---|
| | No Treatment[1] | | NAA at 60 ppm[1] | |
| | Length | Width | Length | Width |
| 0 | 30.5 | 25.5 | 30.5 | 25.5 |
| 14 hrs. | 29.5 | 24.9 | 30.4 | 24.8 |
| 1 day | 30.0 | 24.8 | 33.0 | 27.1 |
| 2 days | 32.7 | 27.7 | 33.2 | 27.1 |
| 3 " | 34.1 | 28.9 | 33.1 | 27.9 |
| 4 " | 34.9 | 29.3 | 35.5 | 29.2 |
| 5 " | 35.3 | 30.1 | 33.1 | 27.1 |
| 6 " | 36.6 | 30.7 | 35.9 | 30.0 |
| 7 " | 36.6 | 31.3 | 33.9 | 29.0 |
| 14 " | 39.2 | 34.4 | 37.6 | 31.5 |
| 52 " [2] | 58.1 | 58.7 | 49.5 | 47.8 |

[1] Average of 10 fruits, measured in millimeters.

[2] Harvest.

Table VI.--Comparison of the length and width of seeds from treated and non-treated Redhaven peaches collected and measured at various intervals--1962.

| Time from Treatment | Seeds from Fruits Receiving | | | |
|---|---|---|---|---|
| | No Treatment[1] | | NAA at 60 ppm[1] | |
| | Length | Width | Length | Width |
| 0 | 11.9 | 7.3 | 11.9 | 7.3 |
| 14 hrs. | 11.5 | 7.4 | 11.7 | 7.6 |
| 1 day | 11.6 | 7.1 | 13.5 | 8.5 |
| 2 days | 13.1 | 8.3 | 13.3 | 8.8 |
| 3 " | 14.0 | 8.9 | 12.3 | 8.0 |
| 4 " | 13.6 | 9.1 | 14.6 | 9.7 |
| 5 " | 13.7 | 9.0 | 13.1 | 9.3 |
| 6 " | 14.4 | 9.9 | 15.2 | 10.5 |
| 7 " | 14.3 | 9.7 | 14.1 | 9.6 |
| 14 " | 15.7 | 10.8 | 16.2 | 11.2 |
| 52 " [2] | 16.7 | 11.6 | 16.0 | 11.2 |

[1] Average of 10 seeds, measured in millimeters.

[2] Harvest.

Table VII.--Comparison of the length and width of embryos from treated and non-treated Redhaven peaches collected and measured at various intervals--1962.

| Time from Treatment | Embryos[1] from Fruits Receiving | | | |
|---|---|---|---|---|
| | No Treatment | | NAA at 60 ppm | |
| | Length | Width | Length | Width |
| 14 hrs. | 68.9 | 40.8 | 78.2 | 48.3 |
| 1 day | 78.2 | 45.6 | 85.5 | 55.4 |
| 2 days | 92.6 | 57.0 | 102.3 | 60.2 |
| 3 " | 103.1 | 66.1 | 93.2 | 54.4 |
| 4 " | 142.1 | 94.5 | 132.6 | 82.2 |
| 5 " | 113.7 | 74.4 | 119.4 | 81.6 |
| 6 " | 138.9 | 87.4 | 176.8 | 118.7 |
| 7 " | 145.5 | 97.9 | 133.9 | 93.1 |
| 14 " | 419.9 | 283.9 | 563.2 | 345.2 |

[1] Measured in microns.

Table VIII.--Comparison of the average cell length and width in embryos from treated and non-treated Redhaven peaches collected at various intervals--1962.

| Time from Treatment | Embryos[1] from Fruits having Received | | | |
|---|---|---|---|---|
| | No Treatment | | NAA at 60 ppm | |
| | Cell Length | Cell Width | Cell Length | Cell Width |
| 14 hrs. | 5.6 | 6.3 | 6.3 | 6.1 |
| 1 day | 5.8 | 6.2 | 6.2 | 6.3 |
| 2 days | 6.1 | 6.4 | 6.2 | 6.1 |
| 3 " | 6.1 | 5.9 | 7.0 | 7.2 |
| 4 " | 6.2 | 5.4 | 6.3 | 7.9 |
| 5 " | 6.2 | 6.6 | 6.5 | 6.2 |
| 6 " | 6.8 | 6.2 | 7.0 | 5.8 |
| 7 " | 6.1 | 6.0 | 6.6 | 6.3 |
| 14 " | 8.9 | 8.1 | 9.7 | 8.0 |

[1] Measured in microns.

Table IX.--Comparison of cell numbers in longitudinal sections of embryos from treated and non-treated fruits collected at various intervals--1962.

| Time from Treatment | Average no. of Cells in Embryos of Fruits having Received | |
|---|---|---|
| | No Treatment | NAA at 60 ppm |
| 14 hrs. | 36 | 35 |
| 1 day | 46 | 56 |
| 2 days | 68 | 79 |
| 3 " | 76 | 73 |
| 4 " | 145 | 135 |
| 5 " | 86 | 118 |
| 6 " | 117 | 231 |
| 7 " | 158 | 153 |
| 14 " | 846 | 1399 |

Table X.--Amount of $C^{14}$ (expressed as cpm) extracted from leaves, fruits and seeds of Redhaven peach at various intervals following the foliar application of NAA-$C^{14}$ on June 16, 1963.

| Days from Treatment | cpm/gm. Dry Weight | | |
|---|---|---|---|
| | Leaf Extract | Pericarp Extract | Seed Extract |
| 2 | - | 21 | 479 |
| 5 | 404 | 18 | 1387 |
| 10 | 368 | 12 | 841 |
| 15 | 785 | 10 | 646 |
| 20 | 232 | 9 | 296 |