THE ABSORPTION, TRANSLOCATION, AND ENZYMATIC DESTRUCTION OF C^{14} LABELED GROWTH REGULATORS WHEN USED AS CHEMICAL THINNING AGENTS FOR THE PEACH (PRUNUS PERSICA, SIEB. ZUCC.) AND THE APPLE (MALU DOMESTICA, BORK.)

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By

CLIVE WELLINGTON DONOHO JR.

AN ABSTRACT

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CLIVE WELLINGTON DONOHO JR. ABSTRACT

A series of studies were conducted during 1959 and 1960 to establish the reason for wide variations in the amount of fruit thinning from year to year and from one location to another when growth regulators are used to chemically thin fruit.

Absorption studies conducted on one-year-old apple and peach trees revealed that preconditioning trees to different environments will affect the absorption capacities of the trees. Peach trees absorbed more ring labeled C^{14} naphthaleneacetic acid (NAA) after growing at air temperatures of 60° to 66° F than when grown at 70° to 75° F. The most favorable relative humidity was 91 to 94 percent. Apple trees absorbed more NAA C^{14} when grown at air temperatures of 60° to 66° F than when grown at 70° to 77° F irrespective of relative humidity (43 to 45 percent or 91 to 94 percent). This could conceivably account for overthinning of apple and peaches when a cool, rainy period of several weeks duration precedes the application of NAA. It appears that the cool weather may be more important in its effects on thinning than the rain.

The amount of cuticle present on the leaves of apple and peach trees grown under the different environments for the absorption studies was nearly the same although differences in absorption of NAA C^{14} for these same trees were significant. From these studies it appears that the amount of cuticle

present on the leaves is not important in absorption. However, it may be that the chemical composition or quality of the cuticle is important in affecting absorption of a growth regulator such as NAA.

Absorption of NAA C^{14} by apple and peach leaves was found to be highly dependent upon the pH of the solutions containing the plant regulator. Absorption of NAA C^{14} decreased as the pH was increased from 3 to 9. In a 24-hour period, apple and peach leaves absorbed 8 percent and 12 percent, respectively, of the applied NAA C^{14} solutions of pH 9, as compared to 70 percent and 74 percent, respectively, when the pH of the solutions was 3. These studies indicated that differences in the pH of the water supply used in making an application of the growth regulator for fruit thinning could be an important source for variation found in thinning experiments.

Translocation studies revealed that little of the applied NAA C^{14} moved from the original site of application until after a 24-hour perod when the material was placed on the upper surface of apple leaves. By contrast, some movement of C^{14} was evident after one hour when the material was applied to the lower surface of apple leaves or to the upper or lower surfaces of peach leaves. C^{14} was found to be translocated into the fruit and seeds of the apple and peach following a foliar application of ring labeled NAA C^{14}.

A study was initiated in 1960 to determine the enzymatic destruction potential of developing peach and apple seeds in destroying carboxyl labeled

IAA and NAA. The enzymatic destruction potential was compared with periods of high and low natural hormone accumulation in the seeds.

Indoleacetic acid (IAA) destruction potential of the apple and peach seed varied greatly with the different stages of seed development. High IAA oxidase activity was found to be closely associated with previously reported low natural hormone content of the seeds. NAA destruction was very slow as compared to IAA destruction and the amount destroyed was of such low magnitude that it probably would not be considered an important influence on the effectiveness of NAA when applied as a thinning agent. From these studies it appears that the effectiveness of a growth regulator to thin peach and apple fruits may be dependent upon the activity of the IAA oxidase enzyme in the seeds. For example, when activity is high, the natural hormone content in the seed is low and little thinning results from the application of a growth regulator. When the enzyme activity is low, the natural hormone content in the seed is high and corresponds to the period when the growth regulator is effective as a thinning agent. This period of low IAA oxidase activity and high hormone content in peach seeds occurred when the fruit approached 30 millimeters in length. This was found also to be the optimum size of the fruit for NAA to be effective as a thinning agent. Applications of NAA to peach trees when the fruit was greater than 30 millimeters did not result in significant fruit thinning.

Field studies using growth regulators as fruit thinning agents indicated that two synthetic growth regulators, 3-chlorophenoxy alpha propionic acid (3-CP) and NAA, may be used effectively as fruit thinning agents for peach trees. Application of 1,000 ppm of maleic hydrazide (MH-30) approximately one month after bloom as suggested for use in South Carolina, resulted in very little fruit thinning. The 3-CP and NAA were applied when the concentration of the natural hormones in the seed was high using the fruit length of 30 millimeters as the index. Either the concentration of maleic hydrazide was too low for Michigan conditions or the timing of the application should be based on a different index for stage of development of the components of the seed to give effective thinning.

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TABLE OF CONTENTS

TABLE OF CONTENTS CONT'D

INTRODUCTION

The application of growth regulators to the foliage of peach and apple trees to bring about the desired reduction of excess fruit has resulted in a wide variation in the amount of thinning from year to year and from one location to another. These variations in amount of thinning may result from a failure of the materials to be absorbed and translocated to the fruits or the materials may be enzymically destroyed after they enter the plant.

There have been numerous reports on the effect of environment on absorption of different compounds but in a majority of the cases, the different environmental conditions were induced only at the time of application of the material and during the absorption period. Relatively few studies have been made to determine the preconditioning effect of environment on plants and how it is related to absorption. It has been conjectured that the environment to which the plant was exposed preceding the period of treatment of a plant regulator may influence the degree in which the plant growth regulator is absorbed.

Since the existence of an enzyme in plants capable of destroying indoleacetic acid (IAA) has been reported (18, 21, 33, 69, 70), it would be of interest to know if this and other enzymes, capable of destroying naphthaleneacetic acid (NAA) are present in peach and apple trees. The knowledge of such enzymatic systems in fruit trees could assist the researcher in interpreting the erratic results encountered when growth regulators are used to bring about fruit abscission.

Several growth regulators have been used to chemically thin both apples and peaches. NAA and naphthalene acetamide have been used successfully for thinning apples and NAA has been partially successful in thinning peaches. Other promising growth regulators used to thin peaches are 3-chlorophenoxy alpha propionic acid (3-CP) and maleic hydrazide (MH-30). However, the performance of all of these chemicals is not consistent in every season. Because of this inconsistency a study was developed to determine: (1) the absorption of ring labeled C^{14} NAA by leaves of apple and peach trees grown under different environments; (2) the amount of leaf cuticle formed under different environments; (3) the effect of pH on absorption of NAA; (4) the pattern of movement within the leaf and rate of translocation of labeled NAA out of the leaf; (5) the presence of NAA C^{14} in the fruit when applied only to the foliage; (6) the presence of enzyme systems in the leaves and developing seed of the peach and apple capable of destroying IAA and NAA, and a comparison of high and low periods of IAA oxidase activity in the developing seed with the periods of high and low natural hormone accumulations in the seed; and (7) which of the most promising growth regulators could best be used commercially to thin peaches. The results of this study are reported herein.

LITERATURE REVIEW

Absorption of Growth Regulators

The ease by which a plant regulator is absorbed and translocated within the plant may well determine the effectiveness of the chemical as a fruit thinning agent. As previously stated, the amount of fruit thinning following application of a growth regulator may vary from year to year and from one location to another. Hoffman (26) stated that these variations seem to be associated with the amount of chemical absorbed by the foliage. It seems, for a growth regulator to be an effective fruit thinning agent, it must first be absorbed by the foliage of the tree, translocated into the fruit and seed, and in turn cause injury to vital fruit and/or seed components. Factors effecting the absorption and translocation of a growth regulator would in turn govern the effectiveness of the growth regulator to bring about fruit abscission. The penetration and movement of a particular plant regulator could be influenced by many physical, chemical, and biological factors of which environment, thickness of the cuticle on the leaves and pH of the dispersion containing the plant regulator could be of prime importance.

Currier and Dybing (9) stated that warm temperature, if not excessive, promoted the penetration of herbicides by affecting (a) physico-chemical processes (increased rate of diffusion, lower viscosity, etc.); and (b) physiological

factors (acceleration of photosynthesis, phloem translocation, protoplasmic streaming, and growth). Schell (58) reported that higher temperatures (92-93° F) increased absorption of IAA by apple leaves two-fold over lower temperatures (38-39° F) and that faster drying rates produced more absorption than slower drying rates. Westwood and Batjer (78), studying the absorption of NAA by apple leaves, found that prolonged drying of the NAA solution at a higher temperature (80° F) gave more absorption than prolonged drying at a lower temperature (50° F). They also noted that preconditioning apple trees at 70° F for one week resulted in greater NAA absorption than preconditioning at 50° F when absorption was measured by "leaf-angle increase". Edgerton and Haeseler (15) preconditioned McIntosh apple trees for eight days at 55° F and found that absorption was more for the trees preconditioned at the 55° F than for the trees grown in the greenhouse which ranged in temperatures from 68 to 70° F.

Smith et al. (62), studying the effect of relative humidity on the absorption of maleic hydrazide, found by holding tomato plants at 75 to 80° F and then varying the humidity, a range from 12 to 62 percent of maleic hydrazide was absorbed, the lowest amount being absorbed at 45 percent relative humidity and the highest amount at 100 percent relative humidity. Schell (58) reported that raising the relative humidity from 60 to 95 percent without changing the temperature increased the absorption of IAA by 18 percent when applied to the upper surface of apple leaves, but only 5 percent when IAA was applied

to the lower surface. Currier and Dybing (9) suggested that relatively high humidity prevents water stress in the plant, favors stomatal opening, delays drying of spray deposit, and in turn may increase cuticular permeability.

There are several reports which have indirect evidence that environment plays an important role in absorption of organic compounds. Weaver and DeRose (75) stated that relatively warm atmospheric temperatures enhance the absorption of growth regulators by leaves. Hoffman (26) stated that weather conditions for one to two weeks previous to an application of NAA on apple trees appeared to indirectly influence the thinning effectiveness of the chemical. He conjectured that humid cloudy weather during the time the apple foliage is developing might conceivably condition the leaves for maximum absorption of NAA. Kelly (29) reported overthinning of peach fruits when the application of NAA was made following a cool, rainy period of several days duration.

The cuticle, a fatty membrane-like layer which covers the entire surface of the above ground portion of plants, presents a barrier through which compounds must pass in the process of absorption. Metcalf (48) stated that growth regulating compounds penetrate through (1) cuticle of hairs of aerial parts, (2) cuticle of epidermal cells, and (3) the stomata and then through cuticle that covers the outer surfaces of walls that bound intercellular spaces. Therefore, as stated by Van Overbeek (73) cuticle is the first barrier which spray chemicals must pass and even when spray chemicals penetrate through the stomata,

they still have to pass through the cutin layer which lines the intercellular spaces of the leaf.

Most investigators, using non-volatile compounds, tend to support the diffusion of compounds through the cuticle of epidermal cells rather than through the stomata. Muzik et al. (53) reported that the thickness of the epidermal cuticle is a much more important factor in determining the effectiveness of a spray than the opening and closing of the stomata. Thimann (71) found that applying 2, 4-D when the largest number of stomata were open did not increase the effectiveness of the spray.

Absorption of growth regulators has been found to be somewhat dependent on the pH of the solution. Hamner et al. (22) were among the first workers to report that pH would influence the effectiveness of regulating compounds. They found that absorption of 2, 4-D was high at low pH and practically non-existent at pH values above six. Other investigators (3, 23) obtained similar results and suggested that growth regulators are undissociated at low pH levels and, consequently are more soluble in the cuticle than when dissociated at higher pH levels. Similar investigations have not been made using NAA.

Translocation of Growth Regulators

Van Overbeek (73) suggested that the translocation of regulators follows a pattern similar to that of most other organic compounds, and once a compound is taken up by the leaves, it is transported out of that leaf via the phloem.

Metcalf (48) reported that once a growth regulator is within the cytoplasm of epidermal cells, it apparently passes from cell to cell through plasmadesmata. He feels that lateral transport, movement from one epidermal cell to another between veins of leaves, may be related to the prevalence of numerous plasmadesmata which occur in the adjacent walls of epidermal cells of this type.

The movement of growth regulators from the leaf seem to be most closely associated with movement of photosynthates (48, 49, 57, 73). Metcalf (48) stated that 2, 4-D was not translocated in detectable amounts from young, rapidly expanding leaves; but, as the leaf matures and photosynthate has moved from it to the main body of the plant, translocation of the regulator became evident. Fang and Butts (16) indicated that the direction of transport and pattern of distribution is associated with areas of intense metabolic activity.

Evidence seems to indicate that foliar applied growth regulators can be translocated through the conductive system of the plant into the developing fruit and seed. Marth et al. (46) found that a quaternary ammonium compound applied to bean plants was translocated into the seeds of these plants in sufficient amounts to greatly alter internodal elongation of the offsprings. Crane (8) showed that 2, 4, 5-trichlorophenoxy acetic acid stimulated the increase in diameter of apricot fruit as early as seven days after treatment. Maxie et al. (47) found C^{14} in the mesocarp three days after treatment when carboxyl-labeled 2, 4, 5-trichlorophenoxy acetic acid was applied to the leaves and

branches of the apricot trees. Furthermore, within 10 days following treatment, C^{14} activity could be detected in both embryos and integuments and the activity increased with time until the fruit was mature.

The assumption has been made that NAA is translocated to the seed when used as a thinning agent for apples and peaches, but no direct evidence has been presented to verify this assumption (37, 42, 70).

Enzymatic Destruction of Growth Regulators

A. Indoleacetic acid oxidase:

IAA oxidase, an enzyme capable of oxidizing IAA in vitro, has been isolated from several different plant species and plant parts (18, 21, 33, 69, 70), the original isolation was made by Tang and Bonner (69) from pea hypocotyls. In this study (69) they showed that the optimum pH for the activity of IAA oxidase was 6.2 to 6.7. They found also that the rate of IAA destruction was proportional to enzyme concentration when the concentrations of enzyme was low, while at higher concentrations, substrate became the limiting factor.

Teubner and Murneek (70) studying indoleacetic acid oxidase systems of apple embryos reported that the activity of the enzyme system was greatest in the rapidly developing embryos. Fang and Butts (17) reported that $C^{14}O_2$ was evolved when carboxyl-labeled IAA was applied to bean, pea and corn plants and that $C^{14}O_2$ production was found to be greater when plants were kept in the light. This would suggest that light caused either a promotive effect on the

enzymatic oxidation or an increase in photooxidation of the IAA.

Certain authors agree that the ultimate destruction of IAA is a peroxidative reaction (18, 33) and that this reaction is enhanced by various monophenols, such as 2, 4 dichlorophenol (20, 33). Waygood et al. (75) pictured the destruction of IAA as first involving a peroxidation of a specific organic co-factor by an intermediate organic peroxide. As described by Kenten and Mann (34) and Maclachan and Waygood (44), the oxidized co-factor oxidizes manganese, and the manganic ions in turn initiates the spontaneous decarboxylation and oxidation of IAA. The reaction seems to be cyclic since the phenolic compound is regenerated during manganese oxidation. Maclachan and Waygood (45) considered the peroxidase substrate to be a skatole peroxide.

A different type of auxin inactivation has been proposed by Siegel and Weintraub (61). These workers found that IAA combines with peroxides to form a complex which can be restored to indoleacetic acid upon removal of the peroxide by catalase.

Briggs et al. (5) concluded that the inactivation of IAA by homogenates or plant parts is an artifact and can occur only when cut or damaged tissue is present. Bonner (4) stated that there probably is no specific IAA oxidase, rather, there are a variety of IAA oxidizing systems that have been produced as a result of cell rupture.

B. Destruction of other Growth Regulators by Plant Tissue:

Weintraub et al. (77) found that less than 7 percent of the C^{14} present in carboxyl-labeled 2, 4-D occurred as $C^{14}O_2$ after inactivation by bean plants. This suggests some kind of a conjugation rather than a decarboxylation destruction. Ray and Thimann (56) found that when 2, 4-D was applied and then translocated out of leaf that the material became inactivated after four days.

Tang and Bonner (69) incubated IAA with various IAA analogs and auxin-like compounds which included naphthaleneacetic acid (NAA). It would be anticipated that, if a substance related to IAA possessed the ability of combining with the enzyme, it might compete with IAA for position on the enzyme surface and lead to a competitive inhibition. None of the 11 auxins tested (including NAA) exerted any influence on the rate of IAA inactivation. This suggests that the IAA-inactivating enzyme may be exceedingly specific for IAA and will not bring about the destruction of NAA. Teubner and Murneek (70) found that the synthetic growth regulator, naphthaleneacetic acid, was not attacked by the IAA oxidase system in apple embryos.

Wagenknecht and Burris (74) found that IAA oxidase from wax bean roots would also bring about the oxidation of indolepropionic and indolebutyric acid. Ray and Thimann (56) reported that the enzyme, IAA oxidase, isolated from Omphalia flavida does not act on indolepropionic or indolebutyric acid, nor on any of a wide variety of indole compounds except for indoleisobutyric acid which

it attacked slowly. No oxygen uptake was obtained with tryptophane, skatole, indoleacetamide, or ethyl indoleacetate.

Many investigations have been conducted regarding the accumulation of natural hormones in the seed and the physiological processes associated with abscission and development of the fruit. However, no attempt has been made to relate periods of high and low natural hormone content of the seeds to periods of high and low enzymatic destruction potential of natural hormone of the seed.

Chemical Thinning of Fruit using Growth Regulators

The observation that growth regulators would chemically thin fruit was first reported in 1946 by Burkholder and McCown (6). They found that naphthalene-acetic acid caused a reduction in fruit set when applied to apple trees during the bloom period. Schneider and Enzie (59, 60) tested 12 different growth regulators to reduce apple set and found that only NAA and naphthalene acetamide were effective in bringing about a reduction in fruit set. Davidson et al. (13) reported in 1945 that NAA was effective as a fruit thinning agent when applied as late as three weeks after petal fall. Since this period numerous reports have appeared in which NAA was used as a chemical thinning agent for apples (1, 2, 10, 11, 12, 27, 28, 41, 43, 50, 52, 64, 65, 66, 67, 70). Teubner and Murneek (70) have made an excellent review of this work.

Since NAA proved to be a successful thinning agent for apples, it was

only a matter of time before it was tried on peaches. In 1947 Southwick et al. (63) reported that NAA at concentrations of 10 to 40 ppm did not reduce the fruit set of Elberta and Valiant peaches when applied at full bloom or 17 days later. However, Hibbard and Murneek (25) reported that NAA at 40 to 60 ppm caused an increase in fruit drop when applied to peaches 35 days after full bloom. Batjer and Hoffman (2) and Kelley (29, 30, 31, 32) also found NAA to favorably thin peaches when used between 30 and 42 days after full bloom. Kelley (32) and Lombard (37) reported that for NAA to be effective as a peach thinning agent, the material should be applied at a certain stage of fruit development. Recent work by Lombard, Mitchell and Cardinell (38) indicated that NAA applied at 30 ppm approximately 30 days after full bloom resulted in little or no foliage injury and gave significant thinning of peach trees.

Thompson and Rogers (72) after screening 25 new compounds for peach thinning activity reported that two compounds, 2-chlorophenoxy alpha propionic acid (2-CP) and 3-chlorophenoxy alpha propionic acid (3-CP), were effective as thinning agents and recommended them worthy of trials in thinning experiments.

Langer in 1952 (35) reported maleic hydrazide as an effective thinning agent for peaches when used at 500 ppm during bloom. It was later found that maleic hydrazide used during bloom in some years would cause the fruit remaining on the trees to have a split pit, making the crop unacceptable for

commercial sale (36). More recent work by Gambrell et al. (19) indicated that maleic hydrazide used at 1, 000 ppm 30 days after bloom brought about desired thinning of peach trees with no apparent injury to the fruit or tree.

Although numerous investigations concerned with the effect of environments and pH on absorption of different compounds are reported in the literature (3, 9, 15, 22, 23, 58, 62, 67), few were conducted using specific growth regulators normally used as thinning agents for apples and peaches. Most absorption studies associated with the effect of environments have been conducted by changing the environments only during the absorption period. They do not relate the preconditioning effects of environments on the plants as it may effect the absorption of the compound. Several investigators (41, 55, 70) have hypothesized that growth regulators used as thinning agents are translocated into the developing seeds damaging the embryo and/or endosperm, thus causing fruit abscission. However, no direct evidence has been presented to verify the movement of growth regulators into the fruit and seed following foliar application. Also Luckwill (41), Nitch (54) and Lombard (37) have related periods of high and low natural hormone content of apple and peach seeds to periods of natural fruit drop and to periods when the growth regulator may be used effectively as a fruit thinning agent. However, no attempt has been made to relate periods of high and low natural hormone content of the seeds to periods of low and high enzymatic destruction potential of natural auxin (IAA) by the seeds. The studies included herein were designed to answer some of the questions not found reported by other workers.

MATERIALS AND METHODS

Absorption Studies

The effect of environment on the absorption of NAA by apple and peach leaves was determined in the greenhouse January through March, 1959 and February through March, 1960. One-year-old apple and peach trees were grown in environmental chamber (4 x 6 x 4 feet) constructed of plastic and supported by a wooden frame in greenhouses maintained at temperatures of 70° F and 60° F. Trees were placed in environmental chambers one week after vegetative growth had started. Relative humidities of 91 and 94 percent were increased in the chambers by bubbling compressed air through a container of water.

In 1959, trees were grown for a period of three or seven weeks in the environmental chambers and then removed and placed on an open bench in the greenhouse operating at 70° F. In 1960, the trees were allowed to grow in the environmental chambers of three weeks before they were removed and placed on an open bench in the greenhouse.

Absorption studies were made the next day in the following manner: .05 ml of C^{14} ring labeled NAA was deposited on the upper surface of five representative leaves of each tree using a microsyringe with a number 24 needle. This amount (.05 ml) contained 4.8 micrograms of NAA C^{14} with a specific activity of .4 microcuries per ml. Twenty-four hours after application

each treated leaf was washed with 10 ml of a 1 percent NAOH solution to remove the NAA C^{14} not absorbed by the leaf. The control represented NAA C^{14} applied to the upper surface of leaves and washed off immediately. A 10 ml syringe with a number 21 needle was used to make the washings. Aliquotes were taken from these washings, dried under infra-red lamps, and counted, using a Chicago nuclear model D-47 gas flow counter[1] with a micro-mil window, and a model 117 ultra scaler. Three apple and three peach trees were used in 1959, and four apple and four peach trees were exposed to each environment in 1960. The same treating and counting procedures were used in 1960 as described for 1959.

Due to changes in operational temperatures of the greenhouses, it was not possible to duplicate the exact desired environmental conditions in 1960 as used in 1959. Thus, the mean temperature and relative humidity used in 1959 were (a) 60° F and 45 percent relative humidity, (b) 60° F and 94 percent relative humidity, (c) 70° F and 45 percent relative humidity, and (d) 70° F and 94 percent relative humidity. While in 1960, the environments were (a) 64° F and 47 percent relative humidity, (b) 66° F and 94 percent relative humidity, (c) 72° F and 43 percent relative humidity, and (d) 77° F and 88 percent relative humidity.

Studies of the leaf cuticle were made to determine the weight and physical appearance of the leaf cuticle as influenced by the different environmental

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[1] Nuclear Instruments and Chemical Corporation, 223 West Avenue, Chicago 10, Illinois.

conditions during the period of development. One cm discs were punched from nontreated leaves at the time absorption studies were made. In each case the discs were removed from the same location of the leaf on which the labeled NAA was applied for the absorption studies. The cuticle was removed from the discs by the enzymatic digestion method described by Orgell (55). Discs were placed in pectinase solutions for a period of 3 to 5 days at 35° C. This resulted in a breakdown of cellular material leaving only the intact thin layer of cuticle. The isolated cuticle disos were then washed in distilled water, placed on a weighed cover slip, and dried for 24 hours in a vacuum oven at 38° C. The discs were then weighed on a micro-balance. Similar discs (not dried) were viewed under the microscope for possible physical differences.

The effect of pH on absorption of NAA by apple and peach leaves was studied using one-year-old apple and peach trees grown in the 72° F and 43 percent relative humidity environments in the greenhouse during March 1960 as previously described. Identical procedures were employed as previously described, except the pH of the NAA C^{14} solution used was adjusted to 1.5, 2.5, 3, 4, 5, 6, 7, 8 and 9 with concentrated sulfuric acid and/or 6 N sodium hydroxide. The nine pH solutions were evaluated using five apple and five peach trees selected for uniformity. Nine uniform leaves were selected on each tree and a different pH solution of NAA C^{14} was applied to each leaf of each tree.

Translocation Studies

Autoradiograms were prepared to follow the pattern of movement of NAA C^{14} within the leaf and translocation out of the leaf following leaf absorption. These studies carried out in 1959 and 1960 were made by applying .05 ml of labeled NAA to both upper and lower surfaces of leaves from apple and peach trees. Following application of NAA C^{14} to foliage, the leaves were removed at different time intervals, dried at 70° C under steel plates, and then exposed to X-ray film for a period of 28 days.

The movement of NAA C^{14} into the developing fruit and seed following leaf absorption was studied by dipping whole apple spur leaves or peach leaves into a NAA C^{14} solution. One hour to six days following the application of labeled NAA to the leaves: the adjacent fruit was harvested, cut into thin slices, dried at 70°C under steel plates, and then exposed to X-ray film for a 28-day period.

Enzymatic destruction of IAA and NAA by the leaves and developing seed of the apple and peach

1. Use of Plant Parts as a Source of the Enzyme.

Leaf discs punched from apple and peach leaves were incubated with IAA and NAA buffered solution (pH 5, .02M phosphate buffer), to determine if intact leaf tissue could cause the breakdown of these growth regulators. Whole seeds of young developing apples and peaches were also assayed in this manner.

2. Measuring Concentration of IAA and NAA.

(a) Chemical determination of IAA.

After various incubation periods, 1.5 ml of IAA reagent was added to 1.5 ml of the IAA solution which originally contained IAA 10^{-3} M/ml buffer (pH 5, .02M phosphate). The IAA reagent was prepared according to the procedure outlined by Tang and Bonner (69) and is as follows: 15 ml of 0.5 M $FeCl_3$, 500 ml of distilled H_20, and 300 ml of H_2SO_4. Tang and Bonner (69) stated that this material is specific for IAA, since tryptophane, indolepropionic acid, indolebutyric, indolecarboxylic, indolepyruvic acid and indole do not give an appreciable reaction over the range of concentrations used. The amide of IAA responds as does IAA itself.

IAA oxidase activity was assayed by determining the residual IAA in aliquots from mixtures of IAA and plant parts after incubation at 26°C. Readings were made after color had developed for 15 minutes on a Coleman junior spectophotometer at 530 mu.

(b) $C^{14}O_2$ Diffusion Method for Determining Destruction of IAA and NAA.

The ability of intact plant tissue to destroy carboxyl-labeled IAA and NAA was studied using the Conway diffusion method (7). Dilley (14) found this to be a very excellent method of studying the hydrolysis of C^{14} labeled urea by the urease enzyme. Leaf discs (0.7 mm) or young developing apple or peach seeds were placed in the outer well of the diffusion units with carboxyl-labeled

IAA or NAA phosphate buffered solutions (2 ml, 0.02M at pH 5). The IAA and NAA solutions contained .1 microcurie of C^{14} per ml. The center well of the diffusion unit contained a stainless steel planchet with .5 ml of .3N sodium hydroxide solution. The lid was greased with lanolin and placed on the diffusion unit until the end of the incubation period. Any $C^{14}O_2$ resulting from the decarboxylation of the labeled IAA or NAA was absorbed by the sodium hydroxide solution in the planchets. Following a 3-hour incubation period, the sodium hydroxide solution in the planchet was dried under infra-red lamps to a very thin film of sodium carbonate and the samples were then counted using the same instrument as described previously for the absorption study.

3. Synthesis of Carboxyl - C^{14} - Labeled Indoleacetic Acid.

It was necessary to synthesize carboxyl-labeled IAA C^{14} since this product could not be purchased from any known commercial source. The method used to synthesize the labeled material was adapted from a procedure used by Stutz et al. (68) and Fang and Butts (17).

In a 50 ml round pyrex flask, 350 mg of gramine, 44 mg of Na C^{14} N (1 millicurie), and 40 mg of NaOH were dissolved in 10 ml of 50 percent methanol solution. The mixture was refluxed for 96 hours. The solution was then evacuated to remove the methanol and extracted two times with 10 ml portions of ether to remove excess gramine. After chilling to 0°C in an ice

bath and adjusted the pH to 3, the IAA precipitated directly from the aqueous solution. The precipitate was filtered immediately, and washed with a small amount of ice water. The product had a melting point of 164° to 167°C and had the same RF as authentic IAA when chromatographed.

Field Studies on Chemical Thinning of Peaches

Studies were conducted during 1959 and 1960 to determine the effectiveness of naphthaleneacetic acid (NAA), 3-chlorophenoxy alpha propionic acid (3-CP), and maleic hydrazide (MH-30) as chemical thinning agents for peaches. In 1959, four mature bearing peach orchards located in Grand Rapids, Linden, South Haven, and Eau Claire, Michigan were selected for this work. In 1960 only two orchards, the one in Grand Rapids and the one in Linden were used.

Degree of thinning for each treatment in each orchard was evaluated by using five entire tree replications. Fruit counts were made on eight branches of each tree selected in different areas with each branch containing a minimum of 50 fruits.

In the 1960 study made at Linden, each treatment was evaluated by using four Redhaven trees as replications. Fruit counts were made on four branches of each tree with each branch containing approximately 80 fruits. The pH of the treatments was adjusted by adding concentrated sulfuric acid and/or 6 N sodium hydroxide to the spray solution.

Fruit counts were made at the time of the treatments, approximately 28

days after full bloom, with only actively growing fruits recorded. Final counts were made the middle of July when fruit drop was complete. The treatments and the varieties used in the thinning studies are recorded in Table I.

TABLE I

Chemical Treatments made Approximately 28 Days after Bloom on Peach Trees in 1959 and 1960 as a means of Fruit Thinning

Year	Location	Variety	Treatment	Conc. (ppm)	Comments
1959	Grand Rapids	Redhaven	Control	---	
			NAA	30	Twilight
			3-CP	250	application
			3-CP	300	
	Linden	Halehaven	Control	---	
			3-CP	300	Applied 36
			NAA	40	days after bloom
	South Haven	Halehaven	Control	---	
			3-CP	300	
	Eau Claire	Kalhaven	Control	---	
			3-CP	300	
1960	Grand Rapids	Redhaven	Control	---	
			MH-30	1000	
			3-CP	250	
	Linden	Redhaven	Control	---	
			NAA pH 2.6	30	Applied one
			NAA pH 6.8	30	hour before
			NAA pH 10.5	30	a rain

In 1959, all thinning treatments except those at Linden were applied with a hand gun using 200 pounds pressure spraying the trees to beyond the drip point. At Linden the trees were sprayed to no more than the drip point and on one side only. The applications at Linden, South Haven, and Eau Claire were made during the day under fast drying conditions while those in Grand Rapids were made between 8:00 p. m. and 9:00 p. m. By contrast, in 1960, all fruit thinning treatments were applied with a hand gun using 500 pounds pressure. The trees were sprayed to the drip point and all applications were made under fast drying conditions.

RESULTS

Absorption Studies - 1959

The tabulated findings resulting from greenhouse studies conducted in 1959 to determine the influence of predisposed environments on the absorption of NAA C^{14} by peach and apple leaves are given in Tables II and III. The greatest amount of NAA C^{14} was absorbed when peach trees were grown in an environment of 60° F and 94 percent relative humidity (Table II). The least amount of NAA C^{14} was absorbed by leaves of trees grown at 70° F and 45 percent relative humidity before treatment. This was evident for the trees that had grown in these environments for a period of either three weeks or seven weeks before absorption studies were made. However, after seven weeks when the leaves became older, the differences in the amount of absorption of NAA C^{14} became less. Trees grown under 60° F and 94 percent relative humidity for seven weeks absorbed greater quantities of NAA than did other trees; but, there was no significant difference in absorption capacities between trees grown at environments of 60° F and 45 percent humidity, 70° F and 45 percent relative humidity, or 70° F and 94 percent relative humidity.

Apple leaves absorbed significantly more NAA C^{14} after being grown in an environment of a low air temperature of 60° F (Table III) as compared to an air temperature of 70° F. The greatest absorption of NAA C^{14} by apple leaves occurred at 60° F and 45 percent relative humidity. Lower temperature

TABLE II

Effect of Environmental Air Temperatures and Relative Humidities on the Absorption of NAA C^{14} by Peach Leaves

Environment	3 weeks		7 weeks	
	Residue CPM[1]/leaf	Abs'b	Residue CPM[1]/leaf	Abs'b
60° F - 45% RH	1922	63%	926	34%
60° F - 94% RH	1058	80	583	58
70° F - 45% RH	2143	59	893	38
70° F - 94% RH	1866	64	963	31
LSD .05	202			
.01	280		160	
Control	5232	0	1390	0

[1]CPM (counts per minute) represents a mean value for five leaves from each of three trees.

TABLE III

Effect of the Environmental Air Temperatures and Relative Humidities on the Absorption of NAA C^{14} by Apple Leaves

Environment	3 weeks		7 weeks	
	Residue CPM[1]/leaf	Abs'b	Residue CPM[1]/leaf	Abs'b
60° F - 45% RH	1698	67%	996	29%
60° F - 94% RH	2012	61	992	29
70° F - 45% RH	2250	57	934	33
70° F - 94% RH	2236	57	902	35
LSD .05	202		N. S.	
.01	280		N. S.	
Control	5232	0	1390	0

[1]CPM (counts per minute) represents a mean value for five leaves from each of three trees.

(60° F) seemed to be the important factor in absorption of NAA C^{14} by apple leaves as trees grown at 60° F absorbed more NAA than trees grown at 70° F regardless of relative humidity. As with peach leaves (Table II) the differences in absorption capacity of the apple leaves diminished appreciably after leaves became older (Table III). Apple leaves grown under different environments for seven weeks before treatment showed no differences in absorption of NAA C^{14}.

When considering only the effect of air temperature, both apple and peach leaves absorbed more NAA C^{14} when grown at 60° F than when grown at 70° F (Table IV). This was true of peach leaves both three and seven weeks old, but was only true for apple leaves three weeks old.

Absorption by apple leaves were found to be influenced somewhat differently by relative humidity than were peach leaves (Table V). Apple leaves absorbed more NAA C^{14} when grown under 45 percent relative humidity than when grown under 94 percent relative humidity. In contrast with this, peach leaves absorbed more NAA C^{14} after being grown under 94 percent relative humidity than under 45 percent relative humidity.

Absorption Studies - 1960

Absorption studies similar to those of 1959 were carried out in 1960, but the trees were grown under the respective environments for only three weeks. In this study peach leaves were found again to absorb significantly

TABLE IV

Absorption of NAA C^{14} by Apple and Peach Leaves Grown in Different Air Temperatures for Three and Seven Weeks

Temperature	3 weeks		7 weeks	
	Residue CPM[1]/leaf	Abs'b	Residue CPM[1]/leaf	Abs'b
Apple: 60° F	1855	64%	994	29%
70° F	2243	57	918	34
Peach: 60° F	1490	72	754	46
70° F	2005	62	928	35
LSD .01	198		113	
Control	5230	0	1390	0

[1] CPM (counts per minute) represents a mean value for five leaves from each of six trees.

TABLE V

Absorption of NAA C^{14} by Apple and Peach Leaves Grown in Different Relative Humidities for Three and Seven Weeks (1959)

Relative Humidity	3 weeks		7 weeks	
	Residue CPM[1]/leaf	Abs'b	Residue CPM[1]/leaf	Abs'b
Apple: 45%	1974	62%	961	31%
94%	2124	59	947	32
Peach: 45%	2033	61	910	36
94%	1466	72	773	45
LSD .05	143			
.01	198		113	
Control	5230	0	1390	0

[1] CPM (counts per minute) represents a mean value for five leaves from each of six trees.

more NAA C^{14} when grown in the environment of low air temperature (66° F) and high relative humidity (94 percent) in contrast to leaves of trees predisposed to environments of 77° F and 88 percent relative humidity which absorbs the least (Table VI). The condition resulting in the least absorption did not agree for both years, for in 1959 leaves grown under 70° F and 45 percent relative humidity absorbed the least.

The 1959 and 1960 studies on the absorption of NAA by apple leaves as related to air temperature are in close agreement (Table VII). Again the low air temperatures (64° and 66° F) seemed to be the important factor in the absorption of NAA C^{14} by apple leaves. Leaves of trees grown at 64° and 66° F temperatures before treatments absorbed more NAA C^{14} than those grown at 72° and 77° F, irrespective of relative humidity.

Without considering the effect of relative humidity, both apple and peach leaves absorbed more NAA C^{14} when grown at 64° F to 66° F air temperatures than when grown under temperatures of 72 to 77° F (Table VIII).

In 1960, relative humidity had little effect on the absorption of NAA C^{14} by peach and apple leaves (Table IX). Absorption of NAA C^{14} by leaves was the same when trees were grown under 45 or 91 percent relative humidities (Table IX).

TABLE VI

Effect of the Environmental Air Temperature and Relative Humidities on the Absorption of NAA C^{14} by Peach Leaves

Environment	Residue CPM[1]/leaf	% Absorbed
64° F - 47% RH	2920	47
66° F - 94% RH	2317	58
72° F - 43% RH	3054	45
77° F - 88% RH	3661	34
LSD .01	372	
Control	5535	0

[1]CPM (counts per minute) represents mean value for five leaves from each of four trees.

TABLE VII

Effect of Environmental Air Temperatures and Relative Humidities on the Absorption of NAA C^{14} by Apple Leaves

Environment	Residue CPM[1]/leaf	% Absorbed
64° F - 47% RH	2292	59
66° F - 94% RH	2208	60
72° F - 43% RH	2842	49
77° F - 88% RH	2865	48
LSD .01	372	
Control	5535	0

[1]CPM (counts per minute) represents a mean value for five leaves from each of four trees.

TABLE VIII

Absorption of NAA C^{14} by Apple and Peach Leaves Grown in Different Air Temperatures for Three Weeks

Temperature	Residue CPM[1]/leaf	% Absorbed
Apple: 65° F	2250	59
75° F	2853	48
Peach: 65° F	2619	53
75° F	3357	39
LSD .01	263	
Control	5535	0

[1]CPM (counts per minute) represents a mean value for five leaves from each of eight trees.

TABLE IX

Absorption of NAA C^{14} by Apple and Peach Leaves Grown in Different Relative Humidities for Three Weeks

Relative Humidity	Residue CPM[1]/leaf	% Absorbed
Apple: 45%	2567	54
91%	2537	55
Peach: 45%	2987	46
91%	2989	46
LSD .01	N. S.	
Control	5535	0

[1]CPM (counts per minute) represents a mean value for five leaves from each of eight trees.

Cuticle Studies

Peach and apple trees used for leaf cuticle studies in 1959 and 1960 were grown under the different environments as described for the absorption study (page 14). In 1959, leaves from peach trees grown in an air temperature of 60° F and 94 percent relative humidity developed slightly more cuticle on their upper surfaces than leaves of trees grown under other environmental conditions (Table X). This is rather surprising in that leaves from trees predisposed to this same environment showed the greatest absorption capacity (Table II). The cuticle on both upper and lower surfaces of the leaves from trees predisposed to all environmental treatments continued to increase as the time increased from three to seven weeks.

Studies made in 1960, indicated that air temperature and relative humidity had little if any effect on the quantity of cuticle produced by the leaves of the apple and the peach as there were very little differences in the weights of cuticle removed from leaves of trees grown in the different environments (Table XI).

As in 1959 the cuticle of the leaves increased with age and for the peach the cuticle from the lower surface of the leaves weighed more than cuticle on the upper surfaces. These results are different from those reported by Orgell (55) for the apricot since he reported the upper cuticle of the apricot leaf weighed much more than the lower cuticle. Cuticle obtained from the leaves of apple and peach trees grown under the different environments were closely examined

TABLE X

Weights* (milligrams) of Cuticle from Leaves of Peach Trees Grown in Different Environments for Three and Seven Weeks (1959)

Environment	Upper Cuticle		Lower Cuticle	
	3 weeks	7 weeks	3 weeks	7 weeks
60° F - 45% RH	.351	.602	.626	.883
60° F - 94% RH	.533	.718	.722	.892
70° F - 45% RH	.403	.600	.632	1.052
70° F - 94% RH	.398	.702	.568	1.071

*Each weight is a composite of 5 one sq. cm. cuticle discs removed from five leaves.

TABLE XI

Weights* (milligrams) of Cuticle from Leaves of Peach and Apple Trees Grown in Different Environments for One, Two and Three Weeks (1960)

Environment	Upper Cuticle			Lower Cuticle		
	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Peaches:						
64° F - 47% RH	.870	.898	.976	1.571	1.670	1.712
66° F - 94% RH	.719	.941	1.014	1.497	1.539	1.750
72° F - 43% RH	.966	.985	1.075	1.635	1.736	1.781
77° F - 88% RH	.813	.947	1.033	1.394	1.592	1.679
Apples:						
64° F - 47% RH	.764	.989	.995			
66° F - 94% RH	.702	.848	.995			
72° F - 43% RH	.885	.968	1.049			
77° F - 88% RH	.743	.890	.979			

*Each weight is a composite of 10 one sq. cm. cuticle discs removed from ten leaves.

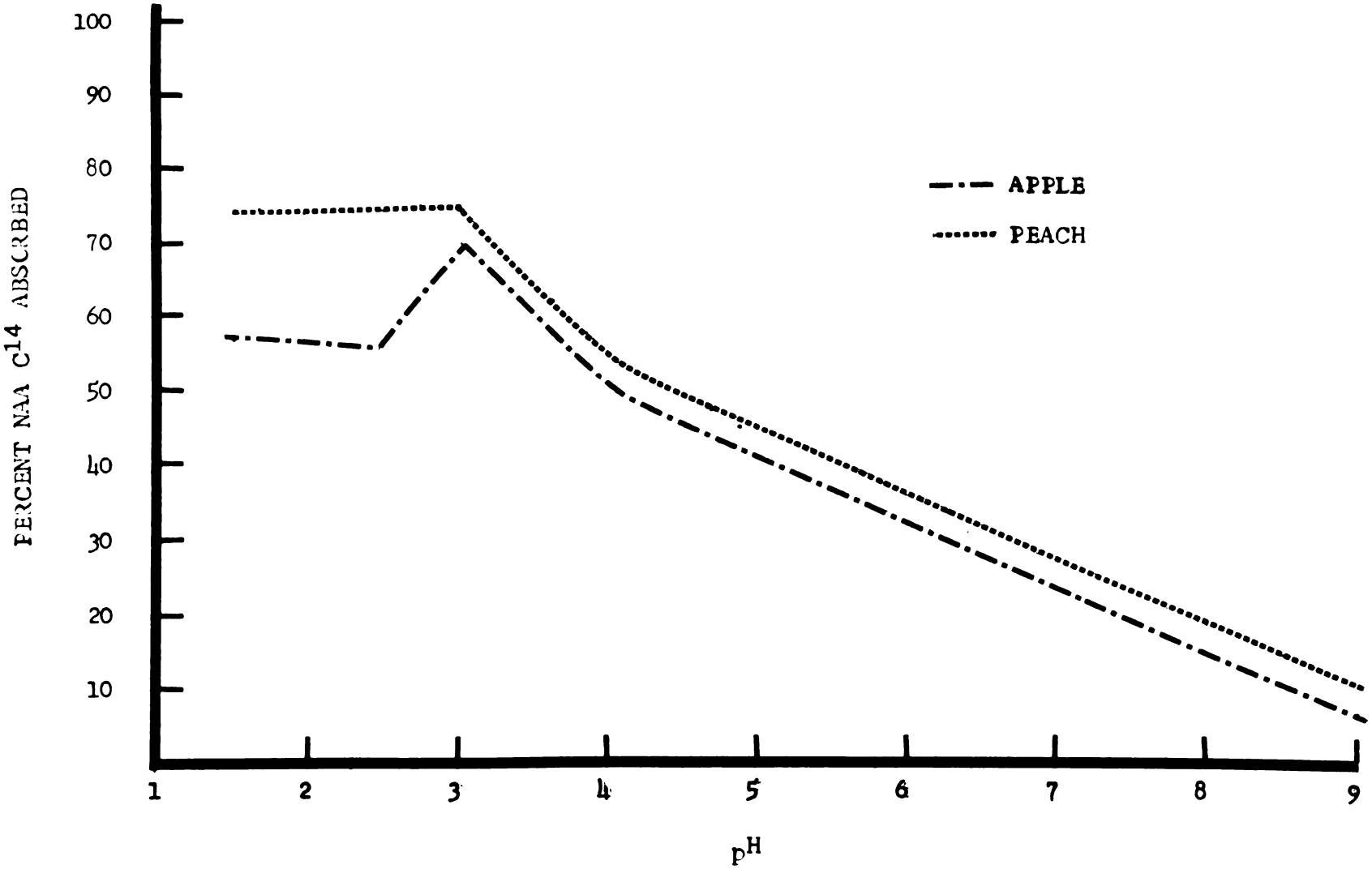
under the microscope, but no differences in structure or thickness resulting from the predisposed environments were evident.

pH Studies

Studies were carried out in 1960 to determine the influence of pH on the absorption of NAA C^{14} by the leaves of apple and peach trees. Nine NAA C^{14} solutions ranging in pH from 1.5 to 9 were applied at the rate of .05 ml as a single large drop to the upper surfaces of peach and apple leaves. Absorption of NAA C^{14} by apple and peach leaves was found to be highly dependent upon the pH of the solution containing the plant regulator (Figure 1). Absorption of NAA C^{14} decreased as the pH was increased from 3 to 9. In a 24-hour period apple leaves absorbed 8 percent of the applied NAA C^{14} solution of pH 9, as compared to 70 percent when the pH of the solution was 3. Similarly, peach leaves absorbed 12 percent of the applied NAA C^{14} when the solution had a pH of 9, as compared to 74 percent when the pH was 3. When the pH of the solution containing the NAA C^{14} was below 3, the absorption of NAA C^{14} remained close to 74 percent for peach leaves, but decreased somewhat below 70 percent for apples leaves. These results are similar to those obtained by Hamner et al. (22) who reported that the effectiveness of 2, 4-D was highly dependent on the pH of the growth regulator solution.

Figure 1

The effect of pH on absorption of NAA C^{14} by apple and peach leaves.


PERCENT NAA C^{14} ABSORBED
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
pH
APPLE
PEACH

Translocation Studies

A. Movement of NAA C^{14} in Apple and Peach Leaves.

NAA C^{14} was applied as a single large drop at the rate of .05 ml to the midrib sections of the upper and lower surfaces of peach and apple leaves to determine its movement within the leaf. Leaves were removed at 1, 12, 24, 36, 48 and 192 hours following treatment and autoradiograms were prepared of the treated leaves (Figures 2, 3).

When NAA C^{14} was applied to the upper surface of apple leaves, very little of the applied material moved from the original site of application during the first 24 hours (Figure 2). After 48 hours, C^{14} was found to be concentrated at the margins of the leaves and in the petiole. After 196 hours very little of the material was found in the margins of the leaf and much less was present in the petiole, indicating that most of the C^{14} had moved out of the leaf. Thus, translocation of C^{14}, when applied to the upper surface of apple leaves as NAA C^{14}, occurred only after a time lapse of 24 hours and maximum translocation occurred at the end of 48 hours.

A different response was obtained when NAA C^{14} was applied to the lower surface of apple leaves as some movement of C^{14} was evident one hour after application (Figure 2). Movement occurred with time until after the 48-hour period; and, after 192 hours, very little of the material was present in the leaf except at the site of the original application.

Figure 2

Autoradiograms illustrating translocation of ring labeled NAA C^{14} in apple leaves. A through B represents movement of C^{14} after 1, 12, 24, 48 and 192 hours respectively, following application of NAA C^{14} to the upper leaf surface. E through J represents movement of C^{14} after 1, 12, 24, 48 and 192 hours respectively, following application of NAA C^{14} to the lower leaf surface.

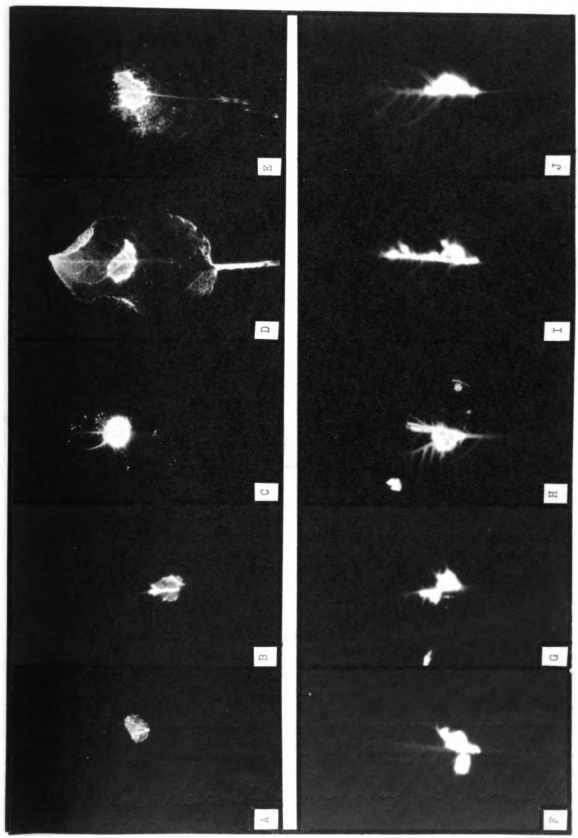
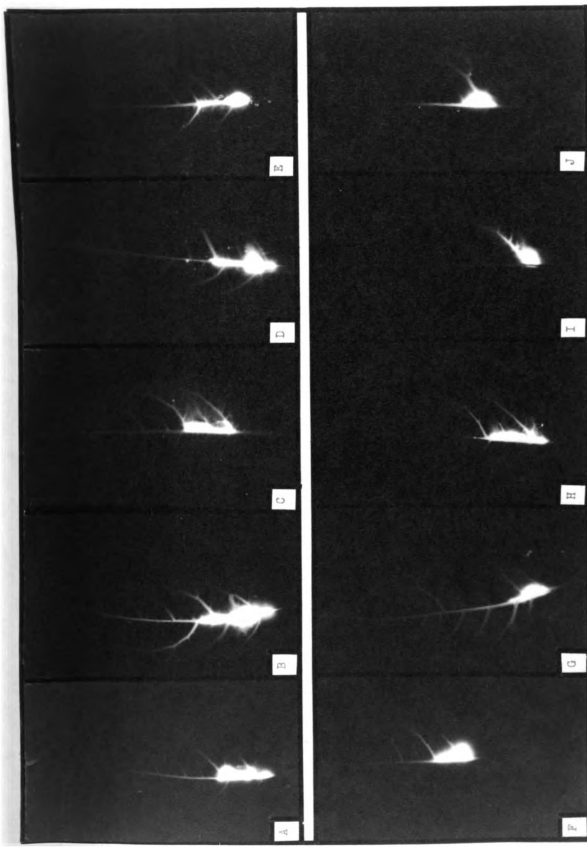

A
B
C
D
E
F
G
H
I
J

Figure 3

Autoradiograms illustrating translocation of ring labeled NAA C^{14} in peach leaves. A through E represents movement of C^{14} after 1, 12, 24, 48 and 192 hours respectively, following application of NAA C^{14} to the upper leaf surface. F through J represents movement of C^{14} after 1, 12, 24, 48 and 192 hours respectively, following application of NAA C^{14} to the lower leaf surface.


A
B
C
D
E
F
G
H
I
J

Movement of C^{14} was apparent one hour after NAA C^{14} was applied to either the upper or lower surfaces of the peach leaves (Figure 3). The material appeared to concentrate more in the vascular tissues with very little movement into the intervenal areas. As indicated by the autoradiograms (Figure 3), maximum movement of C^{14} occurred within 12 hours following application and progressively less of the labeled material was detected in the leaf tissue after this period.

B. Movement and Distribution of C^{14} into the Developing Apple and Peach Fruit Following Application of NAA C^{14} to the Foliage.

C^{14} was detected in the fruit one hour after the entire apple spur leaves were dipped in a labeled NAA solution (Figure 4, B). After 96 hours C^{14} was found distributed throughout most of the fruit with higher concentrations possibly in the vascular areas of the fruit (Figure 4, C). The small seed removed from these fruits and exposed to X-ray film, also showed accumulation of C^{14}. Autoradiograms of the fruit spur, indicated that movement of the C^{14} from the leaves to the fruit was occurring mainly through a narrow portion of the conductive system as very little radioactivity was evident in the woody portion of the cluster base (Figure 4, A).

C^{14} was present in the fruit and seed four to six days following the application of NAA C^{14} to peach leaves with the highest concentration of C^{14}, as indicated by the autoradiograms, in the fruit and less amounts in the developing seed (Figure 5).

Figure 4

Autoradiogram illustrating translocation of C^{14} into the fruit spur, fruit, and seed of the apple following application of ring labeled NAA C^{14} to the spur leaves. A represents distribution of C^{14} in the fruit spur 48 hours after application of NAA C^{14} to spur leaves. B represents distribution of C^{14} in the fruit 1 hour after application of NAA C^{14} to spur leaves. C represents distribution of C^{14} in the fruit 48 hours after application of NAA C^{14} to spur leaves. D represents distribution of C^{14} in the seed 48 hours after application of NAA C^{14} to spur leaves.

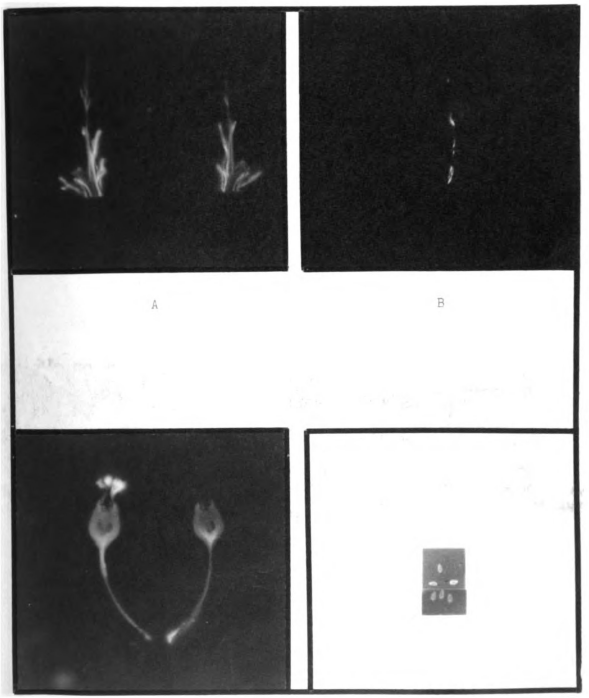
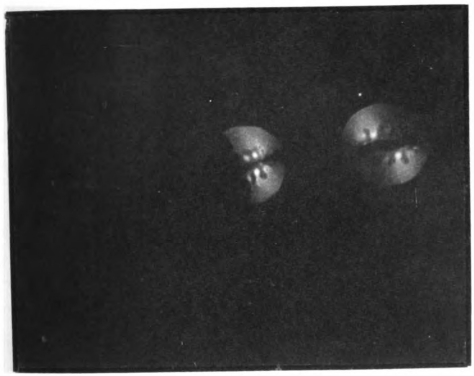

A
B
C
D

Figure 5

A. Autoradiogram illustrating translocation of C^{14} into the fruit and seed of the peach following application of ring labelled NAA C^{14} to the foliage. NAA C^{14} was applied to the foliage six days before fruit was harvested and sectioned for autoradiogram. Note low activity of C^{14} in the seed.

B. Photograph of fruit and seed of the peach used in preparing autoradiogram in A.



A

B

Enzymatic Destruction of IAA by Intact Leaf Discs as Determined Colormetrically.

The colormetric determination of IAA was used to study the destruction of IAA by the enzyme indoleacetic acid oxidase present in the leaves of apple and peach trees. Leaf discs punched from leaves of peach trees degraded IAA at a slower rate than discs from apple leaves (Table XII). Also, there seemed to be an instantaneous loss of IAA when boiled apple discs were added to the IAA solution. Of interest, boiled peach discs had no effect on the IAA present in the solution.

Based on colormetric evaluations, an unknown factor(s) was present in the boiled apple leaf discs that resulted in a reduction of the IAA present in the solution. It is possible that some "factor" was diffusing out of the boiled leaf discs that either destroyed a given amount of the IAA present or interfered with the color reaction between the IAA present and the color reagent.

Because these results were not explainable, a study was made to determine if some "factor" was diffusing out of the boiled leaf discs; and if so, could this "factor" be removed by first placing boiled discs in distilled water for a period of time before incubating with the IAA solution? The results of this study are shown in Table XIII.

Placing the boiled leaf discs in distilled water for one and one-half hours before incubating with IAA solution caused the removal of the "factor" which was giving the low optical density readings in the colormetric determinations.

TABLE XII

Loss of IAA when Solutions were Incubated with Apple and Peach Leaf Discs as Measured by Optical Density

Treatments	Optical Density at 530 mu			
	1 hour	3 hours	6 hours	9 hours
IAA solution	0.80	0.73	0.83	0.81
Peach discs and IAA solution	0.80	0.80	0.68	0.46
Apple discs and IAA solution	0.66	0.60	0.39	0.24
Boiled apple discs and IAA solution	0.44	0.43	0.46	0.47
Boiled peach discs and IAA solution	0.80	0.78	0.74	0.75

The reaction mixture contained peach and apple leaf discs incubated with 10^{-3} M IAA phosphate buffered solutions (0.02 M at pH 5).

TABLE XIII

Effect of Placing Boiled and Non-boiled Apple Leaf Tissue in Distilled Water for one and one-half hours before Incubating with IAA Solutions for one hour, Expressed as Optical Density

Treatments	Optical Density at 530 mu
1. IAA solution	0.60
2. IAA solution and boiled leaf discs	0.28
3. IAA solution and pre-soaked boiled leaf discs	0.57
4. IAA solution and live leaf discs	0.44
5. IAA solution and pre-soaked live leaf discs	0.46

The reaction mixture contained peach and apple leaf discs incubated with 10^{-3} M IAA phosphate buffered solutions (0.02 M at pH 5).

This was not true of the unboiled leaf discs in that live leaf discs placed in distilled water caused the same change in optical density readings as live leaf discs not placed in water.

Following the incubation of boiled leaf discs with IAA solution, aliquots of the IAA solution were removed and diluted to 10^{-5} M IAA, using the control IAA solution as a standard. Avena coleoptiles were placed into the solution for 22 hours and incubated at 26° C to determine if the low optical density readings of IAA solutions incubated with boiled leaf discs caused a reduction in IAA or if it interfered with the color reaction. Results of this study are presented in Table XIV.

The IAA solution incubated with boiled leaf discs did show a reduction in IAA when measured colormetrically, but did not when measured by the Avena test. This indicated that the "factor", diffusing out of the boiled apple leaf discs, interfered with the color reaction and did not bring about the destruction of IAA as the colormetric determination would indicate.

These studies suggest that intact leaf discs of the apple and peach can bring about the destruction of IAA and that boiled apple leaf disc cannot be used as a control for comparison with live leaf disc, since there is some "factor or factors" that diffuse out of the boiled discs which interfere with the colormetric determination of IAA.

TABLE XIV

A Comparison of Optical Density and Avena Coleoptiles Straight Growth Test in Measuring 10^{-5} M IAA Present in a IAA Buffered Solution and a IAA Buffered Solution Plus Boiled Apple Leaf Discs

Treatment	Optical Density	Avg. Coleoptile Length
1. IAA (10^{-5} M) buffered solution	0.55	13.02 mm
2. IAA (10^{-5} M) buffered solution plus boiled apple leaf discs	0.22	13.60 mm

Enzymatic Destruction of Carboxyl Labeled C^{14} IAA and NAA

The ability of leaf tissue and young developing seeds of the apple and peach to bring about the enzymatic decarboxylation of carboxyl labeled IAA and NAA was studied using the Conway diffusion method (7). Apple and peach leaf discs (7 mm in diameter) caused some destruction of carboxyl labeled NAA C^{14} when incubated in buffered solutions of this material, although apple leaf discs appeared to be somewhat more effective in bringing about the destruction of carboxyl labeled NAA than the peach leaf discs (Table XV).

Young developing Redhaven peach seed and Wealthy apple seed obtained from fruit harvested on June 2, 4, 6, 8, 10, 12, 14, 18, 22, 24, 30, and July 8 were assayed for enzymatic activity in destroying C^{14} labeled IAA and NAA (figures 6, 7). These data are recorded in Table XVI.

Peach seeds were found to vary greatly in their ability to decarboxylate

TABLE XV

Decarboxylation of Carboxyl Labeled NAA C^{14} by Fresh Leaf Discs of Apple and Peach

Treatment*	$C^{14}O_2$ evolved (CPM/15 leaf discs) 1 hour	5 hours
1. NAA solution	0	0
2. NAA solution and apple discs	100	149
3. NAA solution and peach discs	71	104

*The reaction mixture contained peach and apple leaf discs incubated in Conway diffusion units for 1 and 5 hours with phosphate buffer 0.02 M, pH 5.0, and carboxyl labeled NAA C^{14} 0.2 microcuries in a total of 2.0 ml.

TABLE XVI

Decarboxylation of Carboxyl Labeled IAA C^{14} and Carboxyl Labeled NAA C^{14} by Young Developing Redhaven Peach and Wealthy Apple Seeds

Sampling Dates	$C^{14}O_2$ evolved, expressed as counts per minute, from 24 peach seeds and 80 apple seeds			
	Peach Seed		Apple Seed	
	IAA Sol.*	NAA Sol.*	IAA Sol.*	NAA Sol.*
June 2	897	150	0	0
June 4	1134	104	0	0
June 6	988	125	0	0
June 8	458	91	0	6
June 10	91	69	0	25
June 12	610	50	439	18
June 14	1014	47	598	8
June 18	72	118	1966	68
June 22	0	84	2280	74
June 24	0	82	3680	35
June 30			6002	0
July 8			3410	0

*The reaction mixture contained peach and apple seed incubated in Conway diffusion units for 3 hours with 0.02 M phosphate buffer at pH 5.0 and carboxyl labeled IAA C^{14} or carboxyl labeled NAA C^{14}. The reaction mixture had a total volume of 2.0 ml and contained 0.1 microcurie of C^{14} per ml.

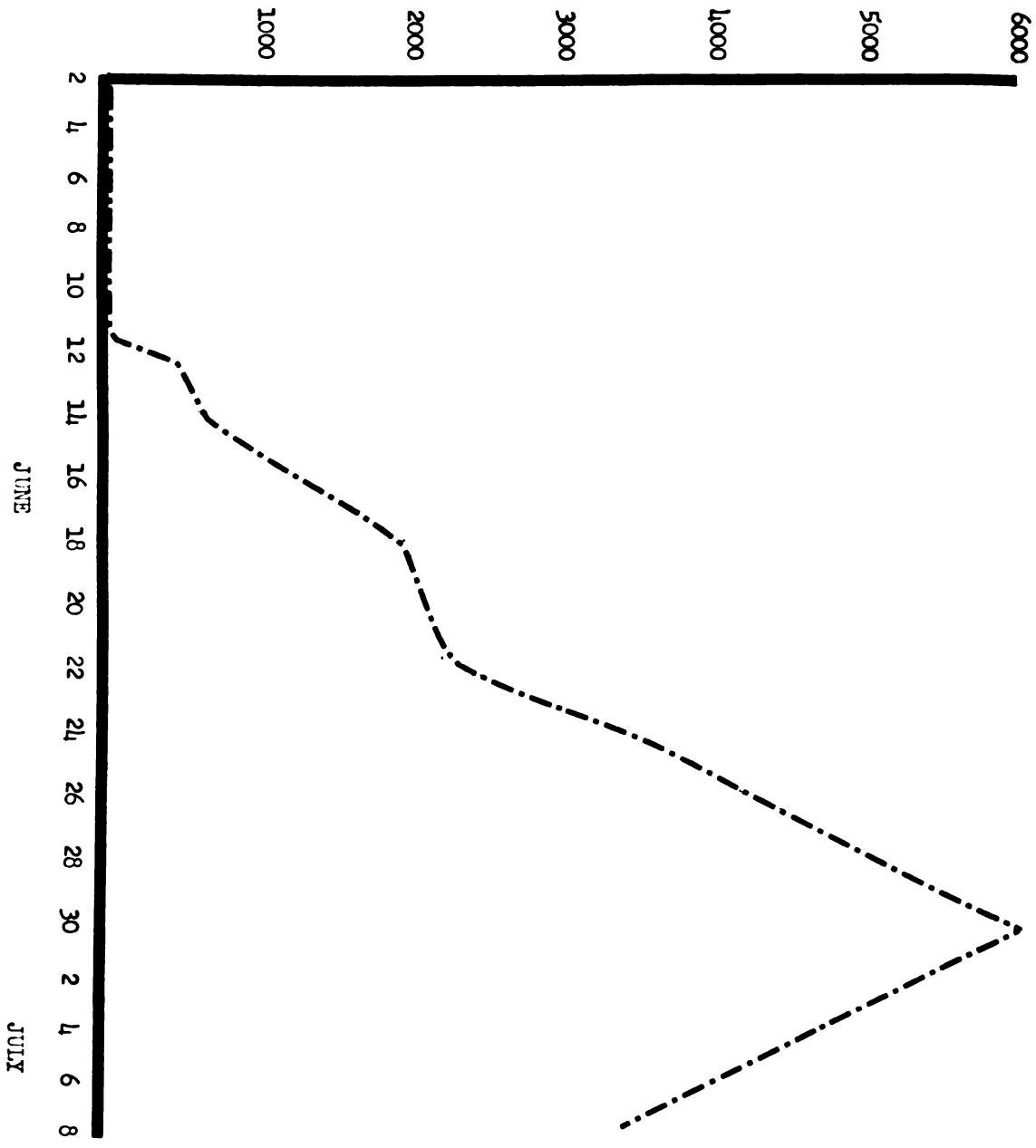
Figure 6

Indoleacetic acid oxidase activity in peach seeds of fruit harvested at intervals beginning June 2 through June 24, 1960.

Figure 7

Indoleacetic acid oxidase activity in apple seed of fruit harvested at intervals beginning June 2 through July 8, 1960.

$C^{14}O_2$ (counts per minute) EVOLVED WHEN APPLE SEED OF 10 FRUIT INCUBATED WITH IAA-1-C^{14} FOR THREE HOURS.



IAA which was related to the different stages of seed development. On June 4, 1960, 18 days after full bloom, the IAA oxidase was highest. The activity decreased thereafter to a very low rate on June 10, approximately 10 days after shuck-off. Following this date, the fruit began to grow very rapidly and by June 12, the length of the pericarp reached 36 mm (Table XVII), which is considered too long for the growth regulator NAA to be effective as a chemical thinning agent (37). With this increase in size there was an increase in IAA oxidase activity until June 14, when the activity of the enzyme decreased very rapidly, and showed no IAA oxidase activity on June 22 and June 24. Assay of peach seeds was stopped after June 24, because seed became so large they would not fit into Conway diffusion units.

Peach seeds were also found to decarboxylate carboxyl-labeled NAA C^{14}, but the activity was much less than the IAA oxidase activity and the activity did not change appreciably with development of the seed. Slightly higher activity was noted on June 2, 1960, but following this date there was a progressive decrease in activity with time until June 14. There was somewhat of an increase on June 18 with another slight decrease followed by a leveling off on June 22 through June 24.

The activity of IAA oxidase in apple seeds was not the same as found for the peach (Figure 7). This is not too surprising since the development of the apple seeds in relation to dates is markedly different, more rapid, from

TABLE XVII

Growth of the Fruit and Seed of the Young Redhaven Peach used in the 1960 Enzymatic Destruction Studies of Carboxyl Labeled IAA and NAA (average of five samples)

Date of Sampling	Days after Bloom	Fruit		Seed	
		length (mm)	diameter (mm)	length (mm)	diameter (mm)
June 2	17	18	13	7	3
June 4	19	21	15	8	4
June 6	21	25	17	10	5
June 8	23	28	21	12	6
June 10	25	30	24	12	7
June 12	27	36	28	13	7
June 14	29	37	31	13	8
June 18	33	42	36	15	10
June 22	37	45	38	18	12
June 24	39	46	38	19	13

the development of the peach seeds (43, 70). The seeds of apples did not cause any decarboxylation of the carboxyl labeled IAA C^{14} until June 12. The procedure for assaying seeds was changed on June 12 and may account for some of the change noted in IAA oxidase activity. Since no breakdown of IAA was detectable using intact apple seed, the seed coats of all apple seeds used in these assays after June 12 were pierced once with a dissecting needle to insure penetration of the solutions of the growth regulators. The IAA oxidase activity continued to increase in the apple seeds from June 12 through June 30, but a substantial decrease in activity was noted by July 8. By visual observations it was determined that June 12 to June 30 was the

period of rapid embryo development in the apple seed and corresponds to the same period of high IAA oxidase activity as previously reported by Teubner and Murneek (70).

Some destruction of carboxyl labeled NAA C^{14} occurred with apple seeds after June 8, but the amount destroyed was not as great as for the peach seeds. The greatest activity of apple seeds on destruction of NAA occurred on June 22 with very little occurring until June 18.

Field Studies on the Chemical Thinning of Peaches

The findings of 1959 and 1960 using 3-chlorophenoxy alpha propionic acid (3-CP), naphthaleneacetic acid (NAA), and maleic hydrazide (MH-30) as thinning agents for Redhaven, Halehaven and Kalhaven peaches are given in Tables XVIII and XIX. Initial fruit counts in the 1959 and 1960 studies were made approximately 28 days after bloom and only actively growing fruits were counted. The variation due to difference in pollination and fruit set between limbs of the same tree was greatly reduced by making the fruit counts at this time.

At Grand Rapids, Redhaven peach trees treated in 1959 with 30 ppm of NAA showed a 27 percent decrease in fruit set over the control trees. Even though the trees were thinned by this chemical, the fruit was somewhat smaller at maturity than the fruit of the control trees (Table XIX). No leaf or fruit damage was noticeable at any time from the use of NAA.

TABLE XVIII

Fruit Set Resulting from the use of 3-Chlorophenoxy Alpha Propionic Acid (3-CP), Naphthaleneacetic Acid (NAA), and Maleic Hydrazide (MH-30) as Thinning Agents

Location	Variety	Pericarp length	Pericarp diameter	Treatment	Conc. (ppm)	Fruit Set (%)
			1959			
Grand Rapids	Redhaven	25	17	Control		87
				NAA	30	60
				3-CP	250	30
				3-CP	300	20
Linden	Halehaven	32	27	Control		51
				3-CP	300	41
				NAA	40	46
South Haven	Halehaven	27	19	Control		90
				3-CP	300	1
Eau Claire	Kalhaven	29	21	Control		78
				3-CP	300	3
			1960			
Grand Rapids	Redhaven	23	18	Control		87
				MH-30	1000	85
				3-CP	250	51
Linden	Redhaven	33	23	Control		93
				NAA pH 10.5	30	92
				NAA pH 6.8	30	91
				NAA pH 42.5	30	89

TABLE XIX

Effect of NAA and 3-CP on the Size of Redhaven Peaches, Grand Rapids (1959)

Treatment	Conc (ppm)	Number and Size per 50 lbs.			
		2" and under	2" - 2 1/4"	2 1/4" - 2 3/4"	Total*
Check		242	63	29	334
NAA	30	340	38	8	386
3-CP	250	102	93	60	255
3-CP	300	98	96	65	259
LSD - .05		75	20	29	
.01		106	28	41	

*Each value is a mean of five 50-pound samples taken from the five replicated trees.

Also at Grand Rapids in 1959 the use of 3-CP caused a 57 to 67 percent decrease in fruit set over the control treatment. The size of the fruit from the 3-CP thinned trees were appreciably larger than the fruit from the control or NAA treated trees (Table XIX). Foliage injury was somewhat severe a short time following application of this material with most of the leaves turning yellow and a large percentage eventually dropping. The injured leaves were rapidly replaced with new green leaves and there was no noticeable damage one month after application without close examination.

The use of NAA and 3-CP on Halehaven trees in Linden, Michigan in 1959 resulted in much less thinning than in other locations. Here the difference in method of application may have been a big factor. In this study

3-CP caused a 10 percent reduction of fruit set over that of the control treatment and little or no leaf damage was evident. However, NAA at 40 ppm gave little thinning in this test. It should be brought out that the NAA was applied one week later than the 3-CP treatments and the fruit had reached an average size of 32 mm in length and 27 mm in diameter. According to studies by Lombard (37), the use of 30 ppm NAA on Redhaven peach trees 49 days after bloom when the fruit was 32.6 mm in length and 30 mm in diameter, resulted in no significant thinning over the control treatment.

The use of 3-CP on Halehaven and Kalhaven trees in South Haven and Eau Claire, Michigan in 1959 caused almost complete removal of the fruit from the trees. The foliage was severely damaged and some twig injury was evident in trees of the South Haven area; however, all trees recovered and no permanent injury was evident by the end of the growing season.

In 1960, the use of 3-CP at 300 ppm in Grand Rapids, caused a reduction of 49 percent in fruit set of Redhaven peaches as compared to a reduction of 13 percent for the control treatment. Again, foliage injury was moderately severe shortly following the application of this material. A large percentage of the leaf surface present at the time of spray application eventually dropped, but new growth and leaves developed.

Application of MH-30 at 1,000 ppm to Redhaven trees approximately 28 days after bloom in Grand Rapids, Michigan resulted in very little thinning with fruit set reduced only 2 percent below that of the control treat-

ment. No apparent foliage injury resulted from the use of this material.

Only a small reduction in fruit set occurred when NAA was used at 30 ppm and at different pH levels, on Redhaven trees at Linden, Michigan. This may be explained in part by the fact that (1) the fruit had reached a size of 33 mm in length, possibly too large for effective thinning, and (2) a hard rain occurred one hour after the application of the material. No apparent leaf or twig injury resulted from the use of these NAA treatments.

Close examination of many peach seeds where growth had been stopped by the use of NAA, 3-CP and MH-30, revealed that the seeds were injured by these materials. The injury was expressed as a severe browning of the tissue with the browning always starting first in the nucellus and endosperm proceeding towards the embryo. Examination of seeds showing only partial browning failed to reveal any browning of the embryo. These visual observations seem to indicate that injury to the embryo occurred only after complete disintegration of the nucellus and endosperm. This suggests the mechanism of injury in fruit thinning of peaches may be first by injury to the nucellus and/or endosperm and not the embryo as previously reported for the apple (42, 70).

DISCUSSION

Preconditioning of one-year-old apple and peach trees by exposure to different environments resulted in a significant difference in the absorption of NAA C^{14} by the leaves. Peach trees grown in a low temperature of 60° to 66° F and a high relative humidity of 94 percent absorbed more labeled NAA than trees grown at a higher temperature of 70° to 77° F. Apple trees were found to be more dependent upon temperature than relative humidity, in that trees grown at 60° to 65° F absorbed more NAA C^{14} than trees grown at 70° to 77° F, regardless of the relative humidity. Westwood and Batjer (78) reported that preconditioning apple trees at 70° F for one week resulted in greater absorption of NAA than preconditioning at 50° F. Whereas, Edgerton and Haeseler (5) found that preconditioning McIntosh apple trees for eight days at 55° F resulted in more absorption of NAA than when trees were grown under normal greenhouse temperatures. The results from this study corroborate the findings of Edgerton and Haeseler (15) but appear contrary to the report of Westwood and Batjer (78) in that the greatest absorption occurred when trees were preconditioned by lower air temperatures.

Preconditioning apple and peach trees to different relative humidities did not effect the absorption of labeled NAA by the leaves with the same degree of uniformity as did the air temperatures. In 1959 peach trees grown in 94 percent relative humidity and apple trees grown in 45 percent

relative humidity absorbed the greatest amount of NAA (Table V). However, in 1960, there were no significant differences in absorption for either apple or peach trees grown at 91 percent or 45 percent relative humidities (Table IX). One difference occurred between the two studies in that the 1960 experiment was carried out approximately one month later than in 1959. Because of this, extremes in air temperature and relative humidity were a little greater as it was impossible to maintain the air temperature at the desired level in the greenhouses during the day. Possibly the interaction of the excessive high temperature and high relative humidity in the 1960 study, conditioned the leaves in such a manner that absorption of NAA C^{14} was decreased. Somewhat different results may have been obtained if the study could have been carried out in absolutely controlled environmental conditions. Of interest, the cuticle development of the leaves was similar for both years, and thus could not account for any differences in absorption.

The overall findings of these studies indicated that the absorption of NAA C^{14} by leaves of apple and peach trees was more affected by preconditioning from air temperatures than by relative humidity and that preconditioning trees to cool air temperatures enhanced the absorption of naphthaleneacetic acid. This could conceivably account for the over-thinning of apples and peaches when a cool rainy period of several weeks duration precedes the application of NAA (26, 29). However, it appears that cool weather rather than rain affects the degree of thinning.

As previously stated the development of the leaf cuticle of the apple and peach was not effected by the environments that produced the difference in absorption. In both 1959 and 1960, there was very little difference in the weights of cuticle removed from leaves of trees grown in the different environments. These findings for apple and peach leaves do not substantiate the conjectures of Loomis (39) who indicated that environment may effect the amount of cuticle produced by the leaves of plants.

As was expected the weight of cuticle from both lower and upper surfaces of the peach leaves and upper surface of the apple leaves increased with the age of the leaf. The development of the cuticle on the lower leaf surface of the apple was not studied because it was impossible to obtain intact cuticle discs due to the presence of the dense pubescence. However, the cuticle removed from the lower surface of the peach leaves in both 1959 and 1960 weighed more than cuticle removed from the upper surface (Tables X and XI). The reverse was true for cuticle removed from apricot leaves in work reported by Orgell (55).

The results from this study indicate that the amount of cuticle produced by leaves of the apple and peach tree is not dependent upon environmental conditions. Also, the quantity of cuticle produced did not appear to be important in the absorption of NAA as trees preconditioned by exposure to different environments absorbed different quantities of NAA C^{14} even though there was little difference in the amount of cuticle present on these

leaves. From these studies it might be conjectured that the quality, chemical composition, or perhaps porosity of the cuticle may be the important factor in absorption, rather than the amount of cuticle present in the leaves.

Absorption of NAA C^{14} by apple and peach leaves was found to be dependent on the pH of the solution of the growth regulator in the same manner as those reported for 2, 4-D (3, 22, 23). It was found that absorption of NAA C^{14} by apple and peach leaves was high at pH 3 and very low at pH 9.

It would seem that this influence of pH on absorption may be caused by the effect of pH on both the compound being absorbed and the cuticle on the leaf. Some researchers have felt that at low pH, the molecules of weak acids such as 2, 4-D and IAA are changed to undissociated forms and consequently are more soluble in the wax and lipid-like substances of the cuticle (3, 23). The increase in hydrogen-ion concentration could also cause the surface acids of the cuticle to become undissociated, thus replacing the negativity of the surface acid present in the cuticle (73). This should, therefore, increase the permeability of the cuticle to undissociate weak acids, such as NAA, at low pH. Another possibility would be the direct effect of the acid used to lower the pH of the solution containing the growth regulator on the cuticle itself. At a pH of 2 to 3, some of the cuticle could be partially dissolved or erroded by the acid, thus reducing a portion of the barrier to the material to be absorbed.

Assuming that a large percentage of the applied NAA may be absorbed

by apple and peach leaves, then it becomes necessary to know if the absorbed material moves out of the leaf into the developing fruit. Also, the period of time required for the material to move from the site of application could be important in determining the length of time required for a compound such as NAA to become effective. By applying NAA C^{14} to the upper and lower surfaces of peach and apple leaves, and by preparing autoradiograms of the leaves after different time intervals, it was possible to study the movement of the material from the site of application (Figures 2, 3). When NAA C^{14} was applied to the upper surface of apple leaves, very little of the applied material moved from the original site of application until after 24 hours. After 48 hours, C^{14} was found throughout the leaf, but concentrated at the margins and in the petiole. Some movement of C^{14} was evident one hour after application of labeled NAA to the lower surface of apple leaves which may be associated with the stomates on the lower surface.

One hour following the application of NAA C^{14} to either the upper or lower surfaces of peach leaves, C^{14} was detected in the vascular tissue of the leaf with maximum movement of C^{14} occurring 12 hours after application (Figure 3).

From these findings, when NAA comes in contact with the peach leaf or the lower surface of the apple leaf, some translocation of the material may occur almost immediately. However, the exact time of movement of C^{14} cannot be accurately determined by autoradiograms, since some self-

absorption of the beta particles by the tissue occurs, but certainly a good indication can be obtained as to when the major portion of the material is translocated.

Evidence obtained from these studies indicate that foliar applied NAA is translocated through the conductive system of the plant into the developing fruit and seed (Figures 4, 5). When entire peach and apple leaves of shoots bearing fruit were dipped in labeled NAA solution, C^{14} was found distributed throughout most of the fruit. The seeds from these fruits were found also to have C^{14} present. Maxie et al. (47) obtained similar results studying the movement of foliar applied carboxyl labeled 2, 4, 5-trichlorophenoxy-acetic acid into the developing fruit and seed of the apricot. He reported C^{14} present in the mesocarp three days after treatment and in the embryo and seed coat ten days following application of the material.

Although the presence of C^{14} in the tissues of the fruit cannot be taken as proof for the presence of NAA C^{14}, it would certainly seem likely that this was the case. Since the NAA C^{14} used in these studies was labeled in the ring position and not the carboxyl group, it would seem likely that at least the naphthalene molecule was being translocated into the fruit. The breakdown of the naphthalene ring by the plant tissues would be unlikely in this short period of time.

Not only must a growth regulator be absorbed by the foliage and translocated to the developing fruit to be effective as a fruit thinning agent, but

it, in all probability, should reach the fruit in its original molecular structure. Any chemical system present in the foliage or fruit, that would bring about the destruction of the growth regulator applied or the destruction of natural auxins already in the fruit, would govern the effectiveness of a thinning agent. Nitch (54) observed that the primary source of native auxin in the fruit was in the developing seed. Luckwill (40) found that in developing apple seeds the greatest portion of hormone production was in the endosperm and suggested that the accumulation of natural hormone in the endosperm could control abscission of the young fruit. Lombard (37) found that the natural hormone content of peach seeds was greater during the first period of "June" drop when the fruit reached a length of 30.8 mm. Murneek (51) suggested that NAA used at a relatively high concentration for apple and peach thinning might disturb the embryo and/or endosperm during a critical period in the developing of the seed. Luckwill (41) and Teubner and Murneek (70) have conjectured that the addition of an exogenous supply of a plant regulator to the already natural hormone saturated embryo would result in inhibition of this organ. Therefore, applications of a growth regulator during periods of low auxin level in the seed may not bring about the desired destruction of the seed and eventually abscission.

The existence of an enzyme, indoleacetic acid oxidase, which use indoleacetic acid (natural auxin) as a substrate, has been isolated from several different plants (18, 21, 33, 69, 70). Teubner and Murneek (70) studying

the indoleacetic acid oxidase of apple seeds, reported that the activity of the oxidase was greater in 40-day old apple embryos than in 55-day old embryos.

As stated earlier, periods of high and low natural hormone accumulation in the seed of the apple and peach are known (40) and (37). A study was made to determine the ability of the developing seed of the apple and peach to destroy indoleacetic acid and naphthaleneacetic acid and to relate periods of high natural hormone accumulation in the seed to periods of low indoleacetic acid oxidase activity. The harvesting of fruit for assaying enzymatic activity of the seed was begun on June 2, approximately 16 days after full bloom.

It was found in this study that both apple and peach seeds vary greatly in ability to destroy IAA during their development. On June 2, 4 and 6, 1960 when the fruit ranged from 18 to 25 mm in length, the IAA oxidase activity was very high in peach seeds and corresponded to the size of fruit having very low natural hormone content in the seed, as reported by Lombard (37). NAA has also been reported to be ineffective as a thinning agent for peaches at this period of fruit development (63).

On June 10, approximately 10 days after "shuck-off", the IAA oxidase activity in the peach seed was very low. The fruit had grown to a length of 30 mm and corresponded exactly to the size of fruit that had the highest natural hormone content in the seed (37). This has also been shown to be the optimum number of days after "shuck-off" for the application of NAA as

a fruit thinning agent (32, 37). After June 10, the fruit began to grow very rapidly and by June 12, the length of the pericarp reached 36 mm, which is considered too large for a growth regulator to be effective as chemical thinning agents (37). With this increase in size, there was an increase in IAA oxidase activity. Lombard (37) found that by the time the fruit had reached 37 mm in length the natural hormone content of the seed was very low. From these studies it would appear that periods of low natural hormone content of the seed would correspond very closely to period of high IAA oxidase activity.

Peach leaf tissue and seed were also found to decarboxylate NAA C^{14}, but not to the extent as IAA. Teubner and Murneek (70) reported that IAA oxidase prepared from apple embryos was incapable of oxidizing NAA, but this would not eliminate the possibility of other enzymatic systems capable of destroying NAA. The rate of NAA destruction in peach seeds was low enough to keep it from being considered as a major factor in retarding the effectiveness of NAA as a thinning agent.

Indoleacetic acid destruction by apple seed was very low from June 2 to June 12, 1960. After June 12, which corresponded to approximately three weeks after petal fall, the activity of the IAA oxidase in the seed increased very rapidly. This period of increased activity corresponded to the period of rapid embryo development, when before June 12, 1960, the embryo was very small and difficult to find in the seed. However, by June 24 the embryo

could be seen easily in the seed, and was found to be in the cotyledonary stage of development. Luckwill (41) reported that, with the growth of the embryo and the rapid digestion of the primary endosperm, auxin accumulation decreased in the seed and "June drop" occurred. Of interest, periods of low auxin accumulation in the seeds as reported by Luckwill (41) corresponded very closely with periods of high IAA oxidase activity in the seeds as found in this study. Teubner and Murneek (70) also found that during the period of rapid embryo development, the IAA destruction potential of the apple seeds was quite high and as the embryo approached maturity the IAA oxidase activity dropped.

Apple seeds were found to have less potential in destroying carboxyl labeled NAA C^{14} than peach seeds. Although some decarboxylation of NAA C^{14} occurred when incubated with apple seeds, the total amount destroyed was very small. In fact, the amount destroyed was of such low magnitude that it would probably not be considered important in changing the effectiveness of NAA when applied as a thinning agent.

Thus, based on this study and the findings of other workers (37, 41, 70), it may be stated that the effectiveness of a growth regulator to thin peach and apple fruits may be dependent upon the activity of the IAA oxidase enzyme in the seeds. When the enzymatic activity is high, natural hormone content in the seed is low and application of a growth regulator may result in little thinning. When the enzyme activity is low, natural hormone content is high

and an application of a growth regulator at this period would be effective as a thinning agent.

Three synthetic growth regulators were used as chemical thinning agents for Redhaven, Halehaven and Kalhaven peaches. In the two-year study, both 3-chlorophenoxy alpha propionic acid (3-CP) and naphthalene-acetic acid (NAA) caused a reduction in fruit set when the materials were applied to fruit that ranged in size from 25 to 30 mm in length. These results conformed closely to work carried out by Lombard (37). He found that NAA was effective as a fruit thinning agent for peaches when NAA applications were made just prior to cellular formation of the endosperm. The cellular formation of the endosperm took place when the Halehaven and Redhaven fruit were approximately 30 mm in length. Kelly (32) suggested that the application of NAA to give effective thinning of peaches should be timed from "shuck-off", the period when the hypanthium is shed from the young fruit, rather than from bloom. He felt that the growth of the fruit was much more uniform following "shuck-off" and that environmental influence on the growth and development of the fruit would not be as great following this period.

Results from this study, however, indicated that the bloom date or "shuck-off" date cannot be used accurately to predict optimum time for application of NAA or other growth regulators. According to data reported by Lombard (37), full bloom of Redhaven peaches occurred on May 3, 1958 and

the fruit reached a size of 22 mm in length by June 7, 1958, which was approximately one week after "shuck-off". One week later, June 14, 1958, the fruit had grown to 30.8 mm in length, and the endosperm was in the early cellular stage of development.

In 1960, full bloom of Redhaven peaches did not occur until May 15, but even after this delayed bloom date the fruit reached 25 mm in length by June 8, 1960, which is 3 mm longer than on June 7, 1958. By June 12, 1960 the fruit had expanded to 36 mm in length, 6 mm longer than fruit harvested on June 14, 1958. This would indicate that the size and rate of growth of fruit is not the same every year after a given number of days from full bloom or "shuck-off". The one criteria that was found to be satisfactory for selecting dates for applying NAA as a thinning agent for peaches was the length of pericarp. Applications of NAA made on Redhaven peach trees in 1959 and 1960 were effective in thinning only when applied before the pericarp of the fruit reached 30 mm in length. This same effect was reported earlier by Lombard (37). From these results, it is suggested that linear measurements of young fruit would be a better criteria for determining the time of NAA applications for effective fruit thinning, than by counting the number of days following bloom or "shuck-off". The findings herein indicated that when the linear dimension of the fruit reached between 24 and 29 mm, it would be the optimum state of fruit and seed development for effective thinning of peaches using NAA. Application of NAA after the fruit has reached

a linear size greater than 30 mm, has not resulted in effective thinning. Again, this optimum stage of fruit development for effective thinning corresponded to periods of high natural hormone content of the seed and low IAA oxidase activity.

In 1959 and 1960, the use of 3-CP resulted in a reduction in fruit set on Redhaven, Halehaven and Kalhaven peach trees. Even though foliage injury was somewhat severe following application of this material, the thinning effect on Redhaven trees in many cases was very satisfactory. The material greatly overthinned Halehaven and Kalhaven trees in 1959. Thompson and Rogers (72) were the first to use 3-CP as a thinning agent, but they did not report the foliage injury that was experienced when the material was used in Michigan.

The method of application could prove to be an important factor in the use of 3-CP from the standpoint of injury. When the peach trees were sprayed beyond the drip point, using a high pressure nozzle, excessive foliage injury resulted. However, when trees were sprayed lightly on one side only, little, if any, foliage injury was evident. It could well be that applying 3-CP with an air blast sprayer would reduce the injury to the foliage and yet give the desired thinning of fruit. Complete wetting of the tree by over spraying could be avoided by the use of an air blast sprayer, and possibly less foliage injury would result.

Maleic hydrazide was also used as a fruit thinning agent for peaches,

but little thinning resulted when used at 1,000 ppm one month after bloom. This was contrary to the findings of Gambrell et al. (19), who reported maleic hydrazide to be very effective in thinning peaches in South Carolina when used at this concentration and date following bloom. No foliage injury resulted when used in this manner which suggested that the material could possibly be used at higher concentrations to bring about fruit thinning under Michigan conditions.

Numerous seeds of peach trees sprayed with NAA, 3-CP, and MH-30 were examined one week following the date of applications. It was possible to tell which of the fruit had been affected by these chemicals because the fruit, which would eventually drop, had stopped growing. Visual discoloration of the seeds taken from fruits that had stopped growth was quite apparent upon examination. Browning of these seeds always started in the nucellus and endosperm and then progressed towards the embryo. Those seeds showing only partial browning, did not reveal any visual injury or browning to the embryo. These observations appear to indicate that injury to the embryo occurs only after the complete disintegration of the nucellus and endosperm. Contrary to this, Teubner and Murneek (70) reported that NAA inhibited the growth of apple embryos, and supported the hypothesis that NAA induced embryo abortion and thus caused the fruit to stop growth.

It seems from the results of this study that the chemicals are more injurious to the nucellus and endosperm than the embryo, and that possibly

embryo inhibition and injury occurs only after the nucellus and endosperm has disintegrated. This may, in part, explain why Lombard (37) found that the peach fruits were most susceptible to thinning from an application of NAA just before the endosperm became completely cellular. It appears that the mechanism of fruit thinning of the peach with growth regulators may be brought about first through injury to the nucellus and endosperm followed by injury to the embryo.

SUMMARY

1. Absorption studies carried out in 1959 and 1960 on one-year-old apple and peach trees indicate that preconditioning trees to different environments will affect the absorption capacities of the trees. Peach trees were found to absorb more C^{14} naphthaleneacetic acid (NAA) when grown in environments of cool air temperatures (60° to 66° F) and high relative humidities (91 percent to 94 percent). Preconditioning air temperature was found to be more important in effecting absorption of NAA C^{14} than relative humidities. Apple trees grown under cool air temperatures (60° to 66° F) absorbed more NAA than trees grown under higher air temperatures (70° to 77° F) irrespective of relative humidity (45 percent to 94 percent).

2. Differences in the amount of cuticle developed by the leaves of the apple and peach trees grown under the different environments did not account for absorption differences of leaves grown under the same conditions. There were little if any differences in the weight of cuticle removed from leaves of trees grown in the different environments, although absorption differences were very significant. Cuticle from the lower surfaces of peach leaves weighed more than cuticle from the upper surfaces and the cuticle weights of both apple and peach leaves increased as the leaves matured.

3. Absorption of NAA C^{14} by apple and peach leaves was found to be highly dependent on the pH of the growth regulator solution. Both apple and peach leaves absorbed much more NAA in a 24-hour period at low pH (3) than at neutral (7) or at high pH (9).

4. When NAA C^{14} was applied in the form of a single large drop to the upper surface of apple leaves, little of the applied material moved from the original site of application until after a 24-hour period. Some C^{14} movement was evident after one hour when the material was applied to the lower surface of apple leaves or to the upper or lower surfaces of peach leaves.

5. Following application of foliar applied ring labeled NAA, C^{14} was found to be quickly translocated into the fruit and seeds of the apple and peach. Some C^{14} activity could be detected in the apple fruit one hour after spur leaves had been dipped into a solution of NAA C^{14}.

6. Both the foliage and seeds from developing fruit of the apple and peach were found to be capable of destroying indoleacetic acid (IAA) and NAA. NAA destruction was very slow compared to IAA destruction. IAA destruction potential of the apple and peach seed varied greatly with the different stages of seed development. High IAA oxidase activity was found to be closely associated with previously reported low natural hormone content of the seed.

7. These studies indicate that two synthetic growth regulators, 3-chlorophenoxy alpha propionic acid (3-CP) and NAA, may be used successfully as fruit thinning agents for peach trees. It appears for NAA to be effective as a thinning agent, it should be applied when the pericarp of the fruit reaches 24 to 29 millimeters in length. One week following applications of 3-CP, NAA, and MH-30, affected fruits could be recognized by reduction in growth and the browning of various components of the seed. Visual browning of the nucellus and endosperm appeared first followed by browning of the embryo.

LITERATURE CITED

1. Alderman, Deforest. 1955. Alpha-naphthylacetamide: A chemical fruit thinner. Proc. Amer. Soc. Hort. Sci. 66: 57-64.

2. Batjer, L. P., and M. B. Hoffman. 1951. Fruit thinning with chemical sprays. U. S. Dept. Agr. Circ. No. 867.

3. Blackman, G. E., and R. C. Robertson-Cuninghame. 1953. The influence of pH on the phytotoxicity of 2, 4-dichlorophenoxyacetic acid to Lemna Minor. New Phytologist 52: 71-75.

4. Bonner, W. D. Jr. 1957. Soluble oxidases and their functions. Ann. Rev. Plant Physiol. 8: 427-452.

5. Briggs, W. R., T. A. Steeves, I. M. Sussex, and R. H. Witmore. 1955. A comparison of auxin destruction by tissue extracts and intact tissues of the fern Oxmunda Cinnamonea L. Plant Physiol. 30: 148-155.

6. Burkholder, C. L., and M. McCown. 1941. Effects of scoring and of a-naphthyl acetic acid and amide spray upon fruit set and of the spray upon pre-harvest fruit drop. Proc. Amer. Soc. Hort. Sci. 38: 117-120.

7. Conway, E. J. 1957. Microdiffusion analysis and volume-metric error. 4th edition, Crosby Lockwood and Son, London.

8. Crane, J. C. 1953. Further responses of the apricot to 2, 4, 5-trichlorophenoxyacetic acid application. Proc. Amer. Soc. Hort. Sci. 61: 165-174.

9. Currier, H. B., and C. D. Dybing. 1959. Foliar penetration of herbicides. Review and present status. Weeds 7: No. 2.

10. Davison, R. M. 1955. The use of chemical thinning sprays on apple trees in New Zealand. II. Further experiments with dinitro compounds and synthetic growth substances. New Zealand Jour. Sci. and Tech. Sec. A. 37: No. 1.

11. _____. 1956. The use of chemical thinning sprays on apple trees in New Zealand. III. Effect on fruit storage life. New Zealand Jour. Sci. and Tech. Sec. A. 38: No. 1.

12. Davison, R. M. 1958. The use of chemical thinning sprays on apple trees in New Zealand. IV. Compatibility of growth substances with therapeutant sprays. New Zealand Jour. Sci. and Tech. Sec. A. 38: No. 1.

13. Davidson, J. H., O. H. Hamner, C. A. Reimer, and W. C. Dutton. 1945. Thinning apples with the sodium salt of naphthylacetic acid. Mich. Agr. Exp. Sta. Quart. Bul. 27: No. 3.

14. Dilley, D. A. 1960. The assimilation of isotopic carbon and nitrogen labeled urea into the amino acids of apple and peach leaves. Ph D Thesis, N. C. State College.

15. Edgerton, L. J., and C. W. Haeseler. 1959. Some factors influencing the absorption of naphthaleneacetic acid and naphthaleneacetamide by apple leaves. Proc. Amer. Soc. Hort. Sci. 74: 54-60.

16. Fang, S. C., and J. S. Butts. 1954. Studies in plant metabolism. III. Absorption, translocation and metabolism of radioactive 2, 4-D in corn and wheat plants. Plant Physiol. 29: 56-60.

17. _____. 1957. Studies of carboxyl-C^{14}-labeled 3-indoleacetic acids in plants. Plant Physiol. 32: 253-259.

18. Galston, A. W., J. Bonner, and R. S. Baker. 1953. Flavo-protein and peroxidase as components of the indoleacetic acid oxidase systems of peas. Arch. Biochem. and Biophys. 42: 456-463.

19. Gambrell, C. E., W. H. Rhodes, S. N. Pearman Jr., and T. L. Senn. 1959. Chemical thinning of Dixigem peaches using maleic hydrazide (MH-30). Progress report to Naugatuck Chemical Co.

20. Goldacre, P. L., A. W. Galston, and R. L. Weintraub. 1953. The effect of substituted phenols on the activity of the IAA oxidase of peas. Arch. Biochem. 43: 358-366.

21. Gortner, W. A., and M. Kent. 1953. Indoleacetic acid oxidase and an inhibitor in pineapple tissue. Jour. Biol. Chem. 204: 593-599.

22. Hamner, C. L., E. H. Lucas, and H. M. Sell. 1947. The effect of different acidity levels on the herbicidal action of the sodium salt of 2, 4-dichlorophenoxyacetic acid. Mich. State Univ. Agr. Expt. Sta. Quart. Bull. 29: 337.

23. Hauser, E. W. 1955. Absorption of 2, 4-dichlorophenoxyacetic acid by soybean and corn plants. Agron. Jour. 47: 32-40.

24. Hay, J. R., K. V. Thimann. 1956. The fate of 2, 4-dichlorophenoxyacetic acid in bean seedlings. I. Recovery of 2, 4-dichlorophenoxyacetic acid and its breakdown in the plant. Plant Physiol. 31: 382-387.

25. Hibbard, A. D., and A. E. Murneek. 1950. Thinning peaches with hormone sprays. Proc. Amer. Soc. Hort. Sci. 56: 65-69.

26. Hoffman, M. B. 1957. Chemical Thinning of Apples. Amer. Chem. Paint Co. The hormolog.

27. _____. 1953. Spraying apples. Amer. Fr. Grower 73 (2) 25: 48-51.

28. _____, F. W. Southwick, and L. J. Edgerton. 1947. A comparison of two types of material for the chemical thinning of apples. Proc. Amer. Soc. Hort. Sci. 49: 37-41.

29. Kelley, V. W. 1952. Results of the 1952 chemical peach thinning tests. Trans. Ill. Hort. Soc. 86: 228-231.

30. _____. 1953. Up-to-date in chemical peach thinning. Trans. Ill. Hort. Soc. 87: 95-101.

31. _____. 1954. Chemical thinning of apples and peaches. Trans. Ill. Hort. Soc. 88: 211-219.

32. _____. 1955. Time of application of naphthaleneacetic acid for fruit thinning of the peach in relation to the June drop. Proc. Amer. Soc. Hort. Sci. 66: 70-72.

33. Kenten, R. H. 1955. The oxidation of indole-3-acetic acid by waxpod bean root sap and peroxidase systems. Jour. Biochem. 59: 110-116.

34. _____, and P. J. Mann. 1950. The oxidation of manganese by peroxidase systems. Jour. Biochem. 46: 67-77.

35. Langer, C. A. 1952. The effect of growth regulators on blossom thinning with special reference to apples and peaches. Ph D Thesis, Michigan State University.

36. Langer, C. A. 1953. Unpublished data.

37. Lombard, P. B. 1958. The development of the embryo, endosperm, and pericarp of the peach (Prunus persica, Sieb. Xucc.) as related to fruit thinning with plant regulators. Ph D Thesis, Michigan State University.

38. _____, A. E. Mitchell, and H. A. Cardinell. 1958. Chemical thinning of peaches using naphthaleneacetic acid, N-1-naphthyl phthalamic acid, and 3-chloro IPC. Mich. Agri. Expt. Sta. Quart. Bull. 41: No. 1: 147-156.

39. Loomis, W. E. 1953. Growth and differentiation in plants. The Iowa State College Press.

40. Luckwill, L. C. 1948. The hormone content of the seed in relation to endosperm development and fruit drop in the apple. Jour Hort. Sci. 24: 32-44.

41. _____. 1951. Trials with growth substances as fruit-thinning agents. Ann. Rept. Agr. Hort. Res. Sta., Long Ashton, Bristol. 33-40.

42. _____. 1953. Studies of fruit development in relation to plant hormones. I. Hormone production by the developing apple seed in relation to fruit drop. Jour. Hort. Sci. 28: 14-24.

43. _____. 1953. Studies of fruit development in relation to plant hormones. II. The effect of naphthaleneacetic acid on fruit set and fruit development in apples. Jour. Hort. Sci. 28: 25-40.

44. Maclachlan, G. A., and E. R. Waygood. 1956. Catalysis of IAA oxidation by manganic ions. Plant Physiol. 9: 321-326.

45. _____. 1956. Kinetics of the enzymically catalyzed oxidation of IAA. Can. Jour. Biochem. and Physiol. Nov.

46. Marth, P. C., W. H. Preston Jr., and J. W. Mitchell. 1953. Growth-controlling effects of some quaternary ammonium compounds in various species of plants. Bot. Gaz. 115: 200-204.

47. Maxie, E. C., B. V. Muriel, B. J. Robinson, and A. A. Hewitt. 1959. Distribution of C^{14} from carboxy-labeled 2, 4, 5-trichlorophenoxyacetic acid in fruit tissues of Tilton apricot. Proc. Amer. Soc. Hort. Sci. 73: 134-144.

48. Metcalf, R. L. 1960. Advances in pest control research. Vol. III. Interscience Publishers, Inc., New York. pp 350-436.

49. Mitchell, J. W., and J. W. Brown. 1946. Movement of 2, 4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food material in plants. Bot. Gaz. 107: 393-407.

50. Murneek, A. E. 1950. The relative value of hormone sprays for apple thinning. Proc. Amer. Soc. Hort. Sci. 55: 127-136.

51. _____. 1951. Growth regulating substances in relation to reporduction of some horticultural plants. In F. Skoog., Ed. Plant Growth Substances. Univ. of Wis. Press. Madison. 329-345.

52. _____. 1952. Plant growth-regulators during fertilization and post-fertilization periods. Proc. Amer. Soc. Hort. Sci. 59: 207-217.

53. Muzek, T. J., H. J. Crugade, and A. J. Loustalot. 1954. Studies on the absorption, translocation, and action of C. M. U. Bot. Gaz. 116: 65-73.

54. Nitch, J. P. 1952. Plant hormone in the development of fruits. Quart. Rev. Biol. 27: 33-57.

55. Orgell, W. H. 1955. The isolation of plant cuticle with pectic enzymes. Plant Physiol. 30: 78-80.

56. Ray, P. M., and K. V. Thimann. 1956. The destruction of indoleacetic acid. I. Action of an enzyme from Omphalia flavida. Arch. Bio. Chem. Biophys. 64: 175-192.

57. Rice, E. L. 1948. Absorption and translocation of ammonium 2, 4-dichlorophenoxyacetate by bean plants. Bot. Gaz. 109: 301-318.

58. Schell, R. E. 1956. The study of several factors influencing the absorption of indoleacetic acid and 2, 4, 5-trichlorophenoxy acetic acid by leaves of several apple varieties. M. S. Thesis. Agriculture. Cornell University.

59. Schneider, G. W., and J. V. Enzie. 1943. The effect of certain chemicals on the fruit set of the apple. Proc. Amer. Soc. Hort. Sci. 42: 167-176.

60. Schneider, G. W., and J. V. Enzie. 1944. Further studies on the effect of certain chemicals on fruit set of the apple. Proc. Amer. Soc. Hort. Sci. 45: 63-68.

61. Siegel, S. M., and R. L. Weintraub. 1952. Inactivation of indoleacetic acid by peroxides. Physiol Planetarium 5: 241-245.

62. Smith, A. E., G. M. Stone, J. W. Zukel, and W. L. Hubbard. 1955. Absorption studies of maleic hydrazide by plants. Bethany information sheet No. 68. U. S. Rubber Co.

63. Southwick, F. W., L. J. Edgerton, and M. B. Hoffman. 1947. Studies in thinning peaches with blossom removal sprays. Proc. Amer. Soc. Hort. Sci. 49: 26-32.

64. _____. 1948. Further experiences with chemical thinning of apples and peaches. Proc. Amer. Soc. Hort. Sci. 51: 41-47.

65. _____, and W. F. Weeks. 1949. Chemical thinning of apples at blossom time and up to four weeks from petal fall. Proc. Amer. Soc. Hort. Sci. 53: 143-147.

66. _____. 1950. Some attempts to thin apples with naphthalene acetic type materials after calyx. Proc. Amer. Soc. Hort. Sci. 56: 70-75.

67. Stebbins, T. G., A. L. Neal, and V. R. Gardner. 1946. Thinning apples at blossom time with growth regulating substances and oil-wax emulsions. Proc. Amer. Soc. Hort. Sci. 48: 63-66.

68. Stutz, R. E., D. E. Atkinson, and S. A. Gordon. 1951. Synthesis of 3-indoleacetic acid-2-C^{14}. ANL-4710. 1-2.

69. Tang, Y. W., and J. Bonner. 1947. The enzymatic inactivation of IAA. I. Some characteristics of the enzyme contained in pea seedlings. Arch. Biochem. 3: 11-20.

70. Teubner, F. G., and A. E. Murneek. 1955. Embryo abortion as mechanism of "hormone" thinning of fruit. Mo. Agr. Exp. Sta. Res. Bul. 590.

71. Thimann, K. V. T. 1948. Use of 2, 4-D acid herbicide on some woody tropical plants. Bot. Gaz. 109: 334-340.

72. Thompson, A. H., and B. L. Rogers. 1959. Three years results with new chemicals for thinning of peaches after the blossoming period. Proc. Amer. Soc. Hort. Sci. 73: 112-119.

73. Van Overbeek, J. 1956. Absorption and translocation of plant regulators. Ann. Rev. Plant Physiol. 7: 355-372.

74. Wagenknecht, A. C., and R. H. Burris. 1950. IAA inactivating enzymes from bean roots and pea seedlings. Arch. Biochem. 25: 30.

75. Waygood, E. R., A. Oaks, and G. A. Machlachlan. 1956. The enzymically oxidation of IAA. Can. Jour. Botany. Nov.

76. Weaver, R. J., and H. R. DeRose. 1946. Absorption and translocation of 2, 4-dichlorophenoxyacetic acid. Bot. Gaz. 107: 509-521.

77. Weintraub, R. L., J. W. Brown, M. Fields, and J. Rohan. 1953. Metabolism of 2, 4-dichlorophenoxyacetic acid. I. $C^{14}O_2$ production by bean plants treated with labeled 2, 4-dichlorophenoxyacetic acid. Plant Physiol. 27: 293-301.

78. Westwood, M. N., and L. P. Batjer. 1958. Factors influencing absorption of dinitro-ortho-cresol and naphthaleneacetic acid by apple leaves. Proc. Amer. Soc. Hort. Sci. 72: 35-44.