1976

A Study of Endogenous and Exogenous Growth Regulators on Normal and Abnormal Shuck Opening of Pecan, Carya Illinoensis (Wang) K. Koch.

Chin Kit Ling
Louisiana State University and Agricultural & Mechanical College

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K. KOCH.

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A STUDY OF ENDOGENOUS AND EXOGENOUS GROWTH
REGULATORS ON NORMAL AND ABNORMAL SHUCK OPENING
OF PECAN, CARYA ILLINOENSIS (WANG) K. KOCH

A Dissertation

Submitted to the Graduate Faculty of the
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in
The Department of Horticulture

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ABSTRACT

Studies on levels of endogenous growth promoters and inhibitors that regulated natural shuck opening, regulation of abnormal shuck opening by hormones, and effects of several exogenous growth regulators on shuck opening and kernel yield were conducted.

In the studies on the regulation of natural shuck opening of pecan by endogenous growth substances and the regulation of abnormal shuck opening, the fruits were ether-extracted and fractionated into acidic and neutral/basic fractions which were then partitioned by paper chromatography using n-butanol:ammonium hydroxide:water (10:1:1) as the solvent. Avena coleoptile straight-growth method was employed in the bioassays of growth promoters and inhibitors on the chromatograms. Histograms of coleoptile growth, expressed as percent of the control against 10 RF values, were plotted from the results of the bioassays. Conclusions and suggestions were based on the interpretation of the histograms and statistical analyses of the data.

Indolepyruvic acid, indoleacetic acid, and gibberellin or a gibberellin-like compound were the possible growth promoters in the acidic fraction. Abscisic acid and inhibitor C were the possible growth inhibitors in the acidic fraction. Phenolic acids were the inhibitor(s) present in the neutral/basic fraction.

Data obtained suggest that a promoter/inhibitor balance was involved in the regulation of natural pecan shuck opening. The occurrence of natural pecan shuck opening was first governed by the
disappearance of GA or the gibberellin-like compound and then followed by the disappearance of auxins and increases in abscisic acid and inhibitor C.

The mechanism regulating the natural shuck opening was proposed. The disappearance of GA or the gibberellin-like compound induced indole-acetic acid to be physiologically active in regulating ethylene production. Ethylene induced the production of IAA oxidase or peroxidase which catalysed the oxidation of IAA, and caused a shift in the hormonal balance in favor of abscisic acid making it physiologically active in regulating shuck opening by regulating the synthesis of cellulase which was responsible for the dissolution of cells in the abscission zone between the seed and the shuck and in the sutures of the shuck.

Two situations arised from the studies on the relationship between the endogenous growth hormones and pecan shuck disease. Pecan shuck disease in Situation 1 was probably due to the absence of GA or the gibberellin-like compound accompanied by low levels of auxins and phenolic acids. Pecan shuck disease in Situation 2 was probably due to the presence of growth inhibitor(s) particularly at Rf 0.1-0.4, and the absence of GA or the gibberellin-like compound at Rf 0.8-0.9 in the acidic fraction. In both cases, pecan shuck disease resulted in premature shuck opening.

Several exogenous growth regulators were applied as sprays on foliage and fruits of the cultivar 'Success'. GA and Alar, singly or in combination with one another, significantly reduced the incidence of pecan shuck disease. GA x Alar was most effective in reducing the
incidence of pecan shuck disease. GA was observed to be more effective than Alar.

Synthetic cytokinin (PBA), GA, and/or IAA applications reduced the incidence of pecan shuck disease to a certain extent, but these reductions were not significant. IAA at 100 ppm increased the incidence of pecan shuck disease. Over-dosage of exogenous IAA application, auxin-induced ethylene production, and weak physiological stage of the tree were the reasons suggested for such increase in the incidence of pecan shuck disease.

Kinetin, and combinations of kinetin and Alar among growth substances studied were found to be least effective in reducing the incidence of pecan shuck disease.

Kernel yield was not a good criterion to evaluate the effects of the growth substances studied because fruits were harvested prematurely.
INTRODUCTION

Pecan, *Carya illinoensis* (Wang) K. Koch, is one of the most valuable and popular nut crops in the United States of America. Its production and processing constitute the largest commercial nut industry (22, 218). However, there exists a disease or physiological disorder of pecan fruit (pecan shuck disease), which has been known for many years, but has received little attention until recent years.

Pecan shuck disease is a widespread problem that caused considerable losses to the growers in the south and southwest United States. There are limited research data from which a conclusion concerning the disposition of this disease can be drawn.

The failure in isolating and identifying any possible pathogen from the diseased fruits and the failure of improved cultural practices to reduce or control the disease (132) have switched research emphasis to the internal factors involved in the occurrence of the disease.

According to Lipe and Morgan (193, 194), the concept of dehiscence of fruits appears to have been governed by the endogenous balance of dehiscence promoters and inhibitors resulting in a condition that favors the production of premature shuck-drying promoter(s).

In an attempt to investigate this concept, the following studies were initiated to determine the levels of extractable growth hormones in pecan fruit. An attempt to induce or reduce this disease by the application of synthetic growth regulators was also carried out.

It is hoped that the results of these studies will contribute a
better understanding of the mechanism regulating the occurrence of the pecan shuck disease and will aid in the development of a practical method of controlling this disease.
Pecan Shuck Disease

The Success cv. has demonstrated the highest level of susceptibility to shuck disease, but cultivars having Success as a parent are frequently predisposed to a high level of susceptibility (132). It has also been observed to a lesser extent in the cultivars: Moore, Brooks, Elliot, Mahan, Desirable, Mohawk, Jennings, Bradley, Farley, Barton, Schley, and Stuart (131).

The disease is evidenced by the premature drying of either the apex (distal) end or the stem (proximal) end of the shuck (131). Halliwell and Johnson (132) and Schaller and Kenknight (280) characterized the premature drying of the apex end of the shuck as 'Shuck dieback', and the premature drying of the stem end of the shuck as 'Stem end blight'. The dry area has a burned appearance and has been referred to as 'shuck burning' (131).

Since the date of termination of filling of the nuts noticeably affected nut quality and shelling percentage, shuck disease greatly reduced the weight, density, shelling percentage, and grade of the nuts (277).

Shuck dieback has been observed to be primarily a disease of the cultivar, Success, and those cultivars with Success as parentage. It usually occurs a month to three weeks prior to the normal opening of the shuck. Necrosis occurs at the distal end of the shuck and is soon followed by premature drop (133).
Shuck dieback has not been prevented or alleviated by corrective production practices such as fertilization including macro- and micro-elements, irrigation, and the control of diseases and insects. Nematodes, viruses, fungi, and bacteria were systematically ruled out (133).

Typical shuck dieback symptoms were experimentally produced by girdling the peduncle (fruit stem), treatment with Ethrel and ethylene. Indeed, shuck dieback was prevented and normal shuck opening delayed by treatment with ethylene action suppressants, carbon dioxide and auxin (133).

Stem end blight, known as 'sticky shuck' may first appear in the late summer as a black necrotic spot at or near the proximal end of the immature shuck. This spot enlarges and engulfs the whole shuck. The shuck usually clings to the nut producing a 'stick tight'. It occurs on most cultivars when the nuts are near fully expanded until the nut shell is hardened (133).

Symptoms of stem end blight could be induced experimentally at random by puncturing the soft shell membrane with a needle during this period. Necrosis is followed by formation of the nut abscission zone resulting in premature drop (133).

Based on the observations by Halliwell and Johnson (133) and Schaller and KenKnight (280), stem end blight may be controlled by rigid fungicide and insecticide applications during the late summer.

Observations from Halliwell and Johnson (133) also suggested that copious amounts of ethylene might be responsible for the formation of the pecan shuck disease complex.

Osburn et al. (245) reported that nuts frequently dropped as a
result of stink bug damage.

Halliwell and Johnson (133) also observed that the Success cultivar, was very sensitive to 2,4-D. NAA (α-naphthalene acetic acid) and IBA (Indole butyric acid) were also reported to cause the premature dropping of pecan fruits.

Factors Contributing to the Incidence of Pecan Shuck Disease

Preliminary investigations by Dodge and Schaller (87, 88) indicated that soil type, size of the crop, pruning and fungicide application did not appear to significantly affect the development of shuck disease. They (87, 88) showed that there was a correlation between the length of the shoot and disease severity. They also maintained that there was a difference in the nutritional levels of the different shoot types. The incidence of the disease could be reduced by maintaining a high nutritional level and increasing the percentage of longer, stronger shoots (88).

Excessive crop with sparse and unhealthy foliage, poor tree growth, vein spot infection, shading of the tree, high rainfall, heavy and waterlogged soil favored the incidence of shuck disease (278, 280).

Ethylene: Pecan Shuck Dehiscence

Many workers (44, 45, 253) have reported that ethylene functions as a ripening hormone in fruits. This response of ripening apparently regulated by endogenous ethylene (44, 45, 253) could be induced by the application of exogenous ethylene.

In an attempt to improve pecan harvesting by using ethylene, Finsh (104) in 1936 reported that ethylene treatment of detached pecan fruits caused shuck dehiscence and shedding of the nuts. He also
induced an epinasty characteristic of ethylene in leaves of a potato plant enclosed with pecan shucks.

Ethylene has also been reported to hasten the dehiscence of the shuck of walnut and pecan (58, 300). The promotion of pecan and walnut shuck dehiscence with ethylene required 36 to 40 hours.

Love et al. (205) reported that pecan shuck dehiscence was easier after a post-harvest dip in 6,000 ppm Ethephon for 30 minutes. The observations were made after treatment with Ethephon and after the pecan fruits were stored for six days at temperature of 90°F. Ethephon was reported to release free ethylene gas, chloride and phosphate upon disintegration (333).

Lipe and Morgan (192, 194) made a periodic measurement of ethylene evolution by detached pecan fruits from mid-season until the completion of shuck dehiscence. They found that low ethylene evolution was observed until one week before the initiation of shuck dehiscence, when a maximum rate of almost 20 ul per kg fresh weight per hour was attained. They concluded that internal levels of ethylene rose to dehiscence-stimulating levels a sufficient time before dehiscence for the gas to have initiated the process, and they proposed that ethylene was one of the regulators of natural fruit dehiscence.

Ethylene evolution peak was found to be between September 15 and October 11 under the experimental conditions at Texas A & M University Plantation near College Station (192).

In 1971, Hinrichs et al. (145) also found that Ethephon stimulated fruit loosening on pecan and stimulated leaf abscission. Their data supported the finding of Love et al. (205).

In the study on the location of ethylene synthesis in dehiscing
pecan fruits, Lipe and Morgan (195) found that a large volume (almost 100%) of ethylene came from the seed (kernel). This indicated that the responding tissue (shuck) received its message to dehisce directly as ethylene was made in the kernel. However, the shuck would not dehisce a month or more in advance of natural dehiscence even when treated with up to 1,000 μl/liter ethylene.

**Auxins**

Since the discovery of the growth-promoting substance, indole-acetic acid (IAA) in *Avena sativa* by Went (337) in 1926, and its isolation in various plant tissues (175, 314), the effects of auxins on the growth of plants had been demonstrated by many workers. Devlin (83) has made some comprehensive reviews on the physiological effects of auxin on cell elongation, phototropism, geotropism, apical dominance, root initiation, parthenocarpy of fruits, respiration, and callus formation.

The highest concentrations of auxin has been found in the growing tips of the plant, that is, in the tips of the coleoptiles, buds, and in the growing tips of leaves and roots (83).

Auxin which is found widely distributed throughout the plant, was undoubtedly transported from the meristematic region to the other parts of the plant (83). This widespread occurrence of auxin in the plant has been supported by several studies (313, 315, 326).

Hatcher (138) found that auxin content in the apple twigs rose in the spring as growth got underway, and it subsequently declined through the growing season; trailing after it was a decline in the growth rate until autumn. Long shoots of *Ginkgo* was correlated with
large and continuing supplies of auxin while short shoots developed a diminutive supply (124).

Correlations between auxin content of tissues and growth rates have been found in many instances. Results by Scott and Briggs (285) showed that the growth rate in pea stems dwindled from the apex toward the base of the plant, as did the auxin content.

Thimann (314) demonstrated that cultures of *Rhizopus suinus* could convert the amino acid, tryptophan to IAA. Since then, much work had been done to show the conversion of tryptophan to IAA.

Generally, tryptophan was considered to be the primary precursor of IAA. Labeled tryptophan was converted to $^{14}$C-IAA in watermelon slices (77), cabbage shoots (179), tomato and barley shoots (118), lime fruits (173), tobacco shoots (247), bean shoots (34), and bean roots (224).

Salem and Kenefick (274) had indicated that indirectly zinc was necessary for the synthesis of optimal levels of tryptophan in the tissue of corn seedlings.

Wildman et al. (343) isolated an enzymatic system that was capable of converting tryptophan to IAA in spinach leaves. The presence of an enzyme capable of converting tryptophan to IAA in *Avena* coleoptiles showed a close agreement between the distribution of IAA and the enzyme (344). In 1970, Sherwin (288) showed that tryptophan transaminase, an enzyme capable of converting tryptophan to tryptamine, an intermediate to IAA, was detected in cucumber seedlings and in numerous other plant species (321).
Regulation of Ethylene Production by Auxin

The initial discovery that auxin regulated the production of ethylene was made by a few workers (73, 358). They observed that NAA and IAA produced effects on plants including epinasty, inhibition of growth, root induction, tissue swelling, similar to the characteristics of ethylene. Abeles (1) reported that 2,4-dichlorophenoxyacetic acid and its various analogs and amino acids such as glutamic acid, alanine, and methionine stimulated subscession and simultaneously accelerated ethylene evolution on cotton explant.

The fact that auxin increased ethylene evolution was confirmed by Abeles and Rubinstein (12), and Morgan and Hall (230, 231) by using a more precise analytical technique, gas chromatography. Schneider (281) also reported that NAA spray caused thinning of apple fruits and also increased ethylene evolution.

Later, extended studies by others indicated that auxin-induced ethylene production also played a role in abscission (12), inhibition of flowering in Xanthium (2), inhibition of stem elongation (46, 47), inhibition of root elongation (59, 60), isocoumarin formation in carrots (61), fading of orchid flowers (50, 86), bean hypocotyl hook opening (166, 167), swelling of onion leaf bases (188), induction of phenylalanine ammonium lyase in parsnip root (260), changing the sex of flower in cucurbits (287), and latex flow in rubber tree (78).

Auxin did not affect ethylene production in mature fruits of apple, pear, and tomato (12). They suggested that the absence of an effect on mature fruits might be due to the fact that the potential for ethylene production in these tissues was already fully expressed and limited by factors not under hormonal control.
Morgan and Hall (230) found that ethylene treatment increased both IAA oxidase and peroxidase activity of extracts from ethylene fumigated plants. Schwertner and Morgan (283) supported their idea (230) with the observation that auxin oxidase cofactors (monophenols) speeded abscission.

Induction of synthesis of new RNA and protein by exogenously applied IAA has been shown in a variety of plant tissue. Applied IAA had induced RNA and protein synthesis in Rhoeo leaves (270), green pea stem sections (80), bean endocarp (270), and oat coleoptile section (213).

In a study of the effect of ethylene on the auxin synthesis, Valdovinos et al. (323) found that tryptophan conversion to IAA in Coleus was inhibited by treating plants with ethylene and concluded that ethylene regulated auxin levels by controlling the activity of auxin biosynthesis.

Kang et al. (165) studied the relationship between the rate of ethylene production and the internal levels of auxin in apical and sub-apical portions of pea seedlings. They observed that a rapid rise in ethylene production caused by IAA was followed by a gradual decline and return to pretreatment levels after 24 hours. These results suggested that ethylene production required continuous presence of auxin in a physiologically active concentration. Evidence reviewed by Abeles (4) indicated that the increase in ethylene production induced by auxin was due to an increase in enzymes. These enzymes were associated with the pathway from which ethylene was formed from the precursor, methionine.
Auxin: Regulation of Abscission

Auxin can inhibit and promote abscission depending on the concentration used or on the time of application. With the fact that auxin promoted ethylene production in mind, the dual action of auxin could be resolved. The promotive effects of auxin on abscission could be invariably explained by the presence of increased levels of ethylene under conditions where auxin was unable to get to the separation layer in sufficient time or concentration to delay senescence (12, 267).

Hanson (135) demonstrated that 2,4-D enhanced ripening of pears. LaRue (181) and Shoji et al. (289) demonstrated the delaying effect of auxin on the leaf abscission. Marth and Mitchell's (211) initial work demonstrated that 2,4-D both promoted and inhibited ripening.

Auxin and other similar compounds are known to inhibit the ability of ethylene to promote degreening and abscission of leaves (4). Similarly, auxin has been shown to prevent degreening and softening of banana (211, 328, 329, 331).

Vendrell (329) demonstrated that the ripening of banana was delayed when auxin was infiltrated into the tissue but not when applied as a dip. He explained that the advancement in ripening when the whole fruit was dipped was attributed to the uneven distribution of auxin which caused a localized production of ethylene. Ripening in the peel which contained a high level of auxin was delayed while ethylene diffusion from these cells triggered ripening in the surrounding pulp. Beyer's (31) evidence showed that ethylene functioned in abscission by reducing the transport of auxin from the leaf to the abscission zone.
**Gibberellins**

Kurosawa (178) studied the bakanac disease of rice plants caused by fungus *Gibberella fugikuroi* which caused a characteristic excessive growth in the rice plant. He found that it was the extract from the fungus causing the stimulation. In 1935, Yabuta (354) crystallized the compound and named it gibberellin (GA).

The ability of GA to stimulate plant growth has attracted the attention of many physiologists and has also led to the classification of the molecular structures of several gibberellins (75).

Phinney and West (250) defined gibberellins as substances possessing the same carbon skeleton as gibberellin-A3 (gibberellic acid) or one very closely related to it and biologically active in stimulating cell division, cell elongation, or both, in plants.

GA can be detected in many plant parts and in various stages of growth. Wheeler (340) reported that GA might be present in the early stages of seed germination. GA has been reported to appear in several types of fruits and its content correlated with growth rates in some instances (237). Substantial amounts of GA might be found in roots (54), seeds during seed enlargement (23), and seed maturation (23, 66), and leaves, but its regulatory role over the growth of roots or leaves seemed doubtful (56). GA is known to have general occurrence in the stem and its important role in the regulation of growth has been reported (83).

The detailed pathway for the biosynthesis of gibberellin from mevalonic acid had been described by West and Fall (338, 339).

McComb (216) reported that GA moved in the same pattern as the carbohydrate-translocation system and with similar velocity.
An experiment with radioactive GA by Jacob and Kaldewey (160) has provided convincing evidence that the movement of GA in the plant is without polarity.

Morgan and Mees (234) found that yields of tomatoes, peas, and runner beans were not increased when plants were sprayed with GA during the period of early fruit set, in spite of a marked increase in vegetative growth. Yields of root crops--potatoes, turnips, and carrots--were decreased.

The application of gibberellin to three cultivars of snapbeans, had no effect on total yield but gibberellin applied at flowering increased parthenocarpic fruit set (351).

Application of 10 and 50 ppm gibberellic acid resulted in 1.5 to 2 times more fruits harvested from treated tomato plants (121). Courter and Drinkwater (68) reported that GA reduced total yields of field grown tomato and reduced early yields even to a greater extent.

Gibberellin applied at bloom or shortly after bloom resulted in increased berry set of seedless grape, *Vitis vinifera*, increased size and looser cluster (334). Hull and Lewis (153) reported that post-bloom sprays of GA on bearing cherry and peach trees had no effect on yield in the year studied or the following year.

GA sprayed at rates of 3 to 10 ppm on lettuce plants at the four- and eight-leaf stages of growth significantly increased seed yield (136), but Wittwer et al. (351) reported that lettuce and cabbage treated with gibberellin had no increase in seed yield.

Smith (296) reported that three autumn applications with 10 ppm GA to strawberry plants showed no significant difference in yield between the GA-treated and the control, but higher dosages reduced
yield. In 1961, Smith et al. (297) confirmed that higher concentration of GA caused the reduction in yield.

Application of gibberellin to seed potatoes increased the number of stems, but did not increase yield (294). Guzman (125) reported that gibberellin treated 'Red Lasoda' showed significant increase both in sprouting and in yield. Hawthorne (139) reported that gibberellin had no effect on tuber yield, but there was a reduction in tuber size.

Pecan seeds soaked in 5,000 ppm GA resulted in earlier germination (84, 341) and higher germination percentage (341).

Pecan fruits and foliage were sprayed with a 200 ppm solution of potassium gibberellate either early (June 1) or late (August 8). The sprays were applied one, two, or three times. The late application of GA to fruits and foliage of pecan trees led to no increase in the volume of nuts (303). Shuck weight increased significantly with increasing potassium gibberellate concentration to 400 ppm. Maturity was delayed and the shell weight and kernel weight were increased. However, neither the appearance nor the flavor of the kernel appeared to be affected by potassium gibberellate application.

Taylor (312) and Dhom (84) showed that application of GA3-lanolin mixture to the base of pecan seedling trunks stimulated cambium growth and produced significant increase in trunk diameter. Such increased trunk diameter allowed early grafting of the seedlings. Dhom (84) also reported that foliar spray of GA up to 500 ppm increased growth of pecan seedlings, but did not increase trunk diameter significantly as to allow early grafting.

Bora and Selman (36) reported that foliar application of GA resulted in the best plant growth on tomato. They indicated that GA
might have the capacity to increase the translocation of carbohydrate from leaf to stem. They also suggested that the effect of GA to stimulate nutrient uptake and accumulation might be achieved in part through a stimulation of RNA and protein synthesis which was believed to be directly concerned with ion accumulation by roots, and in part, by increasing the rate of transport of ions.

Hubbell (152) reported that GA significantly reduced the weight of individual 'Pelican' tomato fruit. This reduction in weight caused by GA was in opposition to increase in weight reported by Courter and Drinkwater (68). He also reported that GA delayed harvesting of tomato fruits significantly, reduced the number of tomato, egg plant, and bell pepper fruits, but increased the height of bell pepper and tomato.

GA sprays increased green color on the fruit of 'Valencia' orange and reduced creasing (100). GA increased the number of sprouts per root piece, promoted the growth of enlarged root pieces with few storage roots, but did not influence the percentage of sprout root pieces of sweet potato (318).

Wills (350) reported that preharvest application of GA as spray reduced the incidence of storage breakdown of apple.

Applied GA stimulated cell division in the cambium of woody shoots, but there was little cell expansion, although GA increased cell expansion in xylem fibers in the presence of IAA (85).

Badr et al. (21) reported that endogenous gibberellin acid level was the limiting factor for xylem development and they suggested that a certain balance between auxin and gibberellin was required to stimulate cambial activity with subsequent xylem development. In addition to stimulation of xylem development in olive shoots, they also
demonstrated that GA stimulated cell division and enlargement in the hypodermal cell layers.

McCune and Galston (217) reported that normal and dwarf types of pea and corn treated with gibberellic acid showed the reversal of the dwarf growth and high peroxidase enzyme activity. They suggested that GA, following its application, might in some ways, reduce the formation of excess peroxidase by nana types, thus sparing IAA for normal growth.

Zwar and Jacobsen (359) reported that GA stimulated the incorporation of labeled uridine and adenosine into all species of RNA in both isolated aleurone layers and layers attached to the endosperm. They suggested that GA stimulated RNA synthesis.

Evidence presented by Leopold and Kriedeman (185) indicated that GA appeared to involve an alteration of nucleic acid-directed protein synthesis in some longer-term regulatory actions, but also to involve some other types of activation phenomenon in short-term regulatory actions.

GA retarded maturity of citrus, deferred senescence in leaves, caused a reversal of adult type shoot growth to thorny juvenile type growth (65, 106, 284).

Kahn et al. (164) reported that light requirement for germination of lettuce seeds could be substituted by GA.

Fruit ripening could be altered by GA application (63). Jackson and Coombe (159) found that the rate of fruit growth in some instances was under the control of endogenous GA levels.

In the study of growth responses of wheat coleoptiles to added GA, auxin, and cytokinin, Wright (352) defined distinctive time of responsiveness to the three hormones. GA responsiveness appeared
first in the ontogeny of the coleoptile, followed by cytokinin responsiveness, and then, during the period of maximal cell enlargement, auxin responsiveness. He suggested that these hormones regulated separate aspects of growth and that they were not limiting at the same time during the growth of coleoptiles.

The inhibitory influence of growth retardants such Phosphon D on stem elongation in bean could be entirely counteracted by GA₃ (197). Growth retardants retarded plant growth by inhibiting the biosynthesis of GA (180).

**Gibberellin: Ethylene**

A relatively small but promotive effect on ethylene production by gibberellin was observed in bean, and bean seedlings (12), citrus explant (189), orange fruits and leaves (65), and peanut seeds (172). GA delayed the ripening of tomato and banana by antagonizing the effect of ethylene (89, 329). Fuchs and Lieberman (110) showed that the action of GA was severely interfered by ethylene, but was not completely suppressed in the bean seedlings.

GA had no effect on ethylene production by pea seedlings (110), and rape seeds (311).

Cooper et al. (65) reported that citrus trees treated with GA evolved adequate amounts of ethylene to induce the abscission of old leaves but not the fruits. In contrast, the fruits on GA treated trees developed a green color and showed less fruit drop than the control trees.

GA had also been found to cause a 50% reduction of ethylene production induced by 2,4-D in soybean seedlings (149), and citrus
fruits (189).

Scott and Leopold (284) reported that ethylene could interfere with the gibberellin stimulation of growth, delay of senescence, and a part of the stimulation of α-amylase production in barley. However, GA and ethylene were also reported to have roughly similar rather than antagonistic action in breaking lettuce seed dormancy (40) and stimulating rice seedling growth (309).

The ability of GA to overcome dwarfing of some plant species, to enhance stem elongation by cell division, or by cell enlargement, or by both, to overcome the dormancy of some seeds, to induce flowering of some photoperiod-sensitive and cold requiring species, to regulate the sex of flowers, and to delay leaf senescence or enhance the development of leaf senescence has been reviewed by Leopold and Kriedemann (185).

Cytokinins

In the search for compounds that would induce cells to multiply, Miller et al. (223) isolated a compound from yeast DNA in 1955. This compound was kinetin (6-furfurylamino-purine). In 1965, Skoog et al. (292) designated all compounds with biological activity similar to that of kinetin in enhancing cell division as cytokinins.

In the study of the chemistry and physiology of kinetin-like compound, Letham (186) reported that substances having cytokinin activity had been extracted from numerous species of higher plants and in most cases, the actively dividing tissues of the plant had proven to be the best sources.

Actually, kinetin is formed from deoxyadenosine, a degradation
product of DNA and cannot be considered as a natural product of plant biosynthesis (130). The naturally occurring cytokinin is zeatin isolated from immature maize seeds (186).

The roots may be a major source of cytokinins since cytokinin has been detected in the xylem sap of a number of plants (51, 163, 348). Apical portions of young sunflower (336), and pea (290) roots contained considerably higher cytokinin activity than did proximal portions. These observations supported the hypothesis (51, 163, 348) that roots are the major source of cytokinin.

Although not as extensively studied as in roots, cytokinin activity has been found in leaves, buds, and shoot apices of tree species (101, 109). Steward and Shantz (307) showed that embryo and young fruits had been commonest sources of cytokinins in plant extract. Cytokinins are widespread in plants as components of tRNA as well as free hormonal substances (185).

Some data from Miller (222) indicated that cytokinin levels rose during the early development of corn kernel and then dropped again as the fruit matured.

With the discovery of the action of cytokinins on the delay of Xanthium leaf senescence by Richmond and Lang (262), Mothers (236) found that localized applications of cytokinins to leaves could lead to the mobilization of various nutrients. This mobilization effect of cytokinins was probably the major function in leaf senescence, bud growth (316), apical dominance, and growth of fruits (335).

Cytokinin was able to stimulate cotyledon enlargement of lettuce seed (154), enlargement of radish cotyledons (187) and Xanthium cotyledons (102). The ability of cytokinin to stimulate these tissue
enlargement is characteristic for the bioassay of cytokinin activity. Using senescing tissue of Xanthium for the bioassay of kinetin and kinins, Osborne and McCalla (243) observed that kinetin increased chlorophyll content. This increase in chlorophyll content by kinetin was supported by Stetler and Laetsch (306) in their study on cultures of tobacco tissue. They found that cultures of tobacco callus became green in the light if cytokinin was supplied. They also found that the conversion of protoplasts into chloroplasts with grana was specifically stimulated by cytokinin.

Cytokinin could stimulate the growth of apple axillary buds when applied a month or so before spring growth was initiated and would overcome apical dominance in actively growing apple shoot (348) and in other species (27, 251, 273).

Torrey (320) reported that cytokinin could stimulate nuclear DNA doubling and mitosis, a property it shared with auxin and gibberellin. He indicated that its ability to stimulate cytokinesis was thought to be exclusive with cytokinins. Fittler and Hall (105) showed that the presence of the cytokinin adjacent to the anticodon was essential for tRNA functioning in protein synthesis, but they doubted its regulatory action in this manner.

Some workers (108, 293) reported that the tendency for auxin to inhibit cytokinin action on bud initiation ordinarily imposed a requirement for a balance between the auxin and cytokinin levels to achieve the differentiation of buds. Cytokinin regulated bud growth by suppressing apical dominance induced by IAA (316). Miller (221) also reported that buds could be induced by cytokinin in callus tissue, roots, cotyledons or stem pieces of tobacco.
In contrast to the promotions of stem growth by auxins and GAs, cytokinins ordinarily inhibited elongation of stem sections (108), stimulated leaf enlargement (220), and growth of radish root by swelling (199).

Letham (186) reported that cytokinins reversed the dormancy-inducing effects of auxins and naturally occurring growth inhibitors such as abscisic acid. William and Billingsley (347) reported that cytokinin and gibberellin applied to dormant buds on young apple trees significantly increased the number of growing buds and the angle between the main trunk and the new shoot. Cytokinin-stimulated cell division was probably responsible for the wide crotch angle observed. They also reported that growth on cytokinin plus GA treated trees was almost double that of the control.

Cytokinins were very effective in deferring senescence (244, 262) as well as abscission (244). Sitton et al (291) found that normal development of senescence might be expected to be correlated with a depression of cytokinin supplied by the roots of sunflower plants and they also found that cytokinin produced in the fruit did not have such strong systemic effect in the plant as that supplied by the root.

**Cytokinin: Ethylene**

Increased ethylene production induced by treatment with cytokinins has been observed in many experiments on plants. Plants including bean (7), blueberry (107), radish (255), sorghum (246), banana (331), and pea (110) showed a positive response of ethylene production to cytokinins.

In investigations of the effect of cytokinin-induced ethylene
production, several workers (48, 110) found that cytokinins enhanced the ability of auxin to increase ethylene production, and caused the auxin effect to persist for a longer period of time. Burg and Burg (48) reported that the effect of cytokinins on auxin-induced ethylene production was synergistic since the amount of ethylene produced was greater than the quantities produced by each treatment singly.

Data from several workers (48, 110) suggested that cytokinin might interfere with auxin degradation mechanism. However, little or no interaction was observed when gibberellin and cytokinin were added together (48).

Burg and Burg (48) also reported that kinetin could promote bud growth in the presence of ethylene by reversing the inhibiting action of ethylene on the growth of pea buds.

Fuchs and Lieberman (110) in 1968 reported that the ability of ethylene production to respond to cytokinin in pea plant varied from one part of the seedling to another. Kinetin caused a four-fold increase in ethylene production from pea plumules, but only a two-fold increase from radicles. However, this increase in ethylene production caused by cytokinin could be blocked by an inhibitor of protein synthesis, cycloheximide. They also provided evidence that kinetin-induced ethylene production occurred by the same pathway used normally under conditions of auxin induction which came from observations that methionine enhanced the activity of cytokinin and methionine itself had no direct effect.

Kinetin was able to block ethylene induction of B-1,3-glucanase (8).

Foliar sprays containing 200 to 500 ppm synthetic cytokinin,
ACCEL (Adenine, N-benzyl-9-(tetrahydro-2H-pyran-2-yl-) effectively stimulated the production of new lateral shoots on both intact and pinched carnations from which high quality cuttings and flowers resulted (157).

The effects of cytokinin on cell division, cell enlargement, root initiation and growth, shoot initiation and growth, and breaking of dormancy have also been reviewed by Devlin (83).

Abscisic Acid

Bennet-Clark and Kefferd (28) in a chromatographic study of growth substances in plant extracts found a β-inhibitor on paper chromatograms developed with a mixture of isopropanol:ammonia:water. Its structure was later determined and characterized by Ohkuma et al. (242) in 1965. This compound was named abscisin II.

Abscisic acid (ABA) was isolated from young cotton fruit (Gossypium hirsutum) by Addcott et al. (16).

Abscisic acid is an isoprenoid compound which serves as a suppressor of growth and shares the mevalonic acid-synthesis pathway with gibberellins and cytokinins (185). The synthesis of ABA takes place in the mature leaf and from there this hormone is readily transported to the shoot apex through petioles and stem tissue (249). ABA was also found in seed coats (176).

Some Effects of Abscisic Acid

Since the isolation and characterization of abscisic acid by Ohkuma et al. (242), a broad spectrum of biological activity of this compound has been found. Abscisic acid inhibited flower induction in the long day plant (Lolium temulentum) (103), lettuce seed germination
(275) and the growth of Avena coleoptile segments induced by IAA (258). Thomas et al. (317) found that ABA depressed GA stimulation of growth of dwarf corn and dwarf peas. Reynolds and Thompson (259) reported that inhibition of lettuce seed germination by ABA could be relieved by kinetin.

ABA counteracted gibberellin-induced α-amylase synthesis in isolated aleurone layers of barley (62, 161). Wareing and Phillips (332) reported that the application of ABA lowered the endogenous gibberellin level in maize seedlings, a condition which could be attributed to an inhibition by ABA of gibberellin synthesis.

ABA promoted petiole abscission in explants of cotton seedlings, but its promotion of abscission could be antagonized by IAA (332). Krogman (176) reported that ABA inhibited DNA and RNA synthesis in duckweed and blocked the synthesis of all classes of RNA in radish leaf discs. He suggested that ABA might act as a general gene repressor or might block the activity of RNA polymerase in chromatin.

Galston and Davies (111) reported that GA could overcome the inhibitory effect of ABA on the sprouting of potato buds, and on the elongation of genetically tall corn leaf sections. GA could also overcome the inhibitory effect of ABA on the germination of ash seeds (301).

Abscisic Acid: Ethylene

The advent of abscission was associated with a drop in auxin content not only in leaves, but also in fruits (207) and stem (114). The phenomenon of abscission in plants was influenced by several environmental factors, for example, temperature, moisture, mineral supply and possibly others. These environmental factors appeared to
act through their effect on the synthesis, utilization and/or depletion of nutrient substrates and hormones within the plant (14).

ABA has been shown to promote ethylene production by leaves (1, 71), and fruit (65), a non-specific effect shared by other compounds such as amino acid, potassium iodide (1), cycloheximide (6), ascorbic acid, indoleacetic acid (64), and defoliants (129). Several workers (1, 65, 71) noted that its ability to increase abscission was correlated with an increase in ethylene production.

ABA increased ethylene production (1, 65, 71) and ethylene regulated cellulase synthesis (151). It was thought that ethylene regulated cellulase synthesis by regulating the synthesis of specific messenger RNA and appropriate complementary transfer and ribosomal RNA (148). The ability of ethylene to promote abscission depended on the synthesis of RNA and protein (10).

The application of ABA as a foliar spray produced a measurable increase in ethylene production in apple shoots within 6 hours and in attached 'Early McIntosh' fruits (91). Mayak and Halevy (215) showed that exogenous application of ethylene to rose petals induced an increase in ABA activity. However, Abeles (4) indicated that the increase in ethylene production did not account for all of the ABA's activity because the presence of a saturating dose of ethylene did not completely mask the ability of ABA.

ABA accelerated abscission and senescence in a variety of plant species (20). Davis and Addicott (79) found a large increase in ABA in cotton fruits during the period of fruit dehiscence.

Cracker and Abeles (71) found that there was little or no effect of abscisic acid on senescence or aging of leaf tissue, but
there was an increase in the rate of cellulase synthesis.

Using synthetic substrates, Biggs (32) showed that cellulase was involved in citrus abscission and that they seemed to be correlated with the swelling characteristic of cell walls in the separation zones.

Abeles et al. (10) reported that cellulase induced by ethylene was localized in the separation layer and its synthesis was inhibited by IAA and cytokinins. They also showed that abscission cellulase was an endoenzyme which was capable of degrading modified cellulose.

For the cellulase to act in the process of abscission, it must first move from the site of synthesis, the cytoplasm, through the plasmalemma into its substrate, the cell wall (10). They suggested that ethylene controlled both the synthesis and the release or secretion of cellulase from the cytoplasm to the cell wall.

ABA suppressed IAA-induced ethylene synthesis (191) and also altered the permeability of the cell membrane (119).

Abeles and Rubinstein (12) reported that SADH (Succinic acid-2,2-dimethylhydraxide) was a unique growth regulator which did not induce ethylene evolution in plants. There were no anatomical or morphological changes in the abscission zone which would account for the delay in abscission of SADH treated fruit (95).

Davis and Addcott (79) also found that large increases in ABA in fruits during the period of fruit dehiscence.

Ethylene

In 1868, Girardin first reported that illuminating gas defoliated shade trees of a number of German cities. Nejubov in 1901, showed that ethylene was the active component of the illuminating gas (4).
In 1934, Gane (113) proved chemically that plants produced ethylene, thereby providing evidence that ethylene was a plant hormone. Methionine was the major if not the sole natural precursor of ethylene in higher plant tissues (191).

Some Growth and Developmental Effects of Ethylene

Ethylene stimulated peach pollen germination and pollen tube growth (39, 276), germination of lettuce seeds (11), peanut and witchweed (99), and increased bud development (33, 72, 129, 233).

Ethylene promoted sprouting of gladiolus corms (322), tuber development at the ends of stolons of potato sprouts (57), bulb formation in onion and leek under non-inductive photoperiod (188).

A number of workers (356, 357) had reported that ethylene retarded elongation growth of many species. Inhibition of oat coleoptile elongation was concurrent with lateral expansion. It was suggested that coleoptile cells were capable of attaining a finite volume by the lateral expansion induced by ethylene and at the expense of longitudinal expansion (210).

Roberts (266) reported that inhibition of cell elongation induced by ethylene as an inhibition of cellulase metabolism was ruled out since ethylene had no effect on water uptake, respiration or protein metabolism. The ability of ethylene to control cell elongation was less effective in the light-grown tissue than in the dark-grown tissue (47, 120).

Ethylene could cause the root initiation from stem, leaves, flower stem, and preexisting roots of a variety of plants (356). Roots were formed in the region of elongation in tobacco and hydrangea,
generally over the whole stem in coleus and tomato plants, only around
the nodes in *Cosmos sulphureus*. Prop roots were formed on the first
five nodes above the ground when popcorn plants were treated with
ethylene. Ethylene induced rooting from the leaves of tomato, cosmos,
marigold, and heliotrope. These roots were usually initiated from the
midrib and from veins with increasing exposure. However, the youngest
and the oldest leaves never formed roots (4).

Ethylene increased the formation of root hairs on *Pisum sativum*
(59), and barley (298). Ethrel was used to increase rooting of apple
seedlings (76), and blueberry (171).

Increased ethylene production by plants treated with 2,4-D led
to the use of Ethrel as latex flow promoters. Leopold (184)
reported that ethylene deferred latex coagulation by preventing
flocculation of rubber particles with lutoids, thus permitting larger
flow rates for prolonged periods of time.

Ethylene promoted the changing of the sex of developing flowers
of cucurbits (69, 116, 206, 304). Melons and gourds (206), squash (69),
pumpkins (304), and cucumber (69) were induced to form female flowers.

Many workers (120, 146, 219, 356) showed that ethylene inhibited
leaf expansion of many plants. Burg et al. (43) explained that the
prevention of leaf expansion was due to the inhibition of cell division.

Ethylene induced swelling of stems (4). The swelling of tissue
induced by auxin was due to an increase in ethylene production (60, 147).
Swelling of the bean hypocotyl was due to an expansion of cortical
cells.

Plants were shown to give epinastic response after ethylene
treatment (73, 74, 356), and treatment with compounds that promoted
ethylene production (73, 74, 358).

Crocker et al. (74) reported that the ability of ethylene to cause leaf curvature varied from species to species, but also from one variety to another. They also demonstrated that tomato petiole curvature was due to the swelling of the upper cells of the basal end of the petiole.

Crocker et al. (73) showed that epinasty resulted as an action of auxin that promoted ethylene. Ethylene altered auxin transport patterns in the petiole and epinasty was the result of an inhibition of lateral auxin transport to lower side of the petiole. The upper portion of the petiole contained more auxin after the plant had been treated with ethylene (208).

In 1972, Leather et al. (182) showed that petiole explants consisting of the basal portion of the tomato petiole with subtending stem tissue curved to a greater extent when auxin was applied to the cut surfaces of the petiole tissue.

Ethylene inhibited the hypocotyl hook opening of the mono- and di-cotyledonous seedlings. It was due to an inhibition of cell elongation on the inside of the hook (4). Abeles (4) also reported auxin increased ethylene production and simultaneously promoted hook closure.

Evidence presented by Burg and Burg (48) indicated that ethylene did not regulate dormancy by growth suppression.

Ethylene had little or no effect on growth of isolated coleoptile segments. However, it enhanced the growth induced by auxin (156). Maxie and Crane (214) reported that ethylene inhibited the growth of figs during the cell division stage and promoted growth
during growth and maturation stage.

Stem growth inhibition was not due to the effect of ethylene on stretchability of the tissue (235).

Ethylene was also reported to have no effect on \(^{14}\text{C}\)-glucose incorporation into cell wall material, total RNA content, incorporation of ATP into RNA, uptake of AA, tryptophan, glucose, proline, leucine, ATP, arabinose, thymidine and permeability of tissue to tritiated IAA (21, 43).

Ridge and Osborne (264) observed that there was an increase in cellulase in auxin-treated tissue and there was no change in cellulase when identical swellings were induced with ethylene. They suggested that cellulase did not play a role in cell wall modification during the swelling process.

**Ethylene: Abscission**

Many workers have conducted studies on ethylene effect on membrane permeability and abscission. Ethylene was reported to have no influence on membrane permeability of banana, bean, avocado, and Rhoeo (272). On the other hand, many workers (37, 42, 49, 272) reported that ethylene caused changes in membrane characteristics.

Ethylene is more stable in oil than in water and membranes contain large quantities of lipids, thus the idea that ethylene has a disruptive effect on membranes which causes a change in permeability and alteration of compartmentalization has been investigated by a number of workers and does appear to be valid. Ripening fruits exhibited obvious changes in term of permeability and retention of soluble components. It appeared more likely that ethylene caused
changes in membrane permeability which in turn led to softening and increased respiration (4). However, evidence from many workers (37, 42, 49) suggested that changes in membrane characteristics were the result of ripening rather than a cause.

Studies to determine whether ethylene increases or controls the rate of enzyme secretion or release from cells has been investigated by a number of workers. Jones (168) reported that ethylene increased not solely the release of α-amylase from barley half-seeds, but also inhibited the amount of α-amylase synthesized by the half-seeds when no gibberellic acid was present. However, high concentrations of ethylene had an inhibitory effect on enzyme secretion.

Horton and Osborne (151) found that ethylene increased the synthesis of cellulase, an enzyme that was responsible for the dissolution of cell walls.

In a study of the regulation of peroxidase activity in pea stem tissue, Ridge and Osborne (264) reported that ethylene inhibited leakage of peroxidase activity from apical tissue and, to a lesser extent basal tissue.

The effect of ethylene on other enzymes such as protease, invertase, catalase, reductase etc. has been reported and reviewed by Abeles (4).

De Le Fuente and Leopold (81) reported that following a short lag, ethylene reduced the breakstrength of abscission-zone explants, and the removal of ethylene by flushing the gas phase prevented further reduction in breakstrength.

In the study of the control of breakstrength by ethylene, Abeles and Leather (9) found that cellulase followed the addition of ethylene
but levels of cellulase remained constant after ethylene gas was removed. This finding indicated that ethylene had another effect in addition to the regulation of enzyme synthesis. Reviewed evidence (4) indicated that this additional effect was the control of cellulase secretion and its movement and that this accounted for the maintenance of break-strength once ethylene was removed.

Some Interaction Effects Between Growth Regulators

Review from Leopold and Kriedemann (185) indicated that GA and auxin both acted independently and together, depending upon the species of plant, the conditions under which the plant was growing, and the type of response being measured.

Edgerton (90) reported that auxin applied with Ethrel counteracted the promotion of abscission induced by Ethrel. Cooper et al. (65) in their study with abscisic acid on citrus, showed that an increased ethylene evolution in calamondin fruits and leaves was observed. Leopold and Kriedemann (185) reported that ethylene also exerted an inhibitory effect against some gibberellin actions.

ABA could not be considered as a GA antagonist in view of the fact that its inhibitory effects applied equally well to growth stimulations by auxin, gibberellin, or cytokinin (183).

Halevy (126) found that there was a reduction in the production of ethylene in cucumbers following treatment of SADH.

Some Theories Related to Abscission and Senescence

Hall (128) reported that foliar abscission was regulated by an auxin-ethylene balance. He showed that ethylene was a natural regulator of abscission, working in a balance against auxin.
Using debladed leaf petioles to study the physiology of abscission, Addicott and Lynch (18) demonstrated that the most important factor controlling abscission is the condition of the auxin gradient across the abscission zone. Auxin and the establishment of auxin-gradient across the abscission zone (18) were not the only factors controlling abscission however. It has been shown that leaf abscission in cotton explants was accelerated by a natural growth-inhibiting hormone, abscisic acid (19). In addition to this, a rise in ABA level has also been reported during the senescence of nasturtium leaves (249), and during the period of fruit dehiscence (79).

Abscission of leaves, flowers, and fruits has been shown to be influenced strongly by auxin and other hormones as well (15).

Wheat coleoptile straight growth bioassays of extracts from pecan fruits established the presence of growth promoting and inhibiting substances. Indoleacetic acid and gibberellin were believed to be among the growth promoting substances. A growth inhibiting substance, possibly abscisic acid, promoted the abscission of cotton cotyledonary node explants. The absolute level of growth substances did not appear to be correlated with the seasonal shedding pattern of fruits (196).

The findings of Addicott (15) and Lipe and Morgan (194) supported the concept of endogenous balance of growth hormones in regulation of abscission.

Lipe and Morgan (192, 194) proposed that ethylene was the hormonal regulator of the dehiscence of pecan shuck.
Alar (2,2-dimethylhydrazide succinic acid) or SADH

Alar, also known as B-995 or B-nine, was discovered in the Agricultural Chemical Laboratories of the UniRoyal Chemical Division of UniRoyal, Inc. It was originally classified as a growth retardant because it suppressed stem elongation. Due to its great variety of responses, it was later called a growth regulator (226).

Edgerton and Hoffman (94) showed that there was an important relationship between potassium gibberellate (KGA) and growth retardant, Alar. The growth response of apple shoots to combinations of Alar and GA had indicated that Alar blocked the action of GA. The application of Alar at 1,000 ppm with KGA at 200 ppm reduced the length of the growth of the shoots to less than 50% of the growth made by shoots treated with GA alone, but influence on diameter was not significant. Treatment with Alar at 1,000 ppm counteracted the stimulus of a high KGA concentration of 500 ppm and reduced the length growth of shoots to 70% of that made by shoots treated with KGA alone while the diameter growth of shoots was actually greater.

Drop prevention or delay in fruit abscission indicated that B-995 might stimulate the action of a growth promoter or auxin in controlling the abscission and preharvest drop of matured apple fruits. Changes occurred in the abscission zone which might inhibit separation of fruit pedicel from spur beyond the stage of maturity characteristic of the control fruits at abscission.

Application of SADH increased skin color of peaches, thus resulting in more uniformly colored fruits (52) and SADH treated peaches matured early (53, 54) and abscised more readily from the stem and left less fruits remaining on the tree when harvested.
mechanically (112).

Storey et al (308) reported that 4,000 ppm Alar reduced growth, increased bud forcing, precociousness, growth density, leaf color, and yield of pecan nuts but delayed maturity and reduced the quality so that there was no difference in value between the yield and quality.

Alar was reported to be quite mobile as an intact compound even after long periods of metabolism (212). There was some translocation of Alar from the treated branches to the adjoining branches and also from the surface of the fruit to the flesh and seeds of apple (93). However, there appeared to be little or no translocation of SADH from treated to untreated branches during the growing season (282). SADH at 2,000 ppm spray had controlled fruit drop and upper shoot growth, reduced fruit size and increased firmness (137). Based on evidence of SADH translocation in apple trees (93, 282), Hatch (137) suggested that SADH controlled growth and development by changing the hormone balance(s) in the tree.

Results of numerous in vitro and in vivo experiments using different plant species indicated that SADH inhibited gibberellin synthesis (24, 197, 345) but Staby (305) reported that SADH showed little or no inhibitory effects on the biosynthesis of neutral terpenes including gibberellin precursors regardless of the concentrations used or the test system used. SADH should be considered as anti-metabolite and not 'anti-gibberelin' (305).

Knapp et al. (174) suggested that SADH treatment possibly increased proteolysis leading to degradation of o-Diphenol. Murr and Morris (238), using in vitro tests, indicated that SADH competed with proline for quinones produced by enzymatic or non-enzymatic oxidation
of o-Diphenols. These observations (238) proposed that in vivo SADH exerted a dual effect in reducing mushroom discoloration first by inducing degradation of o-Diphenol oxidase through an increase in proteolytic activity and second by binding quinones thereby removing intermediates which led to pigment formation.

Regulation of Ethylene Production by Alar

Abeles and Rubinstein (12) reported that N-dimethyamino succinamic acid (Alar) reduced ethylene production and growth of bean seedlings by 50% when Alar was applied as soil drench.

A number of investigators have shown that Alar delays harvest date, ethylene peak, and decreases post-peak and ethanol content after 21.5°C holding period (35).

Forsyth and Hall (107) reported that they failed to observe an effect of Alar on ethylene production by lowbush blueberry flowers, and Alar did not affect auxin transport and quality.

Observations (4, 200, 201, 202, 261) showed that Alar suppressed ethylene production and the ripening of apple fruits.

Alar delayed the respiratory climacteric (202) and might account for the delay in fruit abscission (202). Inhibition of the climacteric by B-nine was overcome by exogenous ethylene but latter treatment failed to completely reverse the B-nine inhibition of endogenous ethylene production. This might suggest that B-nine possibly interfered with endogenous auxin or GA levels (200, 201) because auxin is known to promote fruit ripening and ethylene production (42).

Application of SADH 50 days before harvest in combination with application of either NAA or 2,4,5-T two weeks before harvest totally
eliminated the abscission-inducing effects of Ethephon (202). SADH treatments were more effective than 2,4,5-T treatment in reducing accumulation of ethylene in stored fruits of 'McIntosh' apple (252).

Abeles and Rubinstein (12) and Looney (200, 201) summarized research by others showing that plants treated with Alar usually contained less auxin. Since endogenous auxin levels can control ethylene production, they proposed that reduction in ethylene production following application of growth retardant (including Alar) was an indirect result of reduced auxin level (4).
MATERIALS AND METHODS

This research consisted of five parts and involved studies on:

I. Levels of endogenous growth promoters and inhibitors that regulate natural pecan shuck opening.

II. Regulation of abnormal shuck opening of pecan (pecan shuck disease) by hormones.

III. Effects of gibberellic acid, Alar, and their combinations on the occurrence of abnormal shuck opening of pecan and kernel yield.

IV. Effects of IAA, GA, PBA (synthetic cytokinin, ACCEL or adenine, N-benzyl-9-(tetrahydro-2-H-pyran-2-yl), and their combinations on the occurrence of abnormal shuck opening of pecan and kernel yield.

V. Effects of kinetin, and combinations of kinetin and Alar on the occurrence of abnormal shuck opening of pecan and kernel yield.

Study I: Endogenous growth promoters and inhibitors

Extraction

The procedure for determining the endogenous levels of the growth hormones in the pecan shuck was adopted and modified from Lipe et al. (196), and Loh (198).

Sampling of the pecan fruits at weekly intervals began when the fruits were 1 1/2 cm in diameter and 2 1/2 cm in length and terminated when the pecan fruits showed natural shuck opening. The tree for this study was located at the Ben Hur Farm, Louisiana Agricultural Experiment.
Station, Baton Rouge, Louisiana. Each fruit served as a sample which was collected from the 30 year-old 'Success' tree. Fruits were randomly selected. Sampling of fruits was in all instances carried out in the evening at 6:00 pm in order to avoid the influence of any diurnal change in the endogenous level of hormones. Immediately after the collection of samples, 2 gm of the fresh pecan shuck were cut from each fruit with the seed being removed. This was done to reduce the possibility of the food reserve stored in the seed affecting the extraction procedure when seed coat was not yet hardened. When the fruits became larger, a 5 gm sample was used.

Each sample was cut into small pieces and then extracted with 40 ml diethyl ether for 12 hours at 0°C. The extract was then filtered through glass wool and washed with three 10 ml aliquots of diethyl ether which was then combined with the original extract. The combined ether extract was then extracted with three 20 ml aliquots of 0.5 M NaHCO₃. The ether fraction was dried with anhydrous sodium sulphate. This comprised the neutral/basic fraction of the extract.

Tannins which were present in the bicarbonate fraction were precipitated out with 10 ml of 20% lead subacetate (196). After precipitation, the bicarbonate solution which had a pH of 8.18 was then acidified with 10% hydrochloric acid to pH 2 to 3. The acidified bicarbonate solution was allowed to settle for 10 minutes without centrifugation. The supernatant recovered was then extracted with three 30 ml aliquots of diethyl ether and dried as before. This ether fraction comprised the acidic fraction of the extract.

The two ether extracts were concentrated to one ml under reduced pressure in a hot water-bath at a temperature of 38°C. A 0.3 ml
aliquot of the extract was taken to streak in a thin line across the Whatman No. 1 chromatographic paper strips, 2 cm wide, using a one ml tuberculin disposable syringe. Using the descending technique, the chromatograms were developed in n-butanol-ammonium hydroxide-water solvent (10:1:1, v/v/v) in chromatocab which was saturated with fumes of the same solvent. The solvent was allowed to descend for about 14 hours at temperature of 23°C. The paper was then removed and dried in the laboratory at a temperature of 25°C. Paper chromatograms not immediately used for bioassay were stored at -5°C in darkness.

Bioassay

The procedure for the bioassay of growth promoters and inhibitors present in the paper chromatograms was adopted from Hendershott and Walker (142), and Loh (198). The hull less oat seeds cv. Brighton (Avena sativa L.) for the bioassay of extracts from pecan shucks were obtained from Research Station, Research Branch, Agriculture Canada, University Campus, Saskatoon, Saskatchewan, Canada. The oat seeds were soaked in distilled water for two hours, and then seeded on moist four-layer tissue papers in germinating boxes. The seeds were subjected to red light treatment from a fluorescent tube wrapped with three layers of red cellulose acetate paper for 36 hours. This red light was used to suppress mesocotyl growth. After this, the seeds were allowed to germinate for another 36 hours in the dark at 20°C. Coleoptiles of 15 to 30 mm were selected. A three mm segment from the tip was removed. The next five mm segment was used for bioassay. The primary leaf was not removed from the coleoptile segments since unthreaded segments grew to a greater extent (30). Cutting of the coleoptile segments was
done with a Wightman cutter (225) under green light. After the cutting, the segments were soaked in distilled water for two to five hours to remove possible endogenous growth regulators.

Each paper chromatogram was divided into 10 equal parts of one inch each plus one part (one inch) away from the streaked origin which served as a control. Chromatogram segments were labeled 1 to 10 with 1 having closest to point of origin and 10 being farthest from point of origin. These individual segments, including the control segment, were cut out and placed in individual 15 x 15 mm glass vials containing one ml of phosphate-citrate buffer with two percent sucrose added. The buffer solution which had a pH of 5.15, contained 1.798 g/liter $K_2$HPO$_4$ and 1.019 g/liter citric acid monohydrate.

Four coleoptile segments were selected at random and placed in each vial for bioassay. The vials containing the coleoptiles were allowed to incubate in the dark room at $20^\circ C$ for 24 hours. The lengths of the coleoptile segments were measured using a transparent and flexible ruler.

The average length of the four coleoptiles in each vial was expressed as a percentage of the average length of those in the control vial. Values greater than 100% indicated the presence of a growth promoter. Values smaller or less than 100% indicated the absence of a growth promoter or the presence of an inhibitor.

**Statistical Analysis**

The data obtained from the bioassays of the extracts from the 'Success' pecan shuck were analysed as a completely randomized design with a split plot arrangement of treatments. The time of sampling
(fruit stage) served as a main plot factor. Each time of sampling consisted of three replicates (fruits). Each replicate consisted of measurement of four coleoptile segments. The acid-neutral/basic fraction and chromatogram segments (Rf) served as subplot factors. Bioassay means were used in the statistical analysis.

Study II: Regulation of Abnormal Shuck Opening of Pecan

This study was initiated when an occurrence of the pecan shuck disease was observed on the 'Success' trees, located at Ben Hur Farm of Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana. The period of sampling was between 8/17/75 and 9/10/75. Fruits on the 'Success' tree were divided into two categories: healthy (non-diseased) and shuck-diseased fruits. Each fruit served as a sample. Samples were randomly collected from each category at the same time of day (6:00 pm) so that there was no variation of endogenous levels of hormones due to time of sampling.

Fruits collected were immediately extracted and the extracts were bioassayed in the same way as in Study I.

The data derived from bioassays of the 'Success' pecan shuck extracts were analysed as a completely randomized design with a split plot arrangement of treatments. Fruit category (non-diseased and shuck diseased) served as a main plot factor while the acidic-neutral/basic fraction and RF of chromatograms served as subplot factors. The main plot factor consisted of 10 replicates. Each replicate consisted of four observations. Bioassay means were used in the statistical analysis.
Study III. Effects of GA, Alar, and Their Combinations

This experiment involved the application of GA, Alar, and their combinations to the foliage and fruits of the 15 year-old 'Success' pecan trees located in the Little Eva Plantation at Chopin, Louisiana. Five branches were randomly selected from each tree, out of which four branches received the same chemical treatment while the fifth branch served as the unsprayed check. A check in each tree was necessary to reduce variation in the incidence of pecan shuck disease among trees. The trees were subjected to 3 different concentrations of 1,000 ml of GA, 2 different concentrations of 1,000 ml of Alar, and 1,000 ml of their various combinations. One unsprayed tree was used as a control. The concentration levels of GA were 200, 350, and 500 ppm. The concentration levels of Alar were 1,000 and 3,000 ppm. A 0.3% surfactant was added to the spray solutions. The percent of pecan shuck disease and the kernel yield were the variables measured. Two observations per branch were made for the score of pecan shuck disease. That is, one observation (80 fruits) was made on 9/27/75 and the other observation (20 fruits) was made on 10/4/75. Two observations (20 nuts each) per branch was made on the score of kernel yield in the same day (9/27/75). The mean data obtained were analyzed as a randomized block design with two replications (dates of spray: 7/26/75 and 8/5/75) by analysis of covariance.

Study IV. Effects of IAA, GA, PBA, and their combinations

This experiment involved the application of IAA, GA, PBA, and their combinations in the same manner as in Study III. The trees were subjected to two different concentrations of 1,000 ml of PBA, one
concentration of 1,000 ml of GA, one concentration of 1,000 ml of IAA, and 1,000 ml of each of their combinations. An unsprayed tree was used as a control. The concentration levels for IAA, and GA were 100 and 500 ppm, respectively. The concentration levels for PBA were 200 and 500 ppm. The chemicals were applied as sprays on 8/5/75.

Two observations were made on the score of pecan shuck disease and the score of kernel yield per branch as in Study III. The mean data were analyzed as a completely randomized design by the analysis of covariance.

Study V. Effects of Kinetin, and Combinations of Kinetin and Alar

This experiment involved the application of kinetin, and the various combinations of kinetin and Alar in the same manner as in Study III. The trees were subjected to two different concentrations of 1,000 ml of kinetin, and 1,000 ml of their combinations with Alar at 1,000 ppm and 3,000 ppm. An unsprayed tree was used as a control. The concentration levels for kinetin were 100 and 400 ppm. The chemicals were applied as sprays on 8/5/75.

Two observations were made on the score of pecan shuck disease and the score of kernel yield per branch as in Study III. The mean data were analyzed as a completely randomized design by analysis of covariance.

In Study III, IV, and V, fruits were harvested immediately after the first score (9/27/1975) on the incidence of pecan shuck disease. This was done not only for the evaluation of the effects of the growth substances on the incidence of pecan shuck disease and the kernel yield, but also for the evaluation of the correlation between the incidence
of pecan shuck disease and kernel yield.
RESULTS AND DISCUSSION

Ia. **Endogenous Growth Promoters and Inhibitors in Pecan Fruits**

The lengths of *Avena* coleoptile segments expressed as percent over the control were used in the statistical analysis. In the analysis of variance for the extracts of pecan fruits, cv. 'Success' in both acidic and neutral/basic fractions, highly significant differences were found among fruit stages (times of sampling), between acidic and neutral/basic fractions, and among the bioassay means of the 10 Rf values. Highly significant difference among fruit stages suggests that the extracts collected from fruit at various stages of fruit growth had different growth substances and different concentrations of these growth substances. Highly significant difference between the acidic and neutral/basic fractions suggests that there was a difference in the type of growth substances present in the acidic and the neutral/basic fraction of the other extracts (Appendix 1).

The highly significant difference in the Rf components suggests the presence of qualitatively different growth substances in the extracts, as represented by their Rf values, or also a quantitative difference within a qualitative entity could be involved in the acidic and neutral/basic fractions. Histograms showing growth activities obtained with *Avena* coleoptile straight-growth test of the acidic and neutral/basic fractions of other extracts from the pecan fruits are shown in Figures 1-24 (Tables 1 and 2)
For the interpretation of growth activities regulated by growth substances, it is assumed that a substance which promotes or inhibits the growth of coleoptiles, but appears at slightly different Rf on different chromatograms, is considered biologically the same substance. This assumption is reasonably justified in view of the fact that the Rf values of known pure substances like IAA, GA, and Indolepropionic acid vary from one chromatogram to another. It is further assumed that substances producing growth activity above 100% are growth promoters, and substances producing growth activity below or less than 100% are either growth inhibitors or a deficiency of growth substances exists.

During the period from 7/13/75 to 10/5/75, there were generally three zones of promotion detected on paper chromatograms in the acidic fraction by the biological assays (Figures 1-12). The first promoting zone appeared at Rf 0.1-0.2. Kefford (169) called this promoting substance an accelerator. Hendershott and Walker (141) and Loh (198), who reported the presence of a growth promoter at Rf 0.15 in n-butanol-ammonia-water solvent, thought it was indolepyruvic acid (IPyA). The presence of IPyA at this Rf was further supported by Zweig and Whitaker (360).

A second zone of promotion was detected at Rf 0.3 to 0.4. The growth activity at this Rf was probably due to indoleacetic acid (IAA). The findings here agreed with the results of Hendershott and Walker (141), Kefford (169), and Zweig and Whitaker (360), who reported that the substance at Rf 0.36 was IAA. This Rf value corresponded with that of pure indole-3-acetic acid which chromatographed in the same solvent.

The third zone of promotion was detected at Rf 0.8-0.9. The
growth substance at this Rf value was probably a gibberellin (GA) or a gibberellin-like compound. Heide (140) reported that gibberellin-like substances were detected at this range of Rf values. Pure GA₃, chromatographed in the same solvent, was at Rf 0.85. This Rf value was higher than that reported by Lipe et al. (196) at Rf 0.7 in ethanol: ammonium hydroxide:water (80:5:15) and at Rf 0.6 in isopropanol: ammonium hydroxide:water (8:1:1).

Except for the chromatogram obtained on 7/13/75, a zone of inhibition was detected throughout the growing season at Rf 0.5 to 0.7. The substance presented in this zone was first identified as inhibitor P by Bonnet-Clark and Kefferd (28). Later, the substance at this Rf value was identified as abscisic acid by Nitsch (239), Cornforth et al. (67), and Gazet and Blumenfeld (115). ABA was also found in a wide range of plants including potato shoots and tubers, pear fruit buds, grape seeds, apple leaves and peach seeds (169, 269) and pecan buds (198).

Another zone of inhibition was detected at Rf 1.0. This zone roughly corresponded to the zone (Rf 0.8-0.9) occupied by the inhibitor, naringenin (5,7,4'-trihydroxy flavanone) in the acidic fraction of the extracts of Loh (198). However, Hendershott and Walker (141) reported that naringenin could not be separated from sodium bicarbonate solution by ether. Therefore, it was unlikely to be naringenin. It was named here as inhibitor G for future discussion.

In a study of endogenous growth hormones in the regulation of pecan fruit shedding, Lipe et al. (196) fractioned the fruit extract only into the acidic fraction. They did not study and report on the neutral/basic fraction.
In the neutral/basic fraction (Figures 13-24), a zone of promotion was detected at Rf 0.1 to 0.2 and occasionally extended into Rf 0.6 (Figures 13, 14, 15, and 18) at the early stages of fruit growth. The nature of this growth promoting substance was not determined. No attempt was made to identify this substance. However, the growth promoting substance found at this Rf range in the neutral/basic fraction could be due to auxin carried over from the acidic fraction into the neutral/basic fraction, since auxin-promoting growth activity was high in the early stages of fruit growth. Nevertheless, more work is needed for the conclusive identification of this substance present in the neutral/basic fraction.

A distinct zone of inhibition from Rf 0.1 to 0.9 and possibly extending to Rf 1.0 was observed throughout the growing season from 8/2/75 to the period of natural shuck opening (Figures 16-24). Its peak of inhibition was located between Rf 0.4 to 0.8. According to Loh (198), this zone could be made up of phenolic substances (phenolic acids). He explained that in the extraction procedure, the pH of the sodium bicarbonate solution used to fractionate organic acids was 8.15 (the pH of sodium bicarbonate solution after precipitation of tannins with 20% lead subacetate was 8.18). In the presence of weak alkali, like sodium bicarbonate, strong plant acids would be fractionated into the acidic fraction. Weaker acids like phenolic acids would be fractionated into neutral/basic fractions. These compounds when dried and crystallized had a golden yellow color.
Table 1. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success at Rf values 0.1-1.0 from 7/15/75 to 10/5/75 at weekly interval. (Each value is the average of three replicates)

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Table 2. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors in the basic fraction of the ether extracts of non-diseased shuck of pecan fruit cv. Success at Rf values 0.1-1.0 from 7/13/75 to 10/5/75 at weekly interval. (Each value is the average of three replicates)

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Figure 1. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/13/75. (Each histogram represents the average of three replicates)
Figure 2. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/20/75. (Each histogram represents the average of the three replicates)
Figure 3. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at RF values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/27/75. (Each histogram represents the average of three replicates)
Figure 4. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/2/75. (Each histogram represents the average of three replicates)
Figure 5. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/10/75. (Each histogram represents the average of three replicates)
Figure 6. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/17/75. (Each histogram represents the average of three replicates)
Figure 7. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/23/75. (Each histogram represents the average of three replicates)
Figure 8. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/30/75. (Each histogram represents the average of three replicates)
Figure 9. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/6/75. (Each histogram represents the average of three replicates)
Figure 10. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/13/75. (Each histogram represents the average of three replicates)
Figure 11. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/20/75. (Each histogram represents the average of three replicates)
Figure 12. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 10/5/75. (Each histogram represents the average of three replicates)
Figure 13. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/13/75. (Each histogram represents the average of three replicates)
Figure 14. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/30/75. (Each histogram represents the average of three replicates)
Figure 15. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 7/27/75. (Each histogram represents the average of three replicates)
Figure 16. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/2/75. (Each histogram represents the average of three replicates)
Figure 17. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/10/75. (Each histogram represents the average of three replicates)
Figure 18. Responses of *Avena coleoptiles* to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/17/75. (Each histogram represents the average of three replicates)
Figure 19. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/23/75. (Each histogram represents the average of three replicates)
Figure 20. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 8/30/75. (Each histogram represents the average of three replicates)
Figure 21. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at RF values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/6/75. (Each histogram represents the average of three replicates)
Figure 22. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/13/75. (Each histogram represents the average of three replicates)
Figure 23. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 9/20/75. (Each histogram represents the average of three replicates)
Figure 24. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success sampled on 10/5/75. (Each histogram represents the average of three replicates)
Ib. Promoter/Inhibitor Balance in the Regulation of Shuck Opening

In the analysis of variance for the extracts of pecan fruits, cv. 'Success' in both acidic and neutral/basic fractions, there was a significant fraction x fruit stage interaction, RF x fruit stage interaction, and RF x fraction interaction. The significance of these interactions suggest that the difference between the acidic and neutral/basic fractions is not the same for the various stages of fruit growth; the difference among RFs is not the same for the various stages of fruit growth; and the difference among RFs is not the same for the acidic fraction as for the neutral/basic fraction. These further suggest that there was a variation in the growth substances during the fruit growing period.

Figures 25-29 show the fluctuation in quantity of free or physiologically active growth substances from the acidic fractions of pecan fruit extracts taken at weekly intervals from 7/13/75 to 10/5/75. Natural shuck opening was observed on 9/20/75 and 10/5/75, but the shedding of nuts had not occurred at these times (Figure 31). Figure 30 shows the fluctuation in quantity of free or physiologically active growth substances from the neutral/basic fractions of the pecan fruit extracts.

Figure 31 shows the possible relations of IAA, IPyA, ABA, GA, and inhibitor C. Though the levels of auxins (IAA and IPyA) fluctuated during the fruit growth period, they were generally present in the pecan shuck in reasonably high levels during the growth period of fruits until one week before the occurrence of natural shuck opening. Auxins (IAA and IPyA) disappeared at the time of shuck opening.

GA levels in the acidic fractions of extracts, to a certain
extent, depended on the levels of auxins (IAA and IPyA). It was found that GA or a gibberellin-like compound was absent from 7/20/75 to 7/27/75. It was generally known that GA or a gibberellin-like compound played an important role in the fruit development during the early stages (170). It is difficult to explain why this substance was absent since it appears logical that it should have been at a high level at this time. GA or the gibberellin-like compound tended to follow the trend of IAA in that an increase in the level of IAA was followed by a corresponding increase in GA or a gibberellin-like compound from 8/2/75 to 9/6/75. One should be cautious in placing too much emphasis on the Avena straight-growth method for the bioassay of GA or a gibberellin-like compound and its regulatory activity, since this method is mainly for auxins and inhibitors and is not too sensitive to GA or a gibberellin-like compound. Kaufman and Jones (168) reported that ABA was a potent inhibitor of GA-promoted growth in Avena stem segments at physiological concentration. Also, the Rf for GA and the gibberellin-like compound(s) in this study were next to that of ABA on one side and inhibitor C on the other side. These two inhibiting zones were circumscribing the zone containing GA or the gibberellin-like compound. It was very likely that the presence of inhibitors or the carry-over of the inhibitors into the Rf of GA or the gibberellin-like compound in the chromatograms, could mask the action of GA or the gibberellin-like compound, thus possibly accounting for the absence of GA or the gibberellin-like compound or the low levels of GA activity detected.

The levels of inhibitors, ABA and inhibitor C in the acidic fractions of extracts remained high even during the pecan filling stages, but reached their maximum during the onset of the natural shuck
opening. However, the inhibiting activities of ABA and inhibitor C were not observed on 7/13/75. Instead, a zone of promotion was observed. It is therefore in this discussion, assumed that fruit stages before 7/13/75 did not contain ABA and inhibitor C in the acidic fractions of the extracts.

It was observed to be a trend that an increase in the level of IAA was followed by a corresponding decrease in the level of inhibitor C, and that an increase in the level of IPyA was followed by a corresponding decrease in the level of ABA. During the time of natural pecan shuck opening, the levels of ABA and inhibitor C were maximum. This agreed with the results of Lipe et al. (196).

Inhibiting activity possibly by phenolic acids from the neutral/basic fraction was high throughout the growing season with its maximum peak from 8/2/75 to 8/10/75, and second peak on 9/13/75. The level of phenolic acid seemed to correspond with IAA activity from 7/27/75 to the onset of natural pecan shuck opening (9/20/75) (Figure 32). That is, an increase in IAA activity was followed by a corresponding increase in inhibiting activity caused by phenolic acids. According to Leopold and Kriedemann (185), phenolic inhibitors might occur in very high concentrations in plants either as a free phenolic acid or as a derivative such as the glycoside. Its abundance in plants was only second to carbohydrates (254). In 1964, Zinsmeister and Hulmliller demonstrated that phenolic acids commonly showed inhibitory effects on growth only at fairly high concentrations (185). Nitsch and Nitsch (241) classified phenolic acids into monophenols such as p-coumaric acid and kaempferol, and o-diphenols such as quercitin and chlorogenic acid. They also demonstrated that a monophenol was the co-factor for
the enzyme, IAA oxidase, that catalysed the oxidation of IAA. An
o-diphenol was an inhibitor of IAA oxidase, thus bringing about an
enhancement of growth in an auxin system. Reviewed evidence and data
obtained, seemed to indicate that phenolic acids (phenolic inhibitors)
present in the neutral/basic fraction of the extracts were o-diphenols.
The assertion of the presence of o-diphenols in the neutral/basic
fraction is demonstrated in Figure 32 that the growth activity induced
by IAA corresponded with the increased inhibiting activity induced by
the phenolic acids. No attempt was made to identify these phenolic
acids.

Data obtained from bioassays seemed to reveal that natural pecan
shuck opening of 'Success' was governed by the levels of endogenous
growth promoters and inhibitors present in the shuck. Shuck opening
was observed at the time when there was no auxin-regulated activity, no
growth activity regulated by GA or the gibberellin-like compound, and
with a maximum inhibiting activity by ABA and inhibitor C.

Could it be that natural pecan shuck opening was regulated by
ABA alone or with some other factors? It has been noted that the
treatment of 100 ppm abscisic acid of intact pecan fruits of various
varieties by dipping on August 20, 1974, had no effect on premature
pecan shuck opening (unpublished data). These observations indicated
that ABA was not the sole shuck opening regulator. If it was so, its
physiological action must be accompanied by favorable conditions that
were conducive for it to do so. Such conditions could be the physio-
logical age of the fruits, disappearance of growth promoting substances
such as IAA, IPyA, GA or a gibberellin-like compound, and/or even
cytokinin in the shuck.
Based on the bioassay means, the levels of auxins (IPyA and IAA) were negatively correlated with that of inhibitors (ABA and inhibitor C) in the acidic fraction of ether extracts. An increase in auxin levels (IPyA and IAA) was followed by a corresponding decrease in inhibitors (ABA and inhibitor C). It appeared that auxins (IPyA and IAA) and inhibitors (ABA and inhibitor C) in the acidic fraction, acted competitively for a physiological site.

Regardless of the number of inhibitors present in both the acidic and neutral/basic fractions of extracts, it was shown that as long as growth promoting substance was present in the shuck, whether it be auxin, GA or a gibberellin-like compound, no natural shuck opening was observed two weeks before the occurrence of natural shuck opening. It was thought that the growth promoters in the shuck functioned as a juvenile factor in delaying the senescence of shucks.

Results also indicated that auxins suppressed the acidic inhibitors in the regulation of natural shuck opening. This suggestion was supported by the results of Wareing and Phillips (332) who reported that the promotion of abscission by ABA could be antagonized by IAA. GA was also known to antagonize the effect of ABA (111).

Natural shuck opening could also be possibly due to the disappearance of GA or a gibberellin-like compound causing a relatively quick shift of hormonal balance in favor of the high auxin level (9/13/73; one week before the natural shuck opening) which induced ethylene production that caused shuck opening (192, 194).

When a sufficient amount of ethylene had built up in the internal atmosphere of the seed (kernel), it possibly escaped through the air spaces in the shell layers. The endogenous ethylene, in return,
regulated the synthesis of specific miRNA and appropriate complementary transfer and ribosomal RNA (5, 6) that were possibly specific for the synthesis of IAA oxidase or peroxidase which catalysed the oxidation of IAA (324, 229). Under normal condition, ethylene is also produced by ripening fruits (41). Ethylene-induced peroxidase activity has been reported by many workers (144, 287, 324). Then, the physiological age of the fruit accompanied by the destruction of IAA favored ABA which was physiologically active in regulating the synthesis of cellulase in the abscission zone between the seed and the shuck, and in the sutures of the shuck.

Shuck opening (dehiscence) could not be induced as early as one month before normal shuck dehiscence even by treating with a high concentration of ethylene (1,000 ul/liter of air). However, after this period, exogenous ethylene at the rate of as little as 0.1 ul/liter of air was observed to hasten shuck dehiscence (192, 194).

ABA has been reported to have no apparent effect on abscission (134). On the other hand, ABA was reported to promote abscission (79, 332) and senescence in a variety of plant species (20).

It has also been shown in this study that quantitative and/or qualitative changes in endogenous growth regulators occur during the development of pecan fruits. The data from such investigations were complex and do not allow very definite conclusions regarding causal relations of endogenous levels of growth promoters and inhibitors to the natural shuck opening of pecan. However, the experimental evidence is so much in favor of the hypothesis that the balance of endogenous growth hormones plays a key role in the regulation of natural shuck opening or dehiscence.
It could generally be concluded that natural shuck opening in pecan is governed first by the disappearance of GA or a gibberellin-like compound and then followed by the disappearance of auxin in the shuck. With the disappearance of GA or a gibberellin-like compound, high auxin levels are able to induce ethylene production approximately one week before shuck opening at the site (kernel) away from the high auxin activity region (shuck). Shucks received ethylene produced in the kernel as a message for dehiscence (195). When ethylene had built up in the seed, a possible gradient existed between the seed and the shuck. Ethylene escaped from the seed (kernel) through the air spaces in the shell layers (seed coat). This endogenous ethylene regulated the synthesis of specific mRNA, appropriate complementary transfer and ribosomal RNA (5, 6) that are possibly specific for the synthesis of IAA oxidase or peroxidase that oxidized IAA in the shuck. The destruction of IAA by IAA oxidase or peroxidase (disappearance of IAA) changed the hormonal balance in favor of ABA making it physiologically active in regulating shuck opening by regulating the synthesis of cellulase (151). This enzyme, cellulase, was then responsible for the dissolution of cells (151) in the abscission zone between the seed and the shuck, and in the sutures of the shuck, particularly at the apex end of the fruit.

Since the nature of inhibitor C is not known, the role of this inhibitor in the regulation of natural shuck opening is not discussed here. Possibly, it worked together with ABA in the regulation of natural shuck opening or dehiscence.

This experiment is only a preliminary investigation of natural shuck dehiscence regulated by endogenous growth hormones and should be subjected to further investigations to verify the said hypothesis.
Table 3. Responses of Avena coleoptiles to endogenous IPyA, IAA, ABA, GA or gibberellin-like compound, and inhibitor C in the acidic fraction, and endogenous phenolic acids in the neutral/basic fraction of the ether extracts of the non-diseased shuck of pecan fruit cv. Success from 7/13/75 to 10/5/75 at weekly interval. (Each value is the average of three replicates)

<table>
<thead>
<tr>
<th>Possible compounds</th>
<th>Rf values</th>
<th>7/13</th>
<th>7/20</th>
<th>7/27</th>
<th>8/2</th>
<th>8/10</th>
<th>8/17</th>
<th>8/23</th>
<th>8/30</th>
<th>9/6</th>
<th>9/13</th>
<th>9/20</th>
<th>10/5</th>
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</thead>
<tbody>
<tr>
<td>IPyA</td>
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<td>96</td>
<td>106</td>
<td>104</td>
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<td>105</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>IAA</td>
<td>0.3-0.4</td>
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<td>102</td>
<td>103</td>
<td>114</td>
<td>101</td>
<td>105</td>
<td>109</td>
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<td>99</td>
<td>108</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
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<td>97</td>
<td>95</td>
<td>94</td>
<td>100</td>
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<td>99</td>
<td>85</td>
<td>91</td>
<td></td>
</tr>
<tr>
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<td>90</td>
<td>102</td>
<td>100</td>
<td>104</td>
<td>105</td>
<td>96</td>
<td>95</td>
<td>93</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Inhibitor C</td>
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<td>96</td>
<td>96</td>
<td>100</td>
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<td>95</td>
<td>104</td>
<td>85</td>
<td>86</td>
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</tbody>
</table>

*Phenolic acids--these compounds were present in the neutral/basic fraction of the ether extracts
Figure 25. The seasonal fluctuation in quantity of IPyA in the acidic fraction of chromatograms developed in n-butanol-NH₄OH-H₂O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 26. The seasonal fluctuation in quantity of IAA in the acidic fraction of chromatograms developed in n-butanol-NH$_4$OH-H$_2$O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 27. The seasonal fluctuation in quantity of GA or gibberellin-like compound in the acidic fraction of chromatograms developed in n-butanol-NH$_4$OH-H$_2$O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 28. The seasonal fluctuation in quantity of ABA in the acidic fraction of chromatograms developed in n-butanol-NH$_4$OH-H$_2$O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 29. The seasonal fluctuation in quantity of inhibitor C in the acidic fraction of chromatograms developed in n-butanol-NH₄OH-H₂O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 30. The seasonal fluctuation in quantity of phenolic acids in the neutral/basic fraction of chromatograms developed in n-butanol-NH₄OH-H₂O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 31. The seasonal fluctuation in quantities of IPyA, IAA, GA or gibberellin-like compound, inhibitor C, and ABA in the acidic fraction of chromatograms developed in n-butanol-NH₄OH-H₂O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Figure 32. The seasonal fluctuation in quantity of IAA in the acidic fraction as related to phenolic acids in the neutral/basic fraction of chromatograms developed in n-butanol-NH$_4$OH-H$_2$O (10:1:1, v/v/v) solvent. (Each point represents the average of three replicates)
Study II. Regulation of Abnormal Shuck Opening of Pecan

This experiment was conducted to determine the relationship between the endogenous growth substances present in the shucks and the regulation of abnormal shuck opening (pecan shuck disease). Comparisons of growth substances were made between healthy and shuck-diseased fruits.

Growth substances present in the shucks of healthy and shuck-diseased fruits were extracted with diethyl ether separately as in Study I. During the period of study two different types of paper chromatograms in the acidic fraction, developed in n-butanol-ammonium hydroxide-water (10:1:1, v/v/v) solvent, were observed: one showed no coloration at Rf 0.3-0.4 in the acidic fraction, and the other showed pinkish red coloration at Rf 0.3-0.4 in the acidic fraction (Plate I). Therefore, two situations were used to discuss the possible role of endogenous growth substances in the regulation of abnormal shuck opening.

Situation 1. Chromatograms from shuck-diseased fruits showing no coloration at Rf 0.3-0.4 in the acidic fraction

The lengths of Avena coleoptile segments expressed as a percent over the control were used in the statistical analysis. In the analysis of variance for the extracts of healthy and shuck-diseased pecan fruits, cultivar 'Success', in both acidic and neutral/basic fractions, there were highly significant differences among Rfs, and between acidic and neutral/basic fractions. There was a significant Rf x fraction interaction, and fraction x fruit type interaction. However, there was no significant difference between the healthy and shuck-diseased fruits (Appendix 2).
These could be interpreted to mean that there was a difference between the acidic fraction and neutral/basic fraction within fruit type. The difference between acidic and neutral/basic fractions is not the same for fruit type (healthy and shuck-diseased). Qualitatively different growth substances in the extracts, as represented by their Rf values, as well as possibly quantitative differences within a qualitative entity were present in the acidic and neutral/basic fractions. There was no significant difference in the growth activity regulated by growth substances between the healthy and shuck-diseased fruits.

For the interpretation of growth activity regulated by growth substances, the assumptions made in Study I applied equally here. It is further assumed that the growth substances at their respective Rf identical to those described in Study I are the same compounds. Histograms showing growth activity obtained with Avena coleoptile straight-growth test of the acidic and neutral/basic fractions of ether extracts from shucks of healthy and shuck-diseased fruits are shown in Figures 33 and 34 (Table 4).

Though there was no difference in the growth activity regulated by endogenous growth substances between the healthy and shuck-diseased fruits in the statistical analysis, there were differences observed at Rf 0.1-0.2, Rf 0.3-0.4, Rf 0.5-0.7, and Rf 0.8-0.9 in the acidic fraction. No difference was observed at Rf 1.0 in the acidic fraction. There was a difference in the levels of phenolic acid particularly at Rf 0.5-0.7 in the neutral/basic fraction (Table 5 and Figure 35).

Regardless of fruit type (healthy and shuck-diseased fruits), increased growth promoting activity by auxin (IPyA and IAA) was associated with the low growth inhibiting activity by ABA and high
inhibiting activity by phenolic acids. Inhibitor C was present in equal amounts in the extracts of both healthy and shuck-diseased fruits. These results suggested that phenolic acids possibly served as an inhibitor of IAA oxidase (241), thus resulting in a high level of IAA as well as IPyA. Auxins (IPyA and IAA) and ABA reacted competitively for a physiologically active site in the shuck system. This was supported by observations that an increase in the level of auxins was followed by a corresponding decrease in the level of ABA, and vice versa.

It was noted that healthy fruit contained higher levels of auxins and phenolic acids, and a lower level of ABA as compared to that of shuck-diseased fruit.

Growth activity regulated by GA or a gibberellin-like compound was observed in the acidic fraction of extracts of healthy fruits, but was not observed in the acidic fraction of the extracts of shuck-diseased fruits. This was the only distinctive difference between the healthy and shuck-diseased fruits. Results seemed to indicate that GA or a gibberellin-like compound or an inhibitor located at Rf 0.8-0.9 was the key regulator of abnormal shuck opening or dehiscence. The conditions for abnormal shuck opening were not identical with that of natural shuck opening. Previous results indicated that fruit exhibiting natural shuck opening did not contain auxins, but had high levels of ABA (only 85% growth of the control) and inhibitor C (85% growth of the control) (Table 1, 9/20/75). However, fruit showing abnormal shuck opening contained auxins, and low levels of ABA (95% growth of the control) and inhibitor C (95% growth of the control) (Table 3).

When comparing healthy and shuck-diseased fruits, there was no
significant difference in the levels of auxins, and levels of ABA or inhibitor C (Figure 35). There was a difference in levels of phenolic acid particularly at Rf 0.5-0.7 in the neutral/basic fraction of ether extracts. There was also a significant difference at Rf 0.8-0.9 in the acidic fraction where healthy fruits contained GA or a gibberellin-like compound in the shuck, while shuck-diseased fruits contained no GA or a gibberellin-like compound, but rather contained an inhibitor at Rf 0.8-0.9.

Data seemed to be very conclusive that ABA and inhibitor C were not involved in the regulation of abnormal shuck opening, but lack of GA or a gibberellin-like compound or the presence of an inhibitor at Rf 0.8-0.9 in the acidic fraction of the extracts and low level of phenolic acid in the neutral/basic fraction of the extracts triggered abnormal shuck opening.

If this hypothesis is true, what triggers the early disappearance of GA or the gibberellin-like compound or the occurrence of an inhibitor at Rf 0.8-0.9 in the acidic fraction of extracts? It could possibly be due to the low level of phenolic acid in the neutral/basic fraction of the ether extracts that favored IAA oxidase activity. Suppression of IAA enhancing fruit growth before the natural shuck opening period might result in inadequate fruit development, and thus result in the lacking of GA or a gibberellin-like compound or the presence of a growth inhibitor. It is also reasonable to suggest that factors contributing to the optimum growth of the pecan tree may be involved, since Schaller and Dodge (278) reported that excessive crop with sparse and unhealthy foliage, poor tree growth, vein spot infection, shadding of tree, high rainfall, heavy and waterlogged soil
favored the incidence of abnormal shuck opening of pecan. It is also possible that an inhibitor present at Rf 0.8-0.9 in the acidic fraction was introduced by insects, triggered the destruction of GA or gibberellin-like compound, and became directly involved in the regulation of abnormal shuck opening. The latter is unlikely to be the case due to the fact that such inhibition zone at Rf 0.8-0.9 in the acidic fraction was also observed in the fruit showing natural shuck opening. Many workers (192, 194, 300) had reported that ethylene was the regulator responsible for pecan shuck opening. Though shuck-diseased fruits were observed to produce large amounts of ethylene as compared to healthy fruits (unpublished data), abnormal shuck opening was unlikely due to the effect of ethylene since injured tissues always produce ethylene (41, 349). This increase in ethylene production could possibly be due to the prematurely opened shuck or due to the secondary infection by microorganisms or insects that produced ethylene on the prematurely opened shuck.

Situation 2. Chromatograms from shuck-diseased fruit showing pinkish-red coloration at Rf 0.3-0.4 in the acidic fraction

In the analysis of variance for the extracts of healthy and shuck-diseased fruits of cultivar 'Success' in both acidic and neutral/basic fractions, significant differences were observed between fruit types, among Rf components, and between acidic and neutral/basic fractions. Significant interactions were observed for Rf x fraction, Rf x fruit type, fraction x fruit type, and Rf x fraction x fruit type.

Significant differences between the healthy and shuck-diseased fruits, and among the Rf components could be interpreted to mean that qualitatively different growth substances as well as a possible
quantitative difference within a qualitative entity were involved in the regulation of abnormal shuck opening. However, the differences among Rf components are not the same for the acidic fraction as for the neutral/basic fraction; the differences among Rf components are not the same for the healthy fruit as for the shuck-diseased fruit; and the difference between the acidic and neutral/basic fractions is not the same for healthy fruit as for the shuck-diseased fruit.

Histograms showing growth activity obtained with *Avena* coleoptile straight-growth test of acidic and neutral/basic fractions of ether extracts from the shucks of healthy and shuck-diseased fruits are shown in Figures 36-37 (Table 4).

Growth activity was confined to three zones of chromatograms in the acidic fraction of healthy fruit extracts. The zones of promotion were at Rf 0.1-0.2 (IPyA), Rf 0.3-0.4 (IAA), and Rf 0.8-0.9 (GA or a gibberellin-like compound). The two zones of inhibition were at Rf 0.5-0.7 (ABA) and Rf 1.0 (inhibitor C). Growth inhibiting activity was observed at all Rf's in the neutral/basic fraction of healthy fruit extracts (Figure 36).

No growth promoting activity was observed at any Rf in the acidic fraction of shuck-diseased fruit extracts (Figure 36), nor at any Rf in the neutral/basic fraction of the shuck-diseased fruit extracts (Figure 37).

Auxin (IPyA and IAA) and GA or a gibberellin-like compound were present in the fruits showing no abnormal shuck opening. The level of ABA in the healthy fruit extracts was observed to be identical to that found in fruits during the growing period (7/13/75 to 9/13/75) in Study I (Figures 28 and 32). These results could be interpreted to
mean that auxins (IPyA and IAA) and GA or a gibberellin-like compound served as juvenile factors in promoting the growth of the fruits, in delaying the senescence of pecan shuck tissue, and in suppressing the physiological action of ABA.

In the shuck-diseased fruit extracts, no growth promoter was isolated. The zones of promotion at Rf 0.1-0.2 and Rf 0.3-0.4, which were supposedly occupied by IPyA and IAA respectively, were replaced by two zones of inhibition. A low level of inhibition was observed at Rf 0.1-0.2, but a high level of inhibition was observed at Rf 0.3-0.4 from which a pinkish-red compound was located (Figure 38).

It seemed that pecan shuck disease in Situation 2 was not due to ABA, inhibitor C in the acidic fraction and phenolic acids in the neutral/basic fraction, since both healthy and shuck-diseased fruits contained almost equal levels of these inhibitors (Figure 38). With this in mind, it was highly reasonable to assume that growth inhibitors present at Rf 0.1-0.2, Rf 0.3-0.4, and Rf 0.8-0.9 were responsible for the shuck disease of pecan. Could the mechanism involved in the regulation of natural shuck opening in Study I apply to the phenomenon of abnormal shuck opening, since both fruit types showed no growth promoting activity at the same time of shuck opening? That is, the disappearance of GA or a gibberellin-like compound or presence of inhibitor at Rf 0.8-0.9 caused a relatively quick shift of hormonal balance in favor of auxin before shuck opening resulting in ethylene production in the kernel. This endogenous ethylene, in return, regulated the synthesis of specific messenger RNA and appropriate complementary and ribosomal RNA (5, 6) that were possibly specific for the synthesis of IAA oxidase or peroxidase which catalysed the oxidation
of IAA (229, 324). The oxidation of IAA then favored ABA to be physiologically active in regulating the synthesis of cellulase in the abscission zones between the seed and the shuck, and in the sutures of the shuck. This was unlikely to be the case since a high level of growth inhibiting activity (83% growth of the control) was observed at Rf 0.3-0.4 in the acidic fraction of the shuck-diseased fruit (Figure 38). The growth inhibiting activity at Rf 0.3-0.4 was about 96% growth of the control (Figure 31, average of means on 9/20/75 and 10/5/75) in the acidic fraction of fruit showing natural shuck opening in Study 1.

An attempt was made to identify this compound at Rf 0.3-0.4 in the acidic fraction. It developed yellow color with Prochazka's reagent, Salkowski's reagent, cinnamaldehyde-HCl, NaNO₂ + HCl, and developed orange color with ninhydrin (Appendix 10). No conclusive evidence could be drawn, but it was thought to be a compound of an indole derivative linked with glycoside.

Inhibiting activity at Rf 0.1-0.2 could be the same compound as that present in Rf 0.3-0.4. Its inhibiting activity could possibly be due to the residual traces of this inhibitor at Rf 0.3-0.4 in the acidic fraction of the shuck-diseased fruit extracts.

Data of bioassay from healthy and fruit showing natural shuck opening did not indicate such high inhibiting activity at this zone. However, it seemed to suggest that the inhibiting substance at Rf 0.3-0.4 was probably not an endogenous compound. This substance could be possibly introduced into the shuck tissue when insects, especially stink bug and like species, feed on the shuck.

Osburn et al. (245) reported that nuts frequently dropped as a result of stink bug damage. It is therefore highly probable that this
inhibiting substance at Rf 0.3-0.4 in the acidic fraction of extracts from shuck-diseased fruit, introduced by stink bug or other insects, upset the hormonal system in the shuck resulting in the metabolism or disappearance of GA or the gibberellin-like compound. This change in the hormonal system resulted in the premature shuck drying or shuck opening.

Pecan shuck disease in Situation 2 is unlikely to be reduced or alleviated by the application of GA or gibberellin-like compounds due to the presence of this inhibitor at Rf 0.3-0.4 in the acidic fraction of the extracts of the shuck-diseased fruit. These observations and data could be used to possibly explain the reason why rigid fungicide and insecticide applications during the late summer reduced the incidence of pecan shuck disease (stem end blight) (280).

Knowing the relationship between endogenous growth substances and shuck disease of pecan, it is possible to manipulate the application of some exogenous growth substances at the critical time and have an effective control or reduce the occurrence of pecan shuck disease. Results seemed to indicate that pecan shuck disease in Situation 1 could be controlled or reduced by application of GA or gibberellin-like compounds at the critical time where GA or gibberellin-like compounds are believed to be lacking. It is very doubtful that pecan shuck disease in Situation 2 can be controlled or reduced by the application of exogenous growth substances, GA in particular.
Plate 1. Chromatograms from the acidic fraction of pecan fruit extracts: Non-diseased (H); Shuck-diseased (Situation 1) (D); Shuck-diseased (Situation 2) (DR)
Table 4. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors in the acidic and neutral/basic fraction of ether extracts of healthy (non-diseased) and shuck-diseased fruits of cultivar, 'Success' at Rf 0.1 to 1.0. Each value is the average of 10 replicates (above 100% indicated growth promoter and below 100% indicated growth inhibitor or absence of growth promoter)

<table>
<thead>
<tr>
<th>Rf</th>
<th>ACIDIC</th>
<th></th>
<th>NEUTRAL/BASIC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy fruit</td>
<td>Diseased fruit (situation I)</td>
<td>Diseased fruit (situation II)</td>
<td>Healthy fruit</td>
</tr>
<tr>
<td>0.1</td>
<td>105</td>
<td>100</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>0.2</td>
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<td>94</td>
</tr>
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<td>0.3</td>
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<td>81</td>
<td>93</td>
</tr>
<tr>
<td>0.4</td>
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</tr>
<tr>
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</tr>
<tr>
<td>0.6</td>
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</tr>
<tr>
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Table 5. *Rf values for pure compounds and possible growth substances from chromatograms developed in n-butanol-ammonium hydroxide-water (10:1:1, v/v/v) for both acidic and neutral/basic fraction of ether extracts.*

<table>
<thead>
<tr>
<th>Pure compound</th>
<th>Rf of pure compound</th>
<th>Rf obtained</th>
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<th>Neutral/basic fraction</th>
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<tr>
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</tr>
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<td>IAA</td>
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<td>-</td>
<td>-</td>
<td>1.0</td>
<td>Inh. C</td>
<td>-</td>
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<td>o-diphenol</td>
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<tr>
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<td>0.3-0.4</td>
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</tr>
<tr>
<td>ABA</td>
<td>0.60</td>
<td>0.5-0.7</td>
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<td>-</td>
<td>ABA</td>
<td>-</td>
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<tr>
<td>GA</td>
<td>0.85</td>
<td>0.8-0.9</td>
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<td>-</td>
<td>-</td>
<td>1.0</td>
<td>Inh. C</td>
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<td>Inh. C</td>
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<tr>
<td>-</td>
<td>-</td>
<td>0.1-1.0</td>
<td>-</td>
<td>o-diphenol</td>
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</table>
Table 6. Responses of *Avena coleoptiles* to endogenous growth promoters and inhibitors in the acidic and neutral/basic fraction of ether extracts of healthy (non-diseased) and shuck-diseased fruits of cultivar, 'Success' at Rf 0.1-1.0. (Each value is the average of ten replicates within the Rf range of the growth substances)

<table>
<thead>
<tr>
<th>Rf</th>
<th>Healthy fruit</th>
<th>Diseased fruit (situation I)</th>
<th>Diseased fruit (situation II)</th>
<th>Healthy fruit</th>
<th>Diseased fruit (situation I)</th>
<th>Diseased fruit (situation II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1-0.2</td>
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<td>102.5</td>
<td>96.0</td>
<td>97.0</td>
<td>98.0</td>
<td>97.5</td>
</tr>
<tr>
<td>0.5-0.4</td>
<td>102.5</td>
<td>107.0</td>
<td>83.0</td>
<td>92.5</td>
<td>95.5</td>
<td>95.5</td>
</tr>
<tr>
<td>0.5-0.7</td>
<td>96.0</td>
<td>95.0</td>
<td>94.7</td>
<td>84.0</td>
<td>92.3</td>
<td>87.3</td>
</tr>
<tr>
<td>0.8-0.9</td>
<td>102.0</td>
<td>94.0</td>
<td>95.5</td>
<td>82.5</td>
<td>87.0</td>
<td>78.9</td>
</tr>
<tr>
<td>1.0</td>
<td>95.0</td>
<td>95.0</td>
<td>93.0</td>
<td>95.0</td>
<td>90.0</td>
<td>93.0</td>
</tr>
</tbody>
</table>
Figure 33. Responses of *Avena* coleoptiles to endogenous growth promoters and inhibitors at RF values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased and diseased shuck of pecan fruit cv. Success. (Each histogram represents the average of ten replicates) (Situation 1)
Figure 34. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the neutral/basic fraction of the ether extracts of non-diseased and diseased shuck of pecan fruit cv. Success. (Each histogram represents the average of ten replicates) (Situation 1)
Figure 35. Responses of *Avena* coleoptiles to endogenous growth promoters and inhibitors at RF 0.1-1.0 in the acidic and neutral/basic fractions of ether extracts of non-diseased and shuck-diseased fruits, cultivar 'Success'. (Each histogram represents the average of ten replicates) (Situation 1)
Figure 56. Responses of Avona coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the acidic fraction of the ether extracts of the non-diseased and diseased shuck of pecan fruit cv. Success. (Each histogram represents the average of ten replicates) (Situation 2)
Figure 37. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf values 0.1-1.0 in the neutral/basic fraction of the ether extracts of the non-diseased and diseased shuck of pecan fruit cv. Success. (Each histogram represents the average of ten replicates) (Situation 2)
Figure 38. Responses of Avena coleoptiles to endogenous growth promoters and inhibitors at Rf 0.1-1.0 in the acidic and neutral/basic fractions of ether extracts of non-diseased and shuck-diseased fruits, cultivar 'Success'. (Each histogram represents the average of ten replicates) (Situation 2)
Study III. Effects of GA, Alar, and their Combinations

In the analysis of covariance for effects of GA, Alar, and their combinations on the incidence of pecan shuck disease, there was a highly significant difference among treatments. Therefore, orthogonal comparisons among treatments were made in order to find out which chemical treatment gave a significant result. It was found that GA and Alar, singly or in combination with one another, gave a significant reduction in the incidence of pecan shuck disease when the chemicals were applied on 7/26/75 and 8/5/75. Comparisons among GA, Alar, and their combinations indicated that GA x Alar combinations were most effective in reducing or controlling the incidence of pecan shuck disease. GA was found to be more effective than Alar (Appendix 4).

GA applied alone at 500 ppm or in combination with Alar gave more effective reduction in the incidence of pecan shuck disease than lower GA concentrations and their combinations with Alar.

Alar at 1,000 ppm failed to reduce the incidence of pecan shuck disease. This could probably be explained by the fact that trees receiving Alar at 1,000 ppm possibly had low nutritional levels, thus lack of vigor that was not obvious at the time of chemical application. Dodge and Schaller (87, 88) also reported that there was a difference in nutritional levels of different shoots.

In the analysis of covariance for effects of GA, Alar, and their combinations on kernel yield of pecan, there was no significant difference in yield as compared with the control (Appendix 5). All treatments except Alar at 1,000 ppm and GA at 500 ppm x Alar 3,000 ppm, increased kernel yield. Since trees receiving Alar at 1,000 ppm were suspected of having low nutritional levels or lack of vigor, it
was reasonable to expect low yield if the previous proposed condition (low nutritional level) existed. Schaller et al. (279) reported that weak shoots of the tree frequently resulted in poor filling and poor quality of nuts.

GA at 500 ppm x Alar at 3,000 ppm reduced the kernel yield as compared with the control. It might be due to their optimum effect in delaying maturity of fruits since maximum concentrations of these chemicals were used. Storey et al. (308) reported that Alar 4,000 ppm delayed maturity of pecan fruits. Many workers (65, 106, 284) also reported that GA retarded maturity and senescence.

Table 7 shows the effects of GA, Alar, and their combinations on the incidence of pecan shuck disease and kernel yield of pecan, cv. 'Success'.

The application of GA x Alar combinations gave best reduction in the occurrence of pecan shuck disease, especially when a high concentration of GA was used, and generally increased kernel yield. Since Alar inhibited gibberellin synthesis (94, 180), it was very likely that Alar in this study served as a factor in delaying maturity of fruits and tissue senescence. Its delaying effect was probably further enhanced by GA application. Data seemed to suggest that GA not only served as a factor in delaying maturity (as a juvenile factor), but it also increased the translocation of carbohydrates (36) from leaves and shuck to the seed, thus was responsible for the proper filling of the nuts.

Based on the results, it is logical to conclude that GA, Alar, and their combinations delayed the maturity and senescence of pecan fruits and shuck tissue, while GA may have also aided in increasing
the translocation of carbohydrates to the seeds for the proper
development. Also, the application of GA may have served as a source of
GA. Since it is thought that lack of GA might be the cause of pecan
shuck disease (Situation 1) in Study II, it is also logical to suggest
that applications of GA alone or in combinations with Alar, reduced the
incidence of pecan shuck disease.

Since the fruits were harvested about one month before nut
shedding, it is unwarranted to judge the effects of chemical treatments
on kernel yield of pecan, especially GA and Alar. Yields when compared
with the control were greatly affected since GA and Alar both delayed
maturity and senescence. Nevertheless, from the observations, a
treatment of GA at 500 ppm x Alar at 1,000 ppm should be included in
future studies on control of shuck disease of pecan.
Table 7. Effects of GA, Alar, and their combinations on the occurrence of pecan shuck disease and kernel yield

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Occurrence of pecan shuck disease as % of the control</th>
<th>Average yield of kernel (gm/20 nuts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA 500 x Alar 1000</td>
<td>28.69</td>
<td>62.66</td>
</tr>
<tr>
<td>GA 500 x Alar 3000</td>
<td>31.17</td>
<td>57.11</td>
</tr>
<tr>
<td>GA 350 x Alar 1000</td>
<td>37.77</td>
<td>70.73</td>
</tr>
<tr>
<td>GA 500</td>
<td>40.07</td>
<td>71.40</td>
</tr>
<tr>
<td>GA 350 x Alar 3000</td>
<td>40.94</td>
<td>71.76</td>
</tr>
<tr>
<td>GA 200 x Alar 3000</td>
<td>49.91</td>
<td>65.45</td>
</tr>
<tr>
<td>GA 200 x Alar 1000</td>
<td>50.85</td>
<td>67.60</td>
</tr>
<tr>
<td>GA 350</td>
<td>52.25</td>
<td>62.57</td>
</tr>
<tr>
<td>GA 200</td>
<td>60.94</td>
<td>66.58</td>
</tr>
<tr>
<td>Alar 3000</td>
<td>77.05</td>
<td>68.31</td>
</tr>
<tr>
<td>Alar 1000</td>
<td>102.20</td>
<td>62.01</td>
</tr>
<tr>
<td>Control</td>
<td>100.00</td>
<td>62.46</td>
</tr>
</tbody>
</table>
Study IV. Effects of PBA, GA, IAA, and Their Combinations

In the analysis of covariance for effects of PBA, GA, IAA, and their combinations, no significant differences were found among chemical treatments in reducing the incidence of pecan shuck disease as compared with the control. All treatments seemed to reduce the incidence of shuck disease to a certain degree except for IAA at 100 ppm which increased the incidence of pecan shuck disease as compared with the control (Appendix 6).

Though many workers (12, 73, 230, 231, 358) had reported that IAA induced ethylene production and that ethylene served as a regulator for pecan shuck disease (192, 193, 194), could this increased incidence be attributed to the application of exogenous IAA? If IAA at 100 ppm indeed increased the incidence of pecan shuck disease, several reasons could be used to explain the increase. Perhaps, IAA at 100 ppm was far above the physiological concentration of endogenous IAA level, and it possibly created a steep auxin gradient favoring ethylene production which then precipitated premature shuck opening (pecan shuck disease) (192, 194). It was also noted that 'Success' cultivar was sensitive to 2,4-D (133). NAA and IBA also caused premature dropping of pecan fruits (133). These observations could be used to explain the fact that 'Success' was possibly also sensitive to auxin, particularly IAA. IAA-induced premature shuck opening was further supported by the findings that premature shuck opening was greatly enhanced by the application of 2,4,5-T before the shells of the nuts fully hardened (date of application: 7/26/75) (unpublished data).

IAA-induced premature shuck opening could also be due to the fact that the tree receiving IAA at 100 ppm was in a weak physiological
condition and thus lacking GA. This deficiency of GA or a gibberellin-
like compound changed the physiological action of IAA from juvenility
into auxin-induced ethylene production. Ethylene was then the ultimate
regulator of pecan shuck opening (pecan shuck disease).

If an increased incidence of pecan shuck disease was due to
auxin-induced ethylene production, then, why did the application of IAA
at 100 ppm in combination with PBA and/or GA not increase the incidence
of pecan shuck disease?

When IAA at 100 ppm was applied together with GA, GA might not
only serve as a juvenility factor working synergistically with IAA in
promoting fruit growth rather than auxin-induced ethylene production,
but also might suppress the action of IAA in regulating ethylene
production. This was supported by the finding that GA caused 50%
reduction of ethylene production induced by 2,4-D in soybean (149), and
citrus fruits (189). GA might also have delayed fruit maturity and
shuck tissue senescence.

When IAA was applied together with PBA, the incidence of pecan
shuck disease was reduced. Since cytokinin could delay senescence (244,
262), abscission (244), and suppress action induced by IAA (316), IAA
could not play its role in the regulation of ethylene production.

PBA and GA delayed senescence of tissue. It was also noted
that PBA caused mobilization of various nutrients (236) and GA
suppressed ethylene production (148, 149) and served as a juvenility
factor (65, 106, 284). It was logical to suggest that IAA-induced
ethylene production was masked by PBA and GA, thus reducing the
incidence of pecan shuck disease.

In the analysis of covariance for effects of PBA, GA, IAA, and
their combinations, no significant increase in kernel yield was observed for any treatment when compared with the control (Appendix 7).

Table 8 shows the effects of PBA, GA, IAA, and their combinations on the incidence of pecan shuck disease and kernel yield of pecan, cultivar 'Success'.

As was mentioned in Study III, kernel yield was not a good criterion to evaluate the effects of PBA, GA, IAA, and their combinations in this study due to the fact that fruits were harvested approximately one month before nut shedding. Fruits harvested were still in the growing and filling stage. The increasing or decreasing effect of the growth substances was not yet certain. It is therefore unjustified to place too much emphasis on kernel yield to determine the effects of these growth substances in this study.
Table 8. Effects of PBA, GA, IAA, and their combinations on the occurrence of pecan shuck disease and kernel yield.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Occurrence of pecan shuck disease as % of the control</th>
<th>Average yield of kernel (gm/ 20 nuts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBA 200</td>
<td>64.95</td>
<td>75.78</td>
</tr>
<tr>
<td>PBA 500</td>
<td>46.27</td>
<td>60.91</td>
</tr>
<tr>
<td>IAA 100</td>
<td>132.36</td>
<td>62.45</td>
</tr>
<tr>
<td>GA 500</td>
<td>63.21</td>
<td>71.62</td>
</tr>
<tr>
<td>PBA 200 x IAA 100</td>
<td>60.66</td>
<td>63.81</td>
</tr>
<tr>
<td>PBA 200 x GA 500</td>
<td>24.98</td>
<td>69.62</td>
</tr>
<tr>
<td>PBA 500 x IAA 100</td>
<td>31.60</td>
<td>69.76</td>
</tr>
<tr>
<td>PBA 500 x GA 500</td>
<td>48.11</td>
<td>63.42</td>
</tr>
<tr>
<td>PBA 200 x GA 500 x IAA 100</td>
<td>24.91</td>
<td>80.71</td>
</tr>
<tr>
<td>PBA 500 x GA 500 x IAA 100</td>
<td>32.52</td>
<td>65.52</td>
</tr>
<tr>
<td>Control</td>
<td>100.00</td>
<td>69.74</td>
</tr>
</tbody>
</table>
Study V. Effects of Kinetin, and Kinetin x Alar Combinations

In the analysis of covariance for effects of kinetin, and kinetin x Alar combinations on the incidence of pecan shuck disease (Appendix 8), and kernel yield (Appendix 9), there was no significant reduction in the incidence of pecan shuck disease as compared with the control. No increase in yield was observed.

Table 9 shows the effects of kinetin, and kinetin x Alar combinations on the incidence of pecan shuck disease and kernel yield of pecan, cultivar 'Success'. Kinetin, and kinetin x Alar combinations had low reduction in the incidence of pecan shuck disease. These observations could be interpreted to mean that kinetin, and kinetin x Alar combinations were not directly involved in the regulation of the occurrence of pecan shuck disease.

Yield was not a good criterion to evaluate the effects of kinetin, and kinetin x Alar combinations in this study since fruits were harvested approximately one month before nut shedding. The increasing or decreasing effect of these growth substances was not yet certain. It is therefore unjustified to place too much emphasis on kernel yield to determine the effects of these growth substances in this study.
Table 9. Effects of kinetin, and the combinations of kinetin and Alar on the occurrence of pecan shuck disease and kernel yield.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Occurrence of pecan shuck disease as % of the control</th>
<th>Average yield of kernel (gm/20 nuts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetin 400 ppm</td>
<td>74.77</td>
<td>66.75</td>
</tr>
<tr>
<td>Kinetin 100 ppm</td>
<td>74.80</td>
<td>61.65</td>
</tr>
<tr>
<td>Kinetin 400 ppm x A 1000 ppm</td>
<td>106.64</td>
<td>71.35</td>
</tr>
<tr>
<td>Kinetin 400 ppm x A 3000 ppm</td>
<td>86.19</td>
<td>89.90</td>
</tr>
<tr>
<td>Kinetin 100 ppm x A 3000 ppm</td>
<td>71.25</td>
<td>71.00</td>
</tr>
<tr>
<td>Kinetin 100 ppm x A 1000 ppm</td>
<td>76.57</td>
<td>67.50</td>
</tr>
<tr>
<td>Control</td>
<td>100.00</td>
<td>71.20</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSIONS

This research involved the studies on levels of endogenous growth promoters and inhibitors that regulated natural shuck opening of pecan, regulation of abnormal shuck opening (pecan shuck disease) by hormones, and effects of several exogenous plant growth substances on the occurrence of pecan shuck disease and kernel yield.

Chromatographs of the non-diseased fruit extracts collected from 7/13/75 to 9/13/75 indicated the presence of several growth substances. Chromatographs of the acidic fraction of pecan fruit extracts generally had three zones of promotion and two zones of inhibition. The promoters found at Rf 0.1-0.2, Rf 0.3-0.4, and Rf 0.8-0.9 could probably be indolepyruvic acid, indoleacetic acid, and a gibberellin or a gibberellin-like compound respectively. Zones of inhibition were found at Rf 0.5-0.7, and Rf 1.0. The compound at Rf 0.5-0.7 could be abscisic acid. The compound at Rf 1.0 was of an unknown nature and it was named inhibitor C.

Chromatographs of the neutral/basic fraction of pecan fruit extracts generally had a zone of inhibition from Rf 0.1 to Rf 1.0. This inhibition zone could probably be due to phenolic acids.

The levels of Auxins (IPyA and IAA) were high in a fluctuating manner one week before the natural opening of the shuck. The levels of abscisic acid and inhibitor C were consistently high, and reached their maximum levels at the time of natural shuck opening. The level of GA or a gibberellin-like compound was fairly inconsistent, but
followed the trend of IAA from 7/27/75 to 9/6/75. Abscisic acid and inhibitor C followed the trend of IPyA and IAA, respectively. An increase in auxin activity was followed by a corresponding increase in inhibiting activity by abscisic acid and inhibitor C.

Data obtained suggested that a growth promoter/inhibitor balance was involved in the regulation of natural pecan shuck opening. The occurrence of natural pecan shuck opening of cultivar 'Success' was first governed by the disappearance of GA or the gibberellin-like compound and then followed by the disappearance of auxins and increases in abscisic acid and inhibitor C.

The mechanism regulating the natural shuck opening was proposed. The disappearance of GA or the gibberellin-like compound induced indole-acetic acid to be physiologically active in regulating ethylene production. Ethylene regulated the synthesis of specific mRNA, appropriate complementary transfer and ribosomal RNA that were possibly specific for the synthesis of IAA oxidase or peroxidase which oxidized IAA in the shuck. The oxidation of IAA by IAA oxidase or peroxidase changed the hormonal balance in favor of abscisic acid making it physiologically active in regulating shuck opening by regulating the synthesis of cellulase which was responsible for the dissolution of cells in the abscission zone between the seed and the shuck, and in the sutures of the shuck.

The relationship between the endogenous growth substances present in the pecan shucks and the regulation of abnormal shuck opening was investigated. Based on the interpretations of the histograms and the statistical analysis of the data. Two situations were observed to be responsible for the occurrence of abnormal shuck
opening.

Pecan shuck disease in Situation 1 was probably due to the absence of GA or the gibberellin-like compound accompanied by low levels of auxins and phenolic acids. Abscisic acid and inhibitor C were not involved in the regulation of abnormal shuck opening in Situation 1.

Pecan shuck disease in Situation 2 was probably due to the presence of growth inhibitor(s) particularly at Rf 0.1-0.4 in the acidic fraction, and the absence of GA or the gibberellin-like compound at Rf 0.8-0.9 in the acidic fraction. No growth promoter was found in the shuck diseased fruits in Situation 2. In both cases, pecan shuck disease resulted in premature shuck opening and early nut shedding.

In the studies of effects of exogenous plant growth substances on the occurrence of pecan shuck disease and kernel yield, GA and Alar, singly or in combination with one another, significantly reduced the incidence of pecan shuck disease. GA x Alar was most effective in reducing the incidence of pecan shuck disease. GA was observed to be more effective than Alar.

Synthetic cytokinin (PBA), GA, and/or IAA applications reduced the incidence of pecan shuck disease to a certain extent, but these reductions were not significant. IAA at 100 ppm increased the incidence of pecan shuck disease. Over-dosage of exogenous IAA application, auxin-induced ethylene production, and weak physiological stage of the tree were the reasons suggested for such increase in the incidence of pecan shuck disease.

Kinetin, and combinations of kinetin and Alar among chemicals studied were found to be least effective in reducing the incidence of pecan shuck disease. It was suggested that these compounds were not
directly involved in the regulation of pecan shuck disease.

Yield was not a good criterion to evaluate the effects of these growth regulators studies because fruits were harvested approximately one month before nut shedding, and the increasing or decreasing effect of these growth regulators was not certain at the time of harvest.
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auxin, gibberellin-like substances, and inhibitor in developing


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Appendix 1

Analysis of variance table for the endogenous levels of growth promoters and inhibitors from pecan fruit extracts in the regulation of natural shuck opening (dehiscence) of pecan.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit stage</td>
<td>11</td>
<td>1995.22</td>
<td>6.39 **</td>
</tr>
<tr>
<td>Fruit/fruit stage (error a)</td>
<td>24</td>
<td>312.31</td>
<td></td>
</tr>
<tr>
<td>Fraction (acidic-basic)</td>
<td>1</td>
<td>17346.05</td>
<td>246.97 **</td>
</tr>
<tr>
<td>Fraction x fruit stage</td>
<td>11</td>
<td>1121.65</td>
<td>15.97 **</td>
</tr>
<tr>
<td>Rf</td>
<td>9</td>
<td>1810.59</td>
<td>25.78 **</td>
</tr>
<tr>
<td>Rf x fruit stage</td>
<td>99</td>
<td>95.81</td>
<td>1.36 *</td>
</tr>
<tr>
<td>Rf x fraction</td>
<td>9</td>
<td>350.18</td>
<td>4.99 **</td>
</tr>
<tr>
<td>Rf x fraction x fruit stage</td>
<td>99</td>
<td>84.65</td>
<td>1.20 NS</td>
</tr>
<tr>
<td>Residual (error b)</td>
<td>456</td>
<td>70.24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>719</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01  
*  = P less than 0.05  
NS = Non-significant
Appendix 2

Analysis of variance table for the hormonal regulation of abnormal shuck opening (pecan shuck disease) (Situation 1).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit type</td>
<td>1</td>
<td>44.22</td>
<td>0.16 NS</td>
</tr>
<tr>
<td>Fruit/fruit type (error a)</td>
<td>18</td>
<td>270.64</td>
<td></td>
</tr>
<tr>
<td>Rf</td>
<td>9</td>
<td>784.07</td>
<td>12.03 **</td>
</tr>
<tr>
<td>Fraction (acidic-basic)</td>
<td>1</td>
<td>7148.70</td>
<td>109.66 **</td>
</tr>
<tr>
<td>Rf x fraction</td>
<td>9</td>
<td>155.80</td>
<td>2.39 **</td>
</tr>
<tr>
<td>Rf x fruit type</td>
<td>9</td>
<td>71.93</td>
<td>1.10 NS</td>
</tr>
<tr>
<td>fraction x fruit type</td>
<td>1</td>
<td>970.32</td>
<td>14.88 **</td>
</tr>
<tr>
<td>Rf x fraction x fruit type</td>
<td>9</td>
<td>110.19</td>
<td>1.70 NS</td>
</tr>
<tr>
<td>Residual (error b)</td>
<td>324</td>
<td>65.19</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>399</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01

NS = Non-significant
Appendix 3

Analysis of variance table for the hormonal regulation of abnormal shuck opening (pecan shuck disease) (Situation 2).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit type</td>
<td>1</td>
<td>1466.89</td>
<td>5.75 *</td>
</tr>
<tr>
<td>Fruit/fruit type (error a)</td>
<td>18</td>
<td>255.20</td>
<td></td>
</tr>
<tr>
<td>Rf</td>
<td>9</td>
<td>612.01</td>
<td>8.33 **</td>
</tr>
<tr>
<td>Fraction</td>
<td>1</td>
<td>5760.81</td>
<td>78.38 **</td>
</tr>
<tr>
<td>Rf x fraction</td>
<td>9</td>
<td>493.37</td>
<td>6.71 **</td>
</tr>
<tr>
<td>Rf x fruit type</td>
<td>9</td>
<td>219.37</td>
<td>2.98 **</td>
</tr>
<tr>
<td>Fraction x fruit type</td>
<td>1</td>
<td>1584.04</td>
<td>21.55 **</td>
</tr>
<tr>
<td>Rf x fraction x fruit type</td>
<td>9</td>
<td>193.57</td>
<td>2.63 **</td>
</tr>
<tr>
<td>Residual (error b)</td>
<td>342</td>
<td>73.50</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>399</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01
*  = P less than 0.05
NS = Non-significant
Appendix 4

Analysis of covariance table for effects of GA, Alar, and their combinations on the incidence of pecan shuck disease.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check (covariable)</td>
<td>1</td>
<td>5241.56</td>
<td>1.67 NS</td>
</tr>
<tr>
<td>Replication</td>
<td>1</td>
<td>19487.92</td>
<td>6.19 **</td>
</tr>
<tr>
<td>Treatments</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G + A + Comb. vs Control</td>
<td>1</td>
<td>70217.18</td>
<td>22.31 **</td>
</tr>
<tr>
<td>G + A vs Comb.</td>
<td>1</td>
<td>64427.30</td>
<td>20.47 **</td>
</tr>
<tr>
<td>G vs A</td>
<td>1</td>
<td>59043.88</td>
<td>18.76 **</td>
</tr>
<tr>
<td>G 200 + G 350 vs G 500</td>
<td>1</td>
<td>6132.15</td>
<td>1.94 NS</td>
</tr>
<tr>
<td>G 200 vs G 350</td>
<td>1</td>
<td>1193.31</td>
<td>0.37 NS</td>
</tr>
<tr>
<td>A 1000 vs A 3000</td>
<td>1</td>
<td>10512.81</td>
<td>3.34 NS</td>
</tr>
<tr>
<td>G 200 x A + G 350 x A vs G 500 x A</td>
<td>1</td>
<td>9891.13</td>
<td>3.14 NS</td>
</tr>
<tr>
<td>G 200 x A vs G 350 x A</td>
<td>1</td>
<td>4041.71</td>
<td>1.28 NS</td>
</tr>
<tr>
<td>G 200 x A 1000 vs G 200 x A 3000</td>
<td>1</td>
<td>14.53</td>
<td>0.00 NS</td>
</tr>
<tr>
<td>G 350 x A 1000 vs G 350 x A 3000</td>
<td>1</td>
<td>166.70</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>G 500 x A 1000 vs G 500 x A 3000</td>
<td>1</td>
<td>102.03</td>
<td>0.03 NS</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>35112.71</td>
<td>11.16 **</td>
</tr>
<tr>
<td>Treatment x time</td>
<td>11</td>
<td>6415.39</td>
<td>2.04 NS</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>3146.50</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01  
G = Gibberellic acid (GA)  
NS = Non-significant  
A = Alar (SADH)  
Comb. = GA x Alar combination
Appendix 5

Analysis of covariance table for effects of GA, Alar, and their combinations on yields of pecan, cultivar 'Success'.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>15</td>
<td>10172.23</td>
<td>1.61 NS</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>6309.90</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant
Appendix 6

Analysis of covariance table for effects of IAA, GA, PBA, and their combinations on the incidence of pecan shuck disease.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>1</td>
<td>1140.36</td>
<td>0.28 NS</td>
</tr>
<tr>
<td>Treatments</td>
<td>10</td>
<td>10521.34</td>
<td>2.62 NS</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>42790.05</td>
<td>10.66 **</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>4015.57</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01

NS = Non-significant
Appendix 7

Analysis of covariance table for effects of IAA, GA, PBA, and their combinations on yield of pecan, cultivar 'Success'

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>1</td>
<td>1834.70</td>
<td>0.33 NS</td>
</tr>
<tr>
<td>Treatment</td>
<td>10</td>
<td>6316.27</td>
<td>1.14 NS</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>5641.60</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant
Appendix 8

Analysis of covariance table for effects of Kinetin, Alar, and their combinations on the incidence of pecan shuck disease.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>1</td>
<td>21380.65</td>
<td>14.58 **</td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>2032.00</td>
<td>1.39 NS</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>1531.39</td>
<td>1.04 NS</td>
</tr>
<tr>
<td>Error</td>
<td>5</td>
<td>1466.01</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P less than 0.01
*  = P less than 0.05
NS = Non-significant
Appendix 9

Analysis of covariance table for effects of kinetin, and the combinations of kinetin and Alar on kernel yield.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>1</td>
<td>335.50</td>
<td>0.45 NS</td>
</tr>
<tr>
<td>Treatment</td>
<td>6</td>
<td>3540.04</td>
<td>4.72 NS</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>750.74</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant
Appendix 10

Development of color with some specific chemical reagents by pinkish-red unknown inhibiting compound at Rf 0.3-0.4 in the acidic fraction of the ether extracts from the shuck-diseased fruits (Situation 2)

<table>
<thead>
<tr>
<th>REAGENTS *</th>
<th>COLOR DEVELOPED BY UNKNOWN INHIBITOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prochazka's reagent¹/</td>
<td>yellow</td>
</tr>
<tr>
<td>Modified Salkowski's reagent²/</td>
<td>yellow</td>
</tr>
<tr>
<td>Cinnamadehyde-HCl³/</td>
<td>yellow</td>
</tr>
<tr>
<td>NaNO₂ + HCl⁴/</td>
<td>yellow</td>
</tr>
<tr>
<td>Ninhydrin⁵/</td>
<td>orange</td>
</tr>
</tbody>
</table>

¹/ The chromatogram was sprayed with the mixture of formaldehyde (35-40%), conc. HCl and water (1:1:2). The chromatogram was air-dried.

²/ The spray reagent was prepared by mixing 50 parts 5% perchloric acid with one part of 0.05 M FeCl₃. The chromatogram was air-dried.

³/ The chromatogram was sprayed with 1% cinnamdehyde in methanol. The sprayed chromatogram was put in chamber containing equal parts of H₂SO₄ and HCl.

⁴/ The chromatogram was sprayed with solution of NaNO₂ (1 gm) in 1 N HCl (100 ml) and then air-dried.

⁵/ The chromatogram was sprayed with solution of 0.3 gm ninhydrin in 100 ml n-butanol and 3 ml acetic acid. The chromatogram was allowed to air-dried.

Chin Kit Ling was born on June 30, 1946 in Kajang, Selangor, Malaysia. He attended elementary school in Sungai Chua and completed his secondary school at Yu Hua National Type Secondary School, Kajang in 1965. He enrolled in the Further Education Classes at Methodist Boys' Secondary School, Kuala Lumpur in 1966. In 1967, he attended the College of Agriculture Malaya and was awarded a Selangor State scholarship in 1968. He received a diploma in general agriculture in 1970.

He worked as an Agricultural Assistant at the Plant Pathology Division of Department of Agriculture, Kuala Lumpur for one and a half months before being admitted into University of Malaya. After an academic term, he left for Louisiana State University and A & M College, Baton Rouge, Louisiana, United States of America, to pursue his studies. He obtained the B.Sc. (horticulture) in 1972.

Following graduation, he enrolled in the Graduate School of Louisiana State University in June, 1972 as a graduate assistant working toward his Doctor of Philosophy degree in Horticulture. While at Louisiana State University, he was awarded an Out-of-State fee exemption and Dr. Julian C. Miller honorary scholarship in 1971/72 academic year. In 1972, he received a certificate of merit for his outstanding service in the Law Enforcement Training program, Juvenile Officers Institute, Division of Continuing Education of Louisiana State University and was also initiated as a member of Honor...
Societies of Phi Kappa Phi and Gamma Sigma Delta of Louisiana State University Chapter.

In 1973, he married Kam Nyoke Heng and has one son.

He was selected as an outstanding member of Horticultural Club and was awarded Dr. John J. Mikell honorary scholarship in the academic years of 1972/73 and 1974/75.

He is now a candidate for the Doctor of Philosophy degree in Horticulture at Louisiana State University.
EXAMINATION AND THESIS REPORT

Candidate: Chin Kit Ling

Major Field: Horticulture

Title of Thesis: A Study of Endogenous and Exogenous Growth Regulators on Normal and Abnormal Shuck Opening of Pecan, Carya illinoensis (Wang) K. Koch

Approved:

William A. Young
Major Professor and Chairman

James L. Humphreys
Dean of the Graduate School

EXAMINING COMMITTEE:

James E. Forrester
R. P. Garthright
John E. Love
Emmett W. Lambert

Date of Examination: April 20, 1976