EFFECTS OF KINETIN ON DISTRIBUTION AND EXUDATION OF FREE STEROLS AND FREE FATTY ACIDS IN *ARACHIS HYPOGAEA* L. 'ARGENTINE' UNDER AXENIC CONDITIONS

by

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Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment for the degree of
MASTERS OF SCIENCE
in
Plant Physiology

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August, 1978

Blacksburg, Virginia

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to

for his patience and guidance during the course of the study and writing of this thesis. Her appreciation is extended to

for his suggestions during the study and in preparation of this manuscript. Her appreciation is extended to for his support and help in preparation of this manuscript.

The author would also like to thank for her assistance in the laboratory procedures and moral support.

The author's appreciation is extended to the Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia for the scholarships and teaching assistantship which helped make this study possible.

The author wishes to express her gratitude to her parents, and , for their support, understanding, and guidance.

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INTRODUCTION

Application of growth regulators that change the chemical constituents of plants may be a way to make plants resistant to stress. Chemical constituents such as free fatty acids and free sterols have been found to be important in helping the plant tolerate or resist injury from various stress conditions caused by pathogens, drought, freezing, and air pollution. For instance, sterols in plant tissues are inhibitory to some microorganisms and stimulatory to others (8). Resistance to air pollution damage has been correlated with sterol content of plant cells (130). Fatty acids have been found to be effective insecticides (107) as well as effective plant growth regulators (19, 131). Unsaturated fatty acids have also been found to be important in helping plants resist freezing and drought injury (92, 93).

The rhizosphere also plays an important role in host-parasite interactions. In many cases bacteria, fungi, and nematode populations have been found to be greater in rhizosphere soils as compared to non-rhizosphere soils (114). Material exuded from plant roots, which is utilized by microorganisms, is a contributing factor to the increase of rhizosphere organisms (53, 114). Thus control of root exudation may be an important factor in host-parasite interactions.

Growth regulators such as GA_3 (gibberellic acid) and 2, 4-D [(2, 4-dichlorophenoxy) acetic acid] have been found to alter the concentrations of free fatty acids and free sterols in lipids (55). To expand further on this research, applications of the growth regulator kinetin (6-furfurylamino purine) were used. Kinetin was selected for the

following reasons: (1) it is a naturally occurring growth regulator, (2) it has been shown to affect membranes and membrane permeability, (3) it is important in controlling plant senescence, and (4) it has been shown to mobilize plant constituents (16, 23, 78, 126, 127).

The objectives of the present study were: (1) to determine the effect of kinetin on the distribution of free fatty acids and free sterols of leaves, stems, roots in peanut plants grown under axenic conditions, (2) to determine if kinetin alters the exudation of lipid components in peanut plants grown under axenic conditions, (3) to determine if kinetin alters the qualitative and quantitative composition of free sterols and free fatty acids in plant parts and root exudates of axenically grown peanut plants.

LITERATURE REVIEW

Plant Root Exudation

Nature of Exudates.

Root exudation has been defined as the release of substances into the surrounding medium by healthy and intact plant roots (114). The existence of root exudation was noted by DeCandolle in the nineteenth century as a contributing factor to the "soil sickness" problem (cited by Hale *et al.*, 53).. Since that time several plant constituents have been identified in plant root exudates. Such compounds as amino acids, sterols and growth factors are commonly found in plant root exudates (53, 113, 114). In general the metabolites found within plants may be found in the root exudates.

Fatty Acid and Sterol Root Exudates.

Qualitative and quantitative studies concerned with free fatty acid and sterol root exudation has not received much attention. Youssef *et al.* (142) detected several lipid groups (including free fatty acids and sterols) in exudates of bean (*Phaseolus vulgaris* L.). Free fatty acids and free sterols were also detected in the exudates of peanut (*Arachis hypogaea* L.) by Hale *et al.* (55).

Site of Root Exudation.

Root exudation can occur at several sites along the root surface. The area immediately behind the root cap has been shown to be a major site of root exudation. Schroth and Snyder (118) grew bean plants on filter paper and then developed the paper with ninhydrin. They found

the root tips of primary, lateral, and adventitious roots produced high amounts of exuded amino acids. VanEgeraat (143) found the site of exudation of young pea (Pisum sativum L.) roots was located at the root tips. Other areas of root exudation are root hairs and the site of adventitious root emergence (53, 143, 144). Exudates can be produced as a result of physical injury of roots (4, 54). Injury can be caused by cultural practices or infection by pathogens (53, 54).

Effects of Exudates on the Rhizosphere.

The rhizosphere has been described by Hiltner (67) as being the zone of soil in which the microflora are influenced by the plant roots. Several cases have been noted where fungi, bacteria, and nematode populations were substantially higher in rhizosphere soils as compared to non-rhizosphere soils (98, 114, 124). Factors that determine the extent of the rhizosphere include; (1) the quantity of exudates produced by the plants, (2) the chemical nature of the exudates, (3) the ability of the compounds to be absorbed and/or decomposed by soil organisms, and (4) the soil-type, moisture and texture (114).

Several reviews are available on the interactions between root exudates and organisms in the rhizosphere (53, 110, 114, 119). Recent articles have noted the importance of free fatty acids and sterols in the rhizosphere. Youssef et al. (142) found root exudates of bean containing various lipid groups could stimulate fungal spore germination and mycelial growth of Fusarium solani (Mart.) Sacc. Earlier reports indicated that F. solani growth was governed by nutrients available in the root exudates of the host plant (24, 129).

Sterols are needed by nematodes in order to grow and reproduce (12, 91). Azmi and Jairajpuri (5) found substances in root exudates that attracted nematodes. Wang and Bergenson (152) found that nematodes altered the exudates of tomato both quantitatively and qualitatively, possibly through the utilization of the exudate. Kerry and Jenkinson (79) found that root exudates of host cereal plants stimulated the hatching of the cereal cyst nematode (Heterodera avenae Woll.).

Factors Affecting Root Exudation.

Plant Species. There are substantial differences in the quantity and quality of root exudates between different species of plants. Ayers and Thornton (4) noted the difference in amino nitrogen exudates between pea and wheat (Triticum aestivum L.). Balasubramanian and Rangaswami (6) studied the variations in amino nitrogen and hydrocarbons of sorghum (Sorghastrum sp. Nash.), sunnhemp (Crotalaria juncea L.), and tomato (Lycopersicon esculentum Mill.). Smith (128) studied the differences in exudation of carbohydrates, amino acids/amides, and organic acids in birch (Betula alleghaniensis Michx.), beech (Fagus grandifolia Ehrh.), and sugar maple (Acer saccharum Marsh.). These differences among species in the pattern of root exudation can alter the resistance of plants to various pathogens (113). For example, Currier and Strobel (25) noted that two species of Rhizobium spp. (Braun) showed chemotactic responses to certain legume and non-legume plants. In some cases one or both of the Rhizobium spp. showed either positive, negative, or no chemotactic responses to the plants. Kaiser and Gupts (76) noted that Fusarium oxysporum Schlecht. ex Fr.

grew more toward roots of the host plant than non-host plant. They concluded that this movement was due to stiumlatory substances in the host plant root exudates. Shukla (123) demonstrated that root exudates of gram (Cicer arietinum L.), wheat, and barley (Hordeum vulgare L.) (host plants) could stimulate the germination of sclerotia of Ozonium texanum ver. parasiticum Link ex Fr., but exudates from Brassica sp. L. (non-host plant) could not.

Plant Age. Singh (125) noted a slight variation in the rhizosphere fungal population due to variations in the nature of the root exudates by age. Root exudate production decreased with age in the cereal crops studied (125). Vancura and Stanek (150) found a decrease in exudation 15 days after the germination of bean seedlings. As the plants aged exudates began to increase in quantity again. A quantitative change was also observed in the bean root exudates. Goel and Mehrotra (47) observed an increase in the exudation of old okra (Abelmonschun esculentus L.) plants. Again, the change in the exudate was both quantitative and qualitative. Other research on the effects of age on exudation have been reviewed by Rittenhouse (110), Rovira (114), and Shay (119).

Temperature. Bokhari and Singh (12), working with root exudates of wheat grass (Agrophyron sp. Gaertn.), observed that high temperatures (29.5 C day/18 C night) stimulated root exudation. El-Habbasha and Behairy (39) found low temperatures (0 C and 5 C) and high temperatures (40 C) caused an increase in exudation of onion (Allium cepa L.),

tomato, and squash (Cucurbita pepo L.). Other reviewers (110, 114, 119) have indicated that great variability occurs amoung plant species with respect to temperature and root exudation.

Light. Rovira (112) working with tomato and subterranean clover (Trifolium subterraneum L.) found both quantitative and qualitative changes in root exudates under varying light conditions. In both plants the amount of exudate decreased at low light intensities.

Medium Supporting Roots. Most root exudate studies have been conducted using hydroponics (55, 110, 119). This method allows easy collection of the root exudate and minimized root damage. In one case pea seedlings grown in quartz sand showed a seven-fold increase in root exudation compared to hydroponic culture (14).

Root Damage. Damage of roots may occur by culture method or attacking organisms (14, 54, 114). Initiation of lateral root formation can also cause root damage (99, 100, 114). Root damage results in an increase in the quantity of root exudate (4, 54).

Soil Moisture. Flooding the root system of plants with water, results in a decrease in root exudation (17, 72). Conversely, drought conditions cause an increase in exudation (73, 77, 149). When the stress is removed exudation returns to the original level.

Foliar Application of Compounds. Several reviewers found correlations between foliar application of growth regulators and their subsequent detection in the rooting medium (53, 110, 119). It should also

be noted that foliarly applied compounds can alter exudate patterns qualitatively and quantitatively. Yoshida and Takashi (141) found that application of various forms of nitrogen to leaves of rice (Oryza sativa L.) increased root exudation of cytokinins. Jalali (74) studied the response of wheat root exudates to six foliar treatments of growth regulators and herbicides. He found that different treatments caused changes in the quantity and quality of the root exudates which in turn altered the fungal population of the wheat rhizosphere. Lee and Lockwood (89) found that application of chloramben (3-amino-2,5-dichlorobenzoic acid) caused an increase in root exudation which was correlated with increased pathogen attack.

Microorganisms. A large population of organisms (bacteria, fungi, and nematodes) live in the plant rhizosphere. Many of these organisms receive nutrients from the plants in the form of exudates (24, 109, 142). Rovira (114) stated that microorganisms can affect higher plant exudation in three ways: (1) by altering the permeability of root cells, (2) by altering root metabolism, or (3) by metabolizing the compounds and excreting the altered product. A recent study by Martin (98) demonstrated the effect of soil microorganisms on wheat root exudates. The presence of unidentified soil microorganisms significantly increased root exudation into the rhizosphere. Martin (98) found that the presence of microorganisms caused an increase in root lysis without penetration of the plant cell walls which accounted for the increase in exudation (98). Other reviewers have noted the effects of microorganisms on root exudation (110, 114, 119).

Cytokinins. Cytokinins present in the plant and root exudates can alter the chemical constituents of the plant by changing membrane permeability or altering the synthesis of various compounds. Cytokinins are well known for altering the protein and enzyme content of plant cells. Changes in the sterol content of plant cells caused by cytokinins has received little attention. Heble et al. (59) noted that an application of kinetin (2 ppm) - gibberellic acid (1 ppm) solution caused a decrease in the sitosterol content of nightshade (Solanum xanthocarpum Schrad.) tissue cultures. Recently Brain and Lockwood (15) noted the changes in free and bound sterol content in tissue cultures from Trigonella foenumgraeeum L. treated with kinetin and 2,4-D. Brain and Lockwood (15) found various fluctuations in the amounts of free and bound sterols depending on the type of cell culture (static vs. suspension) and the concentration of 2,4-D. Upon addition of kinetin to the static cultures the concentration of bound and free sterol decreased to the point of being negligible. In suspension cultures increasing kinetin concentrations caused an increase in free sterols and a decrease in bound sterols (15). Little has been done with respect to the interactions between cytokinins and free fatty acids in higher plants. Dimalla and VanStaden (38) suggested that cytokinins may play a role in mobilization of lipid food reserves in the plant.

Studies on root exudation have found that the root tip plays a major role in regulation of root exudation. Hong and Lucoff (69) attributed this regulatory role to the presence of cytokinins in the root tip. They proposed that cytokinins control root exudation by

monitoring the water permeability and rate of ion transport in the root tips.

Sterols in the Plant System

It has been well established that sterols are present in plant systems (8, 60). Plants synthesize a number of free and bound forms of sterols. Cholesterol, campesterol, stigmasterol, and sitosterol are the most prevalent free sterols in higher plants (8). Synthesis of sterols occurs by way of the mevalonic acid pathway (46). Heftmann (60) categorized sterols as possibly having three major functions: (1) to act as precursors for other plant sterols and steroids, (2) to function as plant hormones in control of growth and differentiation, and (3) to act as an integral part of the plant cell membrane. A fourth possible function of sterols is in plant protection.

Precursor Role.

Cholesterol is one of the first sterols formed from the mevalonic acid pathway and thus acts as a branch point for the production of other sterols and steroids. Numerous other substances such as sapogenins (diosegenin, yamogenin, and neotigogenin), alkaloids (tomatidine and solanidine), and the insect-molting hormones (ecdysone and ecdysterone) are known to be formed from cholesterol (60).

Hormonal Function.

Little work has been done relating the higher plant sterols to hormonal action and much of the research is contradictory. For example, sitosterol and stigmasterol had no effect on embryo growth of pea and did not alter chrysanthemum (Chrysanthemum sp. L.) stem elongation (10). In contrast sitosterol stiumlated growth of 6-day-old dwarf

pea and caused flower bud initiation in chrysanthemum (10). Cholesterol was inactive in stimulating growth of the 6-day-old dwarf pea or flower bud formation in chrysanthemum (10, 82). Cholesterol applied to the embryo of pea inhibited root growth (62). Leshem (90) found that cholesterol stimulated root growth and flowering of broccoli (Brassica oleracea L. var. cymosa) cuttings growing in culture medium.

Membrane Function.

Finean (43) first devised the relationship between sterols and membranes. He proposed a model to show the binding of plant sterols to phospholipids (in particular phosphatidylcholine). In Finean's model each long-chain lipid molecule in the membrane is stabilized individually by a cholesterol molecule. Darke et al. (26) using NMR spectroscopy, showed that cholesterol and phosphatidylcholine formed a complex in which the molar ratio between the two components was 1:1, thus demonstrating the interaction of one sterol per phospholipid. Hsia and Boggs (70), and Mailer and Taylor (97) demonstrated that by adding cholesterol to phosphatidylcholine, there was a decrease in the amount of movement which the fatty-acyl chains could undergo. Along with this decrease in the mobility of the fatty-acyl chains would come a tighter packing of the phospholipids. Demel et al. (35) and Chapman et al. (20) showed that incorporation of cholesterol into an artificial membrane monolayer of phosphatidyl-choline decreased the area each phosphatidyl-choline molecule occupied. DeKruyff et al. (31, 32) found that the magnitude of this compacting of the phospholipids increased with increasing proportion of cholesterol to the phospholipid. This increase

in "packing" of the phospholipids would tend to decrease the fluidity of the membrane which was similar to the findings of Feinstein et al. (42). Finkelstein and Cass (44) demonstrated that the addition of cholesterol decreased membrane permeability in artificial membrane systems. DeGier et al. (30) noted that the swelling of liposomes in hypotonic solutions was related to a decrease in membrane permeability caused by the presence of sterols in the medium. McElhaney et al. (101) and DeKruyff et al. (31, 32) emphasized the fact that the addition of cholesterol caused a decrease in permeability due to enhanced packing of the phospholipids, and not changes in the fatty acid composition of the phospholipids.

The degree of packing depends on the type of sterol present. The effects of different sterols, besides cholesterol, on the molecular spacing in monolayers and on permeability of liposomes was studied by Demel et al. (33, 34) and DeKruyff et al. (31, 32). In general, cholesterol had the greatest effect in maintaining membrane integrity in all systems (monolayers, liposomes, and cell membranes) followed by campesterol, sitosterol, then stigmasterol. Stowe and Dotts (132), and Grunwald (51) showed that this effect was due to extra carbon atoms in the side chain of the sterol molecule which decreased the ability of the sterols to pack into the phospholipid layers. Grunwald (49) working with phytosterols and their effects on membrane integrity found that cholesterol was more effective than $CaCl_2$ in stabilizing membranes. Grunwald (50) also found that free sterols were more effective than steryl esters or steryl glycosides in maintaining

membrane integrity. The latter two sterol derivatives were suggested as being possible transport forms of sterols.

Plant Protectants.

Levels of sterols have been shown to be correlated with plant stress induced by high salinity, drought, chilling, air pollution, and pathogen attack. Kuiper (83) noted a correlation in grape (Vitis vinifera L.) between the concentrations of sterols present in roots and tolerance to toxic levels of chloride. He found that lower sterol concentrations in root cells were correlated with higher chloride concentrations in the leaves (83). Stuiver et al. (133) using three different plants, bean, barley (Hordeum vulagre Linn.), and beet (Beta Vulgaris L.) found the same relationship between high sterol (free and esterified) content and increased salt tolerance. Sterol levels and salt tolerance were related to differences in membrane structure and permeability to sodium and chloride.

It has been suggested that resistance to temperature changes may be related to changes in sterol content. Madhosingh (95) found the sterol content to increase with decreasing temperature (10 C to 35 C) in Fusarium oxysporum Schl. em Sny. et Hans. Subsequently an increase in temperature caused a decrease of sterol content (95). Betouhim-El et al. (9) also found that the free sterol content was 1.4 to 3.7 times greater at lower temperature ranges (15 C to 18 C) than at higher temperatures (35.5 C to 37 C). Again the sterol changes were believed to be active at the membrane sites where maintenance of membrane integrity

would decrease the amount of cold damage.

Resistance to air pollution damage has also been correlated with the stabilizing effect of sterols on plant membranes. Spotts et al. (130) found that ozone would not cause as extensive damage in cholesterol treated plants as untreated plants.

Plant sterols play an important part in host-pathogen interactions. Two fungal genera, Pythium and Phytophthora, have both been shown to depend on exogenous supplies of sterols for the completion of normal growth and development (18, 22, 40, 41, 58, 63, 64, 65, 88, 116, 117). These fungi are believed to obtain required sterols from their plant hosts. The interaction between insects and their plant hosts can also be related to sterol requirements (13, 75, 111, 117, 151).

Fatty Acids in the Plant System

Fatty acids are widespread throughout the plant and are components of neutral lipids, waxes, glycerophosphatides, phytosphingolipids, and glycolipids. Fatty acids are found in membranes, waxes and as storage products throughout the plant. The most common free fatty acids found in plants are lauric, myristic, palmitic, stearic, arachidic, palmitoleic, oleic, linoleic, and linolenic (134).

Alteration of Plant Fatty Acid Composition.

The fatty acid composition of organisms can be altered by changing various growth conditions. For example, growing Cephalosporium flaciforme Corda and Cephalosporium kiliense Corda (two fungi) at different temperatures induced qualitative changes in the fatty acid composition of each organism (115). Diamantoglou and Meletiou-Christou (37) found the total lipid concentration of the bark and leaves of carob (Ceratonis siliqua L.) changed over a year period due to temperature variations in the different seasons. The total lipid content was highest during the cold winter months, and lowest during the hot summer months. Changes in levels of linolenic acid, were correlated with freezing resistance (37).

Light is also an important factor in determining fatty acid composition in plants (137). Light can change the saturation of fatty acids (either increase or decrease saturation depending on time, length, and quality of light) and also enhance the synthesis of glycerolipids and phospholipids (137). Heise and Stottmeister (61) found diurnal variation in the concentration of unsaturated fatty acids. During the

night saturated fatty acids were more prevalent than unsaturated fatty acids with the opposite situation existing during the day (61).

Plant Protectants.

Several possible roles for fatty acids have been demonstrated in protecting plants against stress. Perhaps the best known relationship between fatty acids and stress is in freezing resistance. In freezing resistance it was found that plants with a higher amount of unsaturated fatty acids were more resistant to injury (94). Lyons and Asmundson (93) found that increasing the unsaturated fatty acid content of an artificial system depressed the freezing point of the fatty acids.

Some striking relationships between host-pathogen interactions have been correlated with the fatty acid content of the host. Aksenova et al. (1) found that cabbage (Brassica oleracea capitata L.) mitochondria resistant to Botrytis cinerea Persoon contain more unsaturated fatty acids and unsaponifiable lipids than mitochondria of susceptible varieties. These fatty acids stabilized the mitochondrial membranes which resulted in increased pathogen resistance (1). Hubbes et al. (71) found that increases in linoleic acid in elm (Ulmus sp. L.) caused an increase in coremia production by Ceratocystis ulmi Jaekel. The linoleic acid caused striking shifts in the fatty acid composition of the mycelium of C. ulmi, which in turn began production of coremia. Zeller et al. (154) found that toxins produced by Pseudomonas phaseolicola Burk. caused changes in the fatty acid composition of young infected beet. The introduction of the toxin into the leaves caused a lowering of the poly-unsaturated fatty acids, which may be important

in stabilization of membranes to resist pathogen attack.

Fatty acids have been shown to be growth regulators. Proctor (106) studying the inhibitory effects of culturing different algae species together, found that certain algae produced fatty acids that inhibited the growth of other species. He noted that palmitic acid was the most toxic of the fatty acids, followed by lauric, myristic, and stearic acids. Oleic acid was found to be the least toxic (106). Poidevin (105) found the unsaturated fatty acids between C_2 and C_{19} inhibited germination of mustard (Sinapsis alba L.) seeds. The inhibition increased with increasing carbon chain lengths up to C_9 (nonanoic acid), and then decreased with further increases in length. Poidevin (105) concluded that germination was inhibited as a result of interference with some aspect of seed metabolism and not an effect on the surface tension or lipid solubility. Tso (138, 139) demonstrated that the meristematic and differentiating cells of axillary buds in tobacco (Nicotiana tabacum L.) were destroyed when treated with certain fatty acid alkyl or methyl esters. Tso found that the methyl esters of fatty acids with 8 to 14 even chain carbon atoms were effective in sucker control in tobacco plants. Separate tests with the uneven fatty acid methyl esters of pelargonic acid (C_9) and undecenoic acid (C_{11}) also showed effective sucker inhibition (138). Methyl esters of the C_{10} fatty acids were the most effective inhibitors (139). The effectiveness gradually diminished with increased (above C_{12}) or decreased (below C_4) carbon chain lengths (138, 139). Steffens et al. (131) found that fatty alcohols varying in carbon chain length from C_4 to C_{18} were

effective in inhibiting terminal and axillary bud growth of tobacco. Komoto et al. (80, 81) found that fumaric, palmitic, and oleic acids were produced in pea grown under red light which in turn inhibited the plant's ability to respond to gibberellic acid. Cathy et al. (19) found that alkyl esters of the C_8 to C_{12} fatty acids and the C_8 to C_{10} fatty alcohols could selectively kill the terminal meristems of a wide variety of plants.

Fatty acids were also effective against certain plant pathogens. Puritch (107) found that fatty acids and their soaps were toxic to the balsam woolly aphid (Adelgis pinceae Retg.). He noted that the most effective fatty acids and soaps for killing the balsam woolly aphid were divided into two groups: those centering around (1) caprylic and capric acids and around (2) oleic acid. Recently, Hankova and Fencl (57) found that fatty acids of carbon chain lengths of 12 or less inhibited the growth of the yeasts Saccharomyces cerevisiae Meyen ex Hansen, Candida utilis Henneberg, and Candida lipiytica Harrison.

Cytokinins in the Plant System

Distribution of Cytokinins in Plants.

Cytokinins are usually found throughout the plant. It has been established that naturally occurring free cytokinins are synthesized mainly in the roots (66, 122) and to a lesser extent in the leaves and fruits (56, 145). Information on the translocation of cytokinins in the plant is contradictory. Cytokinins have been found to move acropetally and basipetally in both the roots and aerial portions of the plant (103, 108). However, the most common situation seems to be basipetal movement in the roots and acropetal movement in the stems and leaves.

Studies done with girdling and radioactive tracers have shown free cytokinins to exist in the roots and, upon translocation, there were metabolic changes in the free cytokinins to glucosides or ribosides. Mozes and Altman (103) applied radioactive 6-benzylaminopurine to the roots of 'Seville' orange (Citrus aurantium L.), and found 51% of the radioactivity remained in the free form after 24 hr. VanStaden and Brown (146) used girdling experiments to determine the distribution of natural cytokinins in the plant. Girdling resulted in a decrease of cytokinin levels in the leaves and an increase in cytokinin levels just above and below the girdle. The decrease in the leaves was mainly due to the drop in the level of cytokinin-glucosides, which were also undetectable in the bark above the girdle. The glycoside form of cytokinin has been hypothesized as being a storage form of cytokinin (148). The cytokinin increase above and below the girdle was due to the presence of zeatin and zeatin riboside. It is now generally accepted that cytokinins, mainly zeatin and zeatin riboside, are the main translocated compounds

and are transported in the transpiration stream (3, 147). Thus, conditions of high transpiration favor enhanced transport of cytokinins from root to shoot. In one case the estimated velocity of cytokinin transport in an intact root was 2.6 to 5.1 mm/hr (103).

Effects on Membranes.

Cytokinins have been associated with various changes observed in plant membranes. Cytokinin ability to delay senescence has been correlated with maintenance of membrane integrity and subsequent reduction of leakage of protein and chorophyll from organelles or cells (56, 153). In the presence of kinetin, leaf disks and detached cotyledons of sunflower (Helianthus annuus L.) showed an increase in uptake of K^+, Rb^+, and Li^+, but not Na^+. This differential uptake altered the K^+/Ca^+ ratio which is important in a number of plant responses. Likewise a decrease in the K^+/Ca^+ ratio in leaves of plants suffering from nitrogen deficiency has been correlated with the simultaneous decrease of cytokinin content of nitrogen deficient leaves (48). Cytokinins can not only alter the transport of various cations across membranes, but they can also control the transport of various plant constituents. This is illustrated by the accumulation of plant constituents around the site of cytokinin application in senescing leaves. Cytokinins are able to control the transport of carbohydrates, lipids, amino acids and organic acids. Shindy et al. (121) found application of cytokinins to the roots of grape caused more carbohydrates to be translocated to the roots. Also the quantity and quality of materials translocated were

altered. LeJohn and Stevenson (87) found that addition of cytokinins to the fungus Achlya sp. Nees caused a displacement of calcium ions associated with a glycoprotein of the cell membrane. Along with this calcium release, the energy-linked transport of sugars, nucleosides and amino acids was inhibited by the cytokinin application. They found that the addition of magnesium counteracted the effect of cytokinins. As a result LeJohn and Stevenson concluded that the calcium and metabolite transport in the fungus depended on the delicate balance between the cytokinin, calcium and magnesium concentrations within the cell. Allfrey and Northcote (2) found that lipids, triglycerides, and starches stored in the cotyledons of peanut were mobilized by the presence of cytokinins. Likewise Dimalla and VanStaden (38) found cytokinins played a role in the mobilization of lipid food reserves in pecan (Carta illinoensis Koch.) nut which supply the energy required for germination. Kinetin was found to have varying effects on the membrane transport and permeability of Funaria hygrometria Hedw. (36). Kinetin affected the intensity and polarity of the luminescent dye transport across F. hygrometria membranes (36). Thus, cytokinins can control membrane permeability by altering membrane characteristics which affect transport of plant components across cell membranes. The actual mechanism by which cytokinins are able to affect membrane characteristics is unknown, however the binding of cytokinins to various membrane proteins may possibly be the mode of action. LeJohn et al. (86) first reported the interaction between cytokinins and calcium ions of glycoproteins. Calcium ions which are stored in a glycopeptide of what was believed

to be the cell wall were released upon the addition of cytokinins to the system. LeJohn and Cameron (85) found the glycopeptide to be a glycoprotein that could bind 20 atoms of calcium per mole of protein. The binding of the calcium was found to be allosterically regulated by cytokinins. LeJohn and Stevenson (87) reported the interaction of calcium and cytokinins on membrane transport as previously mentioned. LeJohn (84) assumed that if plant growth substances were really acting as hormones, then cytokinins must have a membrane target site on which to act. This hypothesis would be in keeping with the current concept of hormone receptors. LeJohn (84) found the calcium-binding glycoprotein also had specific binding sites for cytokinin and auxin. The alteration of metabolic transport by application of cytokinins set up a dual effect on the transport of L-amino acids. First, cytokinins were able to inhibit the energy dependent transport of amino acids, and secondly the uptake or binding of auxin or tryptophan could be stimulated in the same cells that showed inhibition of L-amino acid uptake. Other workers have shown that cytokinins have binding proteins in the higher plants. Takegami and Yoshida (136) isolated a cytokinin binding protein from tobacco leaves. They found the various cytokinin derivatives to be competitive for the protein binding site, therefore suggesting that the protein might play an important role in the regulation of cytokinin action. Sussman and Kende (135), and Yoshida and Takegami (140) have also been able to isolate cytokinin-binding proteins.

In a few cases varying concentrations of cytokinins have radically different effects on the plant. Bittner *et al.* (11) noted that high

concentrations of polysaccharide-bound-cytokinin (low concentration of free cytokinin) caused an increase in soybean (Glycine max L.) callus growth. LeJohn (84) found two different concentration ranges for cytokinin activity. By studying the stimulatory or inhibitory effect of kinetin on the energy-dependent transport of methionine and the binding of tryptophan or auxin by germinated spores of Achlya sp. Nees, LeJohn (84) found that kinetin at concentrations of 10^{-5} M or higher had an inhibiting effect while concentrations lower than 10^{-5} M had a stimulatory effect.

MATERIALS AND METHODS

Peanut plants, cultivar 'Argentine', were grown in liquid nutrient culture under axenic conditions in growth rooms (52, 120). Seeds (from greenhouse grown plants) were surface sterilized by removing the testae and soaking the seeds for 5 min in 20% commerical NaOCl at 50 C (52). Sterilized seeds were transferred to sterilized screw cap vials half full of Sabouraud dextrose agar prepared with ¼ strength Hoagland and Arnon (68) nutrient solution and incubated at 37 C for 48 hr. After seed germination the vials containing the seeds were placed in the growth room and after 6 days the uncontaminated vials were aseptically transferred to the isolator chambers. Seedlings were checked for contamination after 5 days in the isolator chambers; the sterile seedlings were then planted on glass wool in transplanting tubes (120) and transferred to 120 ml glass bottles containing ¼ strength nutrient solution. After 10 days of growth, nine plants in each chamber were selected for uniform growth and transferred to separate 1 L flasks containing ¼ strength nutrient solution. The nutrient solution was changed after 18 days, then weekly for 2 weeks. Kinetin (10^{-6} M and 10^{-4} M) was added to the nutrient solution during the final solution change. Each kinetin concentration was added to three of the nine plants in each of three isolator chambers with three plants left untreated as the control. Plants remained in the treatments for five days then were harvested and nutrient solutions collected. Upon harvesting, the plants were divided into leaves, stems and roots, placed in labeled paper bags, freeze dried, and weighed. The plant parts of

each of the three plants in each treatment were pooled separately, ground using a Wiley mill (40 mesh screen), and stored in desiccators for later analysis. Nutrient solutions from the three plants in each treatment were pooled, filtered through 0.45 u Millipore filters (Millipore Corp., Bedford, Mass.), frozen, freeze dried, weighed, and stored in a desiccator until analyzed.

For analysis, 1 g aliquots of leaf and stem samples and 0.5 g aliquots of root and exudate samples were placed in separate Soxhlet extraction thimbles and extracted 16 hr with 180 ml of chloroform: methanol (2:1 v/v). Samples were evaporated to dryness under vacuum at 40 C. A small amount of chloroform was used to redissolve the dried sample which was filtered and quantitatively transferred to pre-labeled and tared, teflon-lined screw cap test tubes (15 ml size). The chloroform was evaporated to dryness under nitrogen at 40 C and the tubes were re-weighed and weights subtracted from tared weights to obtain the total lipid weight of the samples. Lipid samples were dissolved with a small amount of chloroform and each total sample applied to thin layer silica gel G (0.75 mm thick) plates in a line 2.5 cm from the bottom of each plate. Chromatograms were developed in hexane: diethyl ether:acetic acid (80:20:1 v/v/v) made fresh daily and allowed to equilibrate in the chromatographic tank for 1 hr. Lipid components and appropriate standards were allowed to separate until the solvent front was approximately 2 cm from the top of the plate. The plates were dried, sprayed with dichlorofluorescein (0.2% in ethanol), and placed in a 100 C oven for 1 min. A long wave U.V.

lamp was used to locate the sterol, fatty acid, and hydrocarbon bands which were marked and scraped from the chromatographic plate. Each band was extracted with diethyl ether and filtered through a column containing a sintered glass filter. The diethyl ether extracts were collected in 2 ml test tubes and evaporated to dryness under nitrogen at 40 C. Sterol samples were dissolved with diethyl ether, transferred to reaction vials, evaporated to dryness under nitrogen, and derivatized with 0.06 ml of N,O-Bis (trimethylsilyl) acetimide (BSA, Pierce Chemical Company) for 50 min at 55 C. Fatty acid samples were derivatized by adding 3.0 ml of boron trichloride in methanol (10% v/v) to each test tube and shaken on a Vortex mixer. The samples were heated at 55 C for 5 min, cooled, and extracted three times with 5.0 ml of hexane. The combined hexane extract was evaporated to dryness under nitrogen and redissolved with 0.2 ml hexane.

Qualitative and quantitative analysis of lipid, sterol and fatty acid samples were conducted using a Bendix model 2600 gas chromatograph (Bendix Corp., Lewisburg, West Virginia). A 60 cm X 4 mm glass column packed with 3% SE-30 by weight on 100/120 gas-chrom Q (Applied Science Laboratories Inc., State College, Penn.) was run at 245 C (isothermal) for sterol analysis, and a 60 cm X 4 mm column of 10% EGS on 80/100 Chromosorb (Supelco Inc., Bellefonte, Penn.) was run at 190 C (isothermal) for fatty acid analysis. For sterol analysis the carrier gas (N_2) flow rate was 3.0 ml/min, air flow rate was 1 ml/min, and hydrogen flow rate was 35 ml/min. For fatty acid analysis the carrier gas (N_2) flow rate was 2.5 ml/min, air flow rate was 1.5 ml/min,

and hydrogen flow rate was 2 ml/min. Peak areas for the fatty acids were calculated by the equation (peak height) X (peak width at ½ height). Peak areas for the sterols were calculated using an Autolab Minigrator (Spectra-Physics, Santa Clara, Calif.). External standards of cholesterol, campesterol, stigmasterol, and sitosterol were used for quantitation and qualitation of sterols. Fatty acid quantitation was based on methyl stearate external standard and identification was based on external standards consisting of a series of known fatty acids (16:0, 18:0, 18:1, 18:2, 18:3, and 20:0). Statistical analysis of variance and significance at the 5% level was determined by use of the Duncan's multiple range test in the SAS-76 computer program (7).

RESULTS

Effects of Kinetin on Plant Dry Weights and Lipid Concentration in Plant Parts and Root Exudates.

The freeze dried tissue weight of the individual plant parts showed no significant differences between the control and kinetin treatments for the stems, roots, and root exudates (Table I). Significant increases in freeze dried weights of plant tissue were noted in the 10^{-6} M treatment of the leaves. Leaves decreased significantly in lipid concentration with both kinetin treatments. Lipid concentrations in other plant parts and root exudates did not differ significantly with treatment. It is interesting to note in Table I that the lipid weights in the root exudates between the control and 10^{-6} M treatment showed a three fold increase in concentration, yet the Duncan's multiple range test did not detect a significant difference. These measurements were based on the dry weight of the root exudate which also contained the nutrient salts of the culture solution. Since kinetin tends to change the nutrient uptake of the plant (48), the variability noted in the weights of the root exudates within the same treatments may be related to this observation. Such variability probably led to the rejection of significance in the Duncan's test.

Although the cumulative data from Table I indicates few significant differences in the components measured, certain trends can be observed if the data are expressed as ratios. Expressing the plant components as ratios could provide information with respect to redis-

tribution of metabolites in plant tissues. Thus, the leaf to stem ratio of dry weights and lipid weights (Table II) increased slightly in the 10^{-6} M treatment and decreased in the 10^{-4} treatment. The leaf to root ratio for dry tissue weight increased in the 10^{-6} M treatment and decreased slightly in the 10^{-4} M treatment. Ratios of leaf to root lipid weights decreased in both kinetin treatments. The leaf to exudate ratio and root to exudate ratio for both measurements decreased in the 10^{-6} M and increased in the 10^{-4} M treatments. The stem to root lipid ratio showed slight increases for both kinetin concentrations. The stem to exudate ratio for both measurements decreased in the 10^{-6} M treatment. Dry weight ratios of stem to exudate in the 10^{-4} M treatment increased.

Effects of Kinetin on Sterol Concentrations in Plant Parts and Root Exudates.

The four free sterols identified in the plant tissues and root exudates were cholesterol, campesterol, stigmasterol, and sitosterol. Of the four free sterols identified, sitosterol was present in the greatest amount. Sitosterol made up 66 to 73% of the total in leaves and stems and 46 to 66% of the total in roots and exudates (Table III). Treatments caused little change in the percentages of the individual sterols within the same plant parts, and regardless of treatments there were variations in percentages between plant parts. Individual free sterol concentrations decreased in the order of sitosterol, stigmasterol, campesterol, cholesterol. In the root exudates cholesterol was generally second highest in concentration.

Statistical analysis of the total sterol concentrations expressed as ug/mg dry weight revealed no significant change in free sterol concentration compared to the control (Table IV). In the stems, 10^{-6} M kinetin caused a significant decrease in sterol concentration compared to the 10^{-4} M kinetin treatment. On the basis of ug/mg lipid (Table IV) 10^{-4} M kinetin resulted in a significant increase in total free sterol in the stems. All other changes were not significant. Trends can be observed in the individual concentrations of the sterols and in the ratios of plant materials.

The concentrations of cholesterol and campesterol (ug/mg tissue dry weight) in the control and kinetin treatments (Table V) appeared

to remain fairly consistent. The concentration of stigmasterol in the leaves and stems decreased in the 10^{-6} M treatment and increased in the 10^{-4} M treatment. In the roots stigmasterol concentrations increased in the 10^{-4} M treatment. Sitosterol concentration decreased in the leaves in both treatments. In the stems sitosterol concentrations decreased in the 10^{-6} M treatment and increased in the 10^{-4} M treatment. In the roots sitosterol concentration increased with both kinetin treatments. In the root exudates sitosterol concentrations decreased in the 10^{-6} M treatment. Expressed as ug/mg lipid (TableVI), cholesterol concentrations increased slightly in 10^{-4} M treatment in leaves and 10^{-6} M treatment in stems and roots while it decreased in the 10^{-4} M treatment in the stems. Decreases in campesterol concentrations were noted in the 10^{-6} M treatment in the leaves and both kinetin treatments in the roots. Stigmasterol concentrations increased in both kinetin treatments in the leaves while in the stems and roots stigmasterol concentrations decreased in the 10^{-6} M treatment and increased in the 10^{-4} M treatment. Sitosterol concentration increased in leaves and stems and decreased in roots with both kinetin treatments. In the exudates all sterols decreased in concentrations in the 10^{-6} M treatment. Campesterol and stigmasterol concentrations in the exudates also decreased in the 10^{-4} M treatment, while sitosterol and cholesterol concentrations increased.

Shifts in sitosterol/stigmasterol ratios have been correlated with senescing activity in plant cells (27, 28, 29). Shifts in the sitosterol/stigmasterol ratio may have occurred as a result of

kinetin treatment (Table VII). In both the control and treatments the ratio of sitosterol to stigmasterol decreased in the order of stems, leaves, roots. Kinetin treatment caused slight increases in the ratio in the stems with a greater effect by 10^{-6} M kinetin than by 10^{-4} M kinetin. Both kinetin treatments resulted in slight decreases in the leaves and roots.

The leaf to stem ratio (ug/mg tissue dry weight) decreased in the 10^{-4}M treatment (Table VIII). The ratio leaf to root decreased in both kinetin treatments (ug/mg tissue dry weight) and increased in the 10^{-4} M treatment (ug/mg lipid). The leaf to exudate ratio increased in 10^{-6} M treatment for both measurements. The stem to root ratio increased in the 10^{-4} M treatment in both measurements and decreased in the 10^{-6} M treatment in ug/mg tissue dry weight. In stem to exudate ratio increased in both treatments for both measurements. In the root to exudate ratio increased occurred in the 10^{-6} M treatment for ug/mg tissue dry weight and both treatments for ug/mg lipid.

Effects of Kinetin on Fatty Acid Concentrations in Plant Parts and Root Exudates.

GLC analysis of fatty acids revealed the presence of 12 components in all plant parts and root exudates (Table IX). Five of the major components were used for the quantitative analysis. These five free fatty acids made up 78 to 92% of the total free fatty acids in the plant parts and 58 to 73% in the root exudates (Table XI).

Statistical analysis showed no significant differences to occur in the total free fatty acid concentrations of the various plant parts and root exudates as ug/mg tissue dry weight, and ug/mg lipid (Table X). Although the total free fatty acid concentrations did not show significant differences trends could be observed for some of the individual components and ratios of plant parts.

The relative percentages of each of the five major free fatty acids varied between both the plant materials and kinetin treatments (Table XI). Relative percentages of palmitic, stearic, and oleic acids were generally higher in the roots and exudates, and lower in leaves and stems. Percentages of linoleic acid were generally higher in the stems than the leaves and roots. Linolenic acid was generally higher in the leaves than the stems and roots. Relative percentages of both linoleic and linolenic acid were low in the root exudates. Decreases of 5% or greater (compared to the control) in the relative percentages of palmitic acid were noted in the 10^{-6} M treatment in stems, roots, and exudates, and 10^{-4} M treatment in the stems. A decrease of greater than 5% was noted for stearic acid in root exudates

in the 10^{-4} M treatment. Increases of 5% or greater in linoleic acid were noted in the 10^{-4} M treatment in stems and 10^{-6} M treatment in roots. Increases of 5% or greater in linolenic acid were noted for both treatments in stems, and 10^{-6} M treatment in roots.

Concentrations of the major individual free fatty acids in the exudates (ug/mg dry weight and ug/mg lipid weight, Tables XII and XIII) tended to decrease in the 10^{-6} M treatment. Linoleic and linolenic acid (fatty acids important in membranes) concentrations generally increased in leaves, stems, and roots with the kinetin treatments. Linoleic and linolenic acid concentrations decreased (ug/mg lipid) with the 10^{-4}M treatment (Table XIII).

Leaf to stem ratios of fatty acids were fairly consistent when expressed as a dry weight or lipid weight basis (Table XIV). The leaf to root ratio decreased in 10^{-6} M treatment (ug/mg tissue dry weight) and increased in 10^{-4} M treatment (ug/mg lipid). Increases in the leaf to exudate ratio were observed in the 10^{-6} M treatment on a dry weight or lipid weight basis. The stem to root ratio decreased in the 10^{-6} M treatment (ug/mg tissue dry weight) and increased in the 10^{-4} M treatment on a dry weight or lipid weight basis. The stem to exudate ratio increased in both treatments for dry weight or lipid weight. The root to exudate ratio increased in 10^{-6} M treatment for dry weight or lipid weight.

A comparison was made between unsaturated free fatty acids and free sterols since it has been found that unsaturated fatty acids inhibit the synthesis of sterols (95, 96). The comparison (Table XV)

revealed that concentrations of sterol in tissues were generally higher than unsaturated free fatty acid concentrations. Exudate concentrations of sterols were generally lower than unsaturated fatty acids. Comparison of total sterol to unsaturated fatty acid as a ratio (Table XVI) indicated that in the 10^{-6} M treatment the ratios decreased compared to the control. The total sterol to unsaturated fatty acid ratio decreased in the leaves and stems with both treatments and the roots and exudates with the 10^{-4} M treatment. An increase in the ratio was observed in the roots in the 10^{-6} M treatment.

Table I. Freeze dried weights and extracted lipid weights for plant parts and root exudates of 57-day-old axenically grown peanut plants. Average of three replications.

	Control		10^{-6}M Kinetin		10^{-4}M Kinetin	
Plant part	g tissue	mg lipid/ g dry wt	g tissue	mg lipid/ g dry wt	g tissue	mg lipid/ g dry wt
Leaves	2.83b*	11.77x	3.90a	9.53y	2.96b	9.44y
Stems	1.94c	2.59z	2.40bc	1.86z	2.44bc	2.84z
Roots	0.61d	1.84z	0.65d	1.94z	0.69d	2.17z
Exudates	0.25d**	0.64z	0.42d	2.00z	0.23d	0.70z

*Figures for the same measurements having same letters are not significantly different from each other (horizontal and vertical comparisons for same measurements) at the 0.05 level in the SAS-76 Duncan's multiple range test (7).

**Weights include nutrient salts dried with the exudates.

Table II. Ratios of dry weights and extracted lipid weight between plant parts and root exudates in 57-day-old axenically grown peanut plants. Average of three replications.

	Ratio of dry weights (g)			Ratio of lipid weights (mg lipid/g tissue)		
Plant components	Control	10^{-6}M Kinetin	10^{-4}M Kinetin	Control	10^{-6}M Kinetin	10^{-4}M Kinetin
Leaves/stems	1.4	1.6	1.2	4.5	5.1	3.3
Leaves/roots	4.6	6.0	4.3	6.4	4.9	4.4
Stems/roots	3.2	3.7	3.5	1.4	1.0	1.3
Leaves/exudates*	11.3	9.3	12.9	18.3	4.8	13.4
Stems/exudates	7.8	5.7	10.6	4.0	0.9	4.0
Roots/exudates	2.4	1.5	3.0	2.8	1.0	3.1

*Exudate weights include nutrient salts dried with the exudates.

Table III. Relative percentages of individual free sterols in plant parts and root exudates of 57-day-old axenic peanut plants. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Sterol	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
Cholesterol	1.5	1.2	1.8	1.3	1.2	1.0	0.7	1.0	0.8	22.4	19.0	21.5
Campesterol	3.2	2.8	3.1	10.1	11.4	9.8	11.1	8.3	11.2	13.4	8.4	6.5
Stigmasterol	26.6	29.5	28.8	20.6	14.5	17.6	32.9	43.4	35.3	18.1	25.7	5.6
Sitosterol	68.6	66.5	66.3	68.0	72.9	71.6	55.3	47.3	52.7	46.1	46.9	66.4

Table IV. Total free sterols of plant parts and root exudates of 57-day-old axenically grown peanut plants. Average of three replications.

	Control		10^{-6}M Kinetin		10^{-4}M Kinetin	
Plant part	ug sterol/ mg dry wt	ug sterol/ mg lipid	ug sterol/ mg dry wt	ug sterol/ mg lipid	ug sterol/ mg dry wt	ug sterol/ mg lipid
Leaves	1.189a*	10.314y	1.051a	11.088y	1.154a	12.166y
Stems	0.513bc	19.922x	0.433c	23.359wx	0.754b	26.221w
Roots	0.269cd	14.610y	0.269cd	13.850y	0.305cd	13.804y
Exudates	0.007d**	1.064z	0.002d	0.325z	0.005d	1.218z

* Figures for the same measurements having same letters are not significantly different from each other (horizontal and vertical comparisons for same measurements) at the 0.05 level in the SAS-76 Duncan's multiple range test (7).

** Weights include nutrient salts dried with the exudates.

Table V. Concentration of individual free sterols in ug/mg tissue dry weight in plant parts and root exudates of 57-day-old axenic peanut plants. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Sterol	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
Cholesterol	0.014	0.012	0.020	0.006	0.005	0.006	0.002	0.003	0.003	0.001	trace	0.002
Campesterol	0.038	0.028	0.036	0.053	0.049	0.072	0.029	0.027	0.033	0.002	trace	trace
Stigmasterol	0.327	0.315	0.333	0.100	0.062	0.135	0.089	0.088	0.109	0.001	trace	trace
Sitosterol	0.810	0.696	0.764	0.354	0.317	0.540	0.149	0.151	0.160	0.003	0.001	0.003

Table VI. Concentrations of individual free sterols in ug/mg lipid in plant parts and root exudates of 57-day-old axenic peanut plants. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Sterol	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
Cholesterol	0.157	0.138	0.215	0.250	0.291	0.254	0.110	0.167	0.116	0.238	0.070	0.261
Campesterol	0.336	0.308	0.383	2.011	2.654	2.563	1.618	1.372	1.538	0.142	0.030	0.079
Stigmasterol	2.742	3.267	3.503	4.115	3.377	4.626	4.802	4.507	4.874	0.193	0.093	0.067
Sitosterol	7.080	7.375	8.065	13.545	17.037	18.778	8.080	7.804	7.276	0.491	0.166	0.811

Table VII. Ratio of sitosterol to stigmasterol for each plant part and each treatment in 57-day-old axenically grown peanut plants. Average of three replications.

Treatment	Leaves	Stems	Roots
Control	2.6	3.4	1.7
10^{-6}M Kinetin	2.3	5.0	1.1
10^{-4}M Kinetin	2.3	4.0	1.5

Table VIII. Ratios of total sterol concentrations between plant parts and root exudates in 57-day-old axenically grown peanut plants. Average of three replications.

	Ratio of sterol to dry weight (ug/mg dry weight)			Ratio of sterol to lipid weight (ug/mg lipid)		
Plant component	Control	10^{-6}M Kinetin	10^{-4}M Kinetin	Control	10^{-6}M Kinetin	10^{-4}M Kinetin
Leaves/stems	2.3	2.4	1.5	0.5	0.5	0.5
Leaves/roots	4.4	3.3	3.8	0.7	0.7	0.9
Stems/roots	1.9	1.4	2.4	1.4	1.4	1.9
Leaves/exudates	179.5*	315.9*	179.0*	9.7	30.2	10.0
Stems/exudates	77.0*	130.0*	113.0*	18.7	63.6	40.8
Roots/exudates	45.5*	96.0*	46.0*	13.7	45.0	11.4

*Weights include nutrient salts dried with the exudates.

Table IX. Free fatty acids tentatively identified in peanut tissues and root exudates

Chain length	Chemical name	Trivial name
12:0	dodecanoate	laurate
14:0	tetradecanoate	myristate
16:0*	hexadecanoate	palmitate
16:1	hexadecamonoenoate	palmitoleate
18:0*	octadecanoate	stearate
18:1*	octadecamonoenoate	oleate
18:2*	octadecadienoate	linoleate
18:3*	octadecatrienoate	linolenate
20:0	eicosanoate	arachidate
20:1	eicosamonoenoate	eicosenoate
22:0	docanoate	behenate
24:0	tetracosanoate	lignocerate

*Major free fatty acid used in the calculations for the quantitative analysis.

Table X. Total free fatty acids (F.A.) of peanut parts and root exudates of 57-day-old axenically grown peanut plants. Average of three replications.

	Control		10^{-6}M Kinetin		10^{-4}M Kinetin	
Plant part	ug F.A./ mg dry wt.	ug F.A./ mg lipid	ug F.A./ mg dry wt.	ug F.A./ mg lipid	ug F.A./ mg dry wt.	ug F.A./ mg lipid
Leaves	0.982ab*	8.526yz	0.955abc	9.471yz	1.064a	11.280yz
Stems	0.657bc	24.026vwx	0.622c	33.135v	0.815abc	28.020vw
Roots	0.160d	18.087wxy	0.220d	23.102vwx	0.150d	13.577xyz
Exudates	0.056d**	9.390yz	0.017d	3.780z	0.060d	8.803yz

* Figures for the same measurements having same letters are not significantly different from each other (horizontal and vertical comparisons for same measurements) at the 0.05 level in the SAS-76 Duncan's multiple range test (7).

** Weights include nutrient salts dried with exudates.

Table XI. Relative percentages of major free fatty acids detected in peanut plant parts and root exudates. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Fatty acid	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
16:0 palmitic	20.46	19.86	19.45	29.01	22.67	21.18	35.81	25.37	33.70	31.94	22.47	31.98
18:0 stearic	7.37	8.19	7.14	9.02	7.66	7.10	10.24	7.42	11.19	25.55	19.86	24.12
18:1 oleic	2.74	2.31	2.49	3.82	3.76	3.92	5.38	4.17	5.43	8.62	9.85	9.95
18:2 linoleic	17.99	19.36	19.35	30.30	32.13	35.37	19.84	27.23	17.67	1.85	2.61	2.93
18:3 linolenic	43.42	41.65	40.73	19.27	26.29	26.04	8.52	18.62	10.50	3.96	3.51	4.43
Total	91.98	91.37	89.16	91.42	92.51	93.61	79.79	82.81	78.49	71.92	58.31	73.41

Table XII. Concentration of individual free fatty acids in ug/mg tissue dry weight in plant parts and root exudates of 57-day-old axenic peanut plants. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Fatty acid	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
16:0 palmitic	0.224	0.197	0.231	0.194	0.153	0.185	0.073	0.068	0.063	0.025	0.007	0.027
18:0 stearic	0.079	0.065	0.085	0.062	0.052	0.062	0.021	0.020	0.021	0.020	0.007	0.019
18:1 oleic	0.028	0.067	0.029	0.026	0.025	0.034	0.011	0.011	0.010	0.007	0.002	0.008
18:2 linoleic	0.189	0.196	0.231	0.228	0.216	0.306	0.039	0.072	0.035	0.001	trace	0.002
18:3 linolenic	0.461	0.431	0.488	0.147	0.177	0.228	0.016	0.049	0.021	0.003	0.001	0.004

Table XIII. Concentrations of individual free fatty acids in ug/mg lipid in plant parts and root exudates of 57-day-old axenic peanut plants. Average of three replications.

	Leaves			Stems			Roots			Exudates		
Fatty acid	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M	Contr.	10^{-6}M	10^{-4}M
16:0 palmitic	1.973	2.091	2.461	7.625	8.119	6.344	8.127	7.080	5.833	4.241	1.507	3.890
18:0 stearic	0.683	0.710	0.899	2.366	2.747	2.118	2.323	2.067	1.938	3.406	1.305	2.909
18:1 oleic	0.249	0.241	0.314	1.003	1.325	1.166	1.212	1.158	0.939	1.004	0.560	1.117
18:2 linoleic	1.648	2.038	2.448	7.967	11.523	10.594	4.500	7.600	3.058	0.261	0.146	0.279
18:3 linolenic	3.973	4.389	5.157	5.064	9.420	7.797	1.924	5.197	1.809	0.477	0.262	0.607

Table XIV. Ratios of total fatty acid concentrations between plant parts and root exudates in 57-day-old axenically grown peanut plants. Average of three replications.

	Ratio of fatty acid to dry weight (ug/mg dry weight)			Ratio of fatty acid to lipid weight (ug/mg lipid)		
Plant component	Control	10^{-6}M Kinetin	10^{-4}M Kinetin	Control	10^{-6}M Kinetin	10^{-4}M Kinetin
Leaves/stems	1.5	1.4	1.3	0.4	0.3	0.4
Leaves/roots	6.1	3.9	7.0	0.9	0.8	1.7
Stems/roots	4.1	2.8	5.4	2.6	2.8	4.1
Leaves/exudates	17.0	43.3*	17.2	0.8	2.2	1.2
Stems/exudates	11.4	31.0*	13.2	2.4	7.6	3.1
Roots/exudates	2.8	11.1*	2.4	0.9	2.6	0.7

*Weights include nutrient salts dried with the exudates.

Table XV. Total free sterol concentrations and unsaturated free fatty acid concentrations of plant parts and root exudates of 57-day-old axenically grown peanut plants. Average of three replications.

Plant part	Control				10^{-6} Kinetin				10^{-4} Kinetin			
	ug/mg lipid		ug/mg tissue		ug/mg lipid		ug/mg tissue		ug/mg lipid		ug/mg tissue	
	sterol	unsat. fatty acid	sterol	unsat. fatty acid	sterol	unsat. fatty acid	sterol	unsat. fatty acid	sterol	unsat. fatty acid	sterol	unsat. fatty acid
Leaves	10.31	5.87	1.19	0.68	11.09	6.69	1.05	0.69	12.17	7.60	1.15	0.75
Stems	19.92	14.61	0.51	0.40	23.36	22.27	0.43	0.42	26.22	19.56	0.75	0.57
Roots	14.61	7.64	0.27	0.06	13.85	13.95	0.27	0.13	13.80	5.81	0.30	0.13
Exudates	1.06	1.74	0.01	0.01	0.32	0.97	trace	trace	1.22	2.00	trace	0.01

Table XVI. Ratio of total free sterol and unsaturated fatty acid concentrations in plant parts and root exudates of 57-day-old axenically grown peanut plants. Average of three replications.

	Control		10^{-6}M Kinetin		10^{-4}M Kinetin	
Plant part	ug/mg lipid	ug/mg dry wt.	ug/mg lipid	ug/mg dry wt.	ug/mg lipid	ug/mg dry wt.
Leaves	1.8	1.8	1.7	1.5	1.6	1.5
Stems	1.4	1.3	1.0	1.0	1.3	1.3
Roots	1.9	4.5	1.0	2.1	2.4	2.3
Exudates	0.6	1.0	0.3	0.7	0.6	0.5

DISCUSSION

Statistical analysis showed few significant differences to occur in the total free sterol and free fatty acid concentrations. However, by use of ratios and percentages, trends were observed in the changing concentrations of free fatty acids and sterols with kinetin treatments. The shifts in the quantity and quality of the free fatty acids and free sterols may have been a result of one or a combination of effects which have been attributed to kinetin. In all cases it should be noted that kinetin elicited different responses in the plant at lower concentrations (10^{-6}M) than at higher concentrations (10^{-4}M). Kinetin concentrations lower than 10^{-5}M have been found to have opposite effects in the plant than those concentrations above 10^{-5}M (84).

Kinetin may have affected sterol concentrations through alteration of the senescence process in the peanut plant. Decreases in the ratio of sitosterol to stigmasterol have been correlated with increasing senescence in higher plants (27, 28, 29). Since stigmasterol is synthesized from sitosterol a decrease in the sitosterol/stigmasterol ratio may indicate a reduction in sitosterol synthesis with continued conversion of sitosterol to stigmasterol (45). The sitosterol/stigmasterol ratio between plant parts of the control showed that sitosterol concentration was higher in the stems than roots and leaves. The application of kinetin further increased the sitosterol concentration in the stems. Increases in sitosterol and decreases of stigmasterol were also noted in the stems in the relative percentages of the individual free sterols (Table III) and in the individual free sterol

concentrations in ug/mg lipid (Table VI). Statistically there was an increase in sterol concentration in the stems (ug/mg lipid) in the 10^{-4}M kinetin treatment. Kinetin treatments may possibly have been causing stimulation of cell growth through cell elongation (23, 56, 78) which may have required greater production of sterols for membrane development (43, 49, 50, 51). Increases in the relative percentages and amounts of linoleic and linolenic acid may also reflect growth in the stems (34, 137). An increase in growth of the stems due to the presence of kinetin might be expected since kinetin is transported acropetally from the roots (103, 108).

Kinetin could have caused changes in membrane permeability, thus altering the quantity and quality of free fatty acids and sterols present in the plant parts and root exudates. The ability of kinetin to delay senescence by reducing leakage of proteins and chlorophyll from organelles or cells has been correlated with the maintenance of membranes (56, 153). Kinetin has also been found to control root exudation which has been studied through changes in membrane permeability to water and ion transport by controlling water permeability and ratio of ion transport in the root tips (69). The decreases in the individual free sterols and fatty acid concentrations observed in the root exudates at the 10^{-6}M treatment could have been caused by the presence of kinetin. As stated previously, kinetin concentrations of 10^{-5}M have been found to cause an increase in membrane permeability while those concentrations lower than 10^{-5}M cause a decrease in membrane permeability (84). It is interesting to note that palmitic acid and stearic acid

were higher in concentration in the root exudates. The linoleic and linolenic acids were retained to a greater extent by the plant roots. Since linoleic and linolenic acids are important in membranes it seems reasonable that the retention of these fatty acids in the roots may have reduced exudation through maintenance of membrane integrity. The same may also be true for the increase of cholesterol and decrease of stigmasterol in the root exudates.

Kinetin may have had an effect on the biosynthesis of free fatty acids and free sterols. Kinetin may alter the availability of the biosynthetic intermediates that fatty acids, sterols, and cytokinins have in common. Fatty acids and sometimes naturally occurring cytokinins use malonyl-CoA as a precursor in their biosynthesis (134). Sterols have several mevalonic acid pathway intermediates that may be in common with cytokinin synthesis (60). Several cytokinins have side chains that are derived from the mevalonic acid pathway (21, 56, 102, 104). Application of kinetin to the plant may have reduced plant production of cytokinins thus allowing more substrate to be used in fatty acid and sterol production. Increases in fatty acid and sterol concentrations of kinetin treated plants would then be expected. Sterols and fatty acids were observed to increase in the leaves and stems (ug/mg lipid), and in one case (10^{-4}M treatment of ug/mg lipid) the increase of sterols was significantly higher than the control.

Kinetin has been shown to control the translocation of hydrocarbons, lipids, amino acids, and organic acids (2, 38, 87, 121). The changes in free sterol and fatty acid concentrations (both individual and total)

observed in the kinetin treated plants may have been the result of altered translocation patterns. In the 10^{-6}M kinetin treatment both total free sterols and fatty acids decreased in concentration in the leaves and stems with a subsequent increase in the roots. These trends would be expected since kinetin was applied to the roots. Different trends were observed in the total free fatty acids and free sterols for the 10^{-4}M kinetin treatment. Free sterols increased in stems and roots and decreased in leaves, while free fatty acids increased in leaves and stems, and decreased in roots.

Interactions between sterol and unsaturated fatty acid synthesis have been observed in other organisms (96, 97). It has been found that unsaturated fatty acids inhibit the action of hydroxymethylglutaryl CoA reductase (EC 1.1.1. 34, HMGCoA reductase), an enzyme involved in sterol production (46, 96, 97). If kinetin were to alter the unsaturated fatty acid concentrations in the plant, then the total sterol concentrations in the plant may likewise be altered. A comparison of the total free sterol concentrations to the unsaturated fatty acids (Table XV) was found to change depending on the plant material. Generally the total sterol concentrations were higher than the unsaturated fatty acid concentrations in the leaves, stems, and roots. In the root exudates the unsaturated fatty acid concentrations were higher than the total sterol concentrations. The ratios between the sterols and unsaturated fatty acids generally decreased, indicating a relative increase of unsaturated fatty acids over sterols (Table XVI). This could mean that kinetin may be affecting the interaction between the

unsaturated fatty acids and sterols. There are two possible ways in which kinetin may be acting. First, kinetin may have changed membrane transport causing a decompartmentalization of the sterols and unsaturated fatty acids. If unsaturated fatty acid concentrations became great enough, they may have affected the HMGCoA reductase enzyme. Secondly kinetin may have acted at the mRNA level to change HMGCoA reductase synthesis, thereby altering the synthesis of sterols. At this point the interaction of unsaturated fatty acids, sterols, and kinetin can only be speculated.

In conclusion, trends can be observed in the changing concentrations of the individual and total free fatty acids and sterols with kinetin treatment. Kinetin may have caused increases in the stem growth which required greater production of certain free fatty acids and sterols for membrane synthesis. Other effects of kinetin may have caused trends which were the result of: (1) mobilization of certain free fatty acids and free sterols to new locations in the plant, (2) alteration of membrane permeability which may have altered root exudation. Kinetin may have also had an effect on altering the sterol concentration in the plant through changes in HMGCoA reductase activity by unsaturated fatty acids.

The observed alterations in constituents of the various plant parts and root exudates may be important in helping the plant resist drought, freezing, air pollution, and pathogen injuries or stress. Fatty acids and sterols have been known to affect the plant's resistance to several stress conditions. Through alteration of plant constituents

by exogenous application of growth regulators such as kinetin more stress resistant plants may be produced.

SUMMARY

The effects of kinetin on the distribution of free fatty acids and free sterols in the leaves, stems, roots, and root exudates of peanut plants grown under axenic conditions were measured.

Peanut plants (Arachis hypogaea L. 'Argentine') were cultured axenically in nutrient solution under controlled conditions in controlled environmental growth rooms. Kinetin concentrations of 10^{-6}M and 10^{-4}M were applied to the roots through the culture medium five days before harvest. Upon harvesting, the plants were divided into leaves, stems, and roots, freeze dried, and dry weight measurements taken. The root exudates were likewise collected, freeze dried, and weighed. The lipid fraction of known aliquots of the plant material was obtained by Soxhlet extraction. Partitioning of the lipid fraction was accomplished by silica gel thin-layer chromatography. Quantitative and qualitative analysis for free fatty acids and free sterols was accomplished by isothermal gas-liquid chromatography with the appropriate external standards.

Kinetin applications caused a significant increase in the tissue weight of the leaves with 10^{-6}M kinetin treatment. Significant decreases of the total lipid weight in the leaves were noted with both kinetin treatments.

In the free fatty acid fraction twelve fatty acids were tentatively identified by comparing retention times with external standards. Five of the major peaks were used for quantitation and qualitation. On the

basis of ug fatty acid/mg tissue dry weight and ug fatty acid/mg lipid the kinetin treatments resulted in no significant changes in the total free fatty acid concentrations. A trend was detected for an increase in five fatty acids at 10^{-6}M kinetin treatments (ug/mg dry weight) in roots with decreases in the leaves and stems. In 10^{-4}M kinetin treatments (ug-mg lipid) free fatty acid concentrations increasd in all plant parts and decreased in exudates.

In the free sterol fraction, cholesterol, campesterol, stigmasterol, and sitosterol were identified. Stigmasterol and sitosterol occurred in the highest concentration in the leaves, stems, and roots, while cholesterol was higher in the root exudates. On the basis of ug sterol/mg tissue there were significant differences in total sterols in stems treated with 10^{-6}M kinetin and 10^{-4}M kinetin. Significant increases in total free sterol concentrations (ug sterol/mg lipid) were noted in stems when plants were treated with 10^{-4}M kinetin.

The percentage of individual fatty acids varied between plant parts, root exudates, and treatments. Treatments caused little change in the relative percentages of individual sterols within plant parts. Determination of the sitosterol/stigmasterol ratio and individual sterol concentrations showed a possible correlation between kinetin application and increased sitosterol synthesis. Interaction between unsaturated fatty acids and free sterols may also occur with kinetin treatments.

On the basis of qualitative and quantitative changes in the plant materials it was suggested that kinetin may alter membrane permeability,

constituent transport, and/or senescence processes through changes in free fatty acid and sterol concentrations.

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EFFECTS OF KINETIN ON DISTRIBUTION AND EXUDATION OF FREE STEROLS AND FREE FATTY ACIDS IN ARACHIS HYPOGAEA L. 'ARGENTINE' UNDER AXENIC CONDITIONS

by

Laura Kathryn Thompson

(ABSTRACT)

The effect of kinetin concentrations (10^{-6}M and 10^{-4}M) on distribution of free fatty acids and sterols in plant parts and root exudates of 57-day-old peanut plants grown in nutrient solutions under gnotobiotic conditions was studied. Kinetin was applied to the roots in the culture medium five days before harvest. Upon harvesting root exudates were collected and plants divided into leaves, stems, and roots. The extracted lipid fraction was partitioned using silica gel thin-layer chromatography. Quantitation and identification of free fatty acids and sterols were accomplished by isothermal gas-liquid chromatography.

On the basis of ug fatty acid/mg tissue and ug fatty acid/mg lipid kinetin caused no significant changes in total fatty acid concentration. The only significant increase in total sterol concentration was observed in the 10^{-4}M treatment (ug sterol/mg lipid) in the stems. Use of individual component concentrations, relative percentages, and ratios helped distinguish trends.

Shifts in quantity and quality of the free fatty acids and sterols may have been a result of one or a combination of effects attributed to

kinetin. Decrease in root exudation in the 10^{-6}M treatment may have been due to altered membrane integrity. Increase in the sitosterol/ stigmasterol ratio in stems of treated plants may have been a result of delayed senescence. Trends showed transport of free fatty acids and sterols to the roots in 10^{-6}M treated plants. Kinetin may have effected sterol concentration through changes in HMGCoA reductase activity by unsaturated fatty acids.