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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

EFFECT OF NITROGEN, POTASSIUM, AND SULFUR DEFICIENCIES ON THE CONCENTRATION OF CHLOROGENIC ACIDS AND SCOPOLIN IN SUNFLOWER

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EFFECT OF NITROGEN, POTASSIUM, AND SULFUR DEFICIENCIES

ON THE CONCENTRATION OF CHLOROGENIC ACIDS

AND SCOPOLIN IN SUNFLOWER

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EFFECT OF NITROGEN, POTASSIUM, AND SULFUR DEFICIENCIES ON THE CONCENTRATION OF CHLOROGENIC ACIDS AND SCOPOLIN IN SUNFLOWER

CHAPTER I

INTRODUCTION

The widespread occurrence of chlorogenic acid and scopolin has been reported in a great variety of plants (Sondheimer 1964, Winkler 1967). Despite this, few quantitative studies have been conducted involving changing environmental conditions.

Best (1944) reported on the accumulation and distribution of scopoletin in tobacco infected with the spotted tomato wilt virus. Scopoletin and its glucoside, scopolin, have also been reported to increase significantly in tobacco and sunflower treated with 2,4-dichlorophenoxyacetic acid (2,4-D), (Dieterman et al. 1964 a, b) and in tobacco treated with maleic hydrazide (Winkler 1967). Koeppe et al. (1969) found an increase in scopolin in both tobacco and sunflower when treated with increased U. V. intensities. Einhellig et al. (1970) found a significant increase in scopoletin and scopolin when tobacco, sunflower and pigweed seedlings were treated with scopoletin. This suggested a direct conversion of scopoletin to scopolin, within a plant.

Since its detection by Robiquet and Boutran in 1837, chlorogenic acid has attracted continuous attention regarding its physiological roles, biosynthesis, translocation, distribution and phytotoxic effects. A distinct decreasing gradient of chlorogenic acid was found from tip to base of tobacco leaf which contains 75% more chlorogenic acid than stem and root tissue (Zucker and Ahrens 1958). Koeppe et al. (1970) reported an increase of chlorogenic acid with age in leaves of sunflower. Koeppe et al. (1969) found that low U. V. intensities increase chlorogenic acid concentration in tobacco and sunflower.

Quantitative studies of effects of mineral deficiencies on phenolic compounds have been limited. Watanabe et al. (1961, 1964) reported a twenty-fold increase in scopolin in boron deficient tobacco and sunflower. Chouteau and Loche (1961, 1965) found an increase in scopolin and a decrease in chlorogenic acid in magnesium, calcium and phosphorus deficient tobacco leaves. However, in nitrogen deficient tobacco leaves, they found a significant increase in chlorogenic acid. Chlorogenic acid and scopolin were found to increase in concentration in tobacco when treated with varying nitrogen fertilizers (Tso et al. 1967). Armstrong (1968) found pronounced increases in chlorogenic acid in nitrogen deficient tobacco leaves and slight increases in scopolin in potassium, magnesium and nitrogen deficient tobacco leaves.

Since previous research clearly showed that various stress conditions affect the concentration of phenolics in plant tissue, this study was initiated to investigate mineral deficiency as a stress condition. Therefore, experiments were designed to determine the effects of nitrogen, potassium and sulfur deficiencies on the

distribution and amounts of scopolin, chlorogenic acid and its isomers within the sunflower plant and the possible effects of age on phenolic levels.

CHAPTER II

MATERIALS AND METHODS

Sunflower seeds (Helianthus annuus L. var. Russian Mammoth) were soaked for 24 hours in distilled water and were planted in thoroughly leached quartz sand in 4-inch glazed pots. Seed germination and subsequent growth were carried out in a Percival growth chamber (16 hour photoperiod; 8,000-10,000 lux; 28°C light period and 18°C dark).

Seven days after germination seedlings were thinned to one plant per pot. The pots were thoroughly leached with distilled water before treatment with nutrient solutions. Control plants were watered with complete Fe-EDTA Hoagland's nutrient solution (Hoagland and Arnon 1950). Experimental plants were made deficient in nitrogen, potassium and sulfur by watering with nutrient solution made deficient as suggested by Hoagland and Arnon (1950). The pots were leached every seven days with 3,000 ml of distilled water.

A control plant and a plant from each treatment were harvested at the end of 1, 2, 3, 4, and 5 weeks. The experiment was repeated ten times so that ten control plants and ten deficient plants were available for analysis from each harvest time. Each plant was separated into old leaves (over 6 cm from apex to base of blade), young leaves (less than 6 cm from apex to base of blade), stem and the entire root system. After recording fresh weights the tissues were boiled in isopropyl azeotrope

(88% isopropanol: 12% water) for 5 minutes to stop enzymatic activity. The fixed plant tissue was ground in a blender and extracted by the procedure used by Wilson et al. (1968). This method consisted of washing the plant tissue with approximately four times (ml) its fresh weight with boiling isopropyl-water (1:1, v/v), five times its fresh weight with IBMW (isopropanol-benzene-methanol-water; 2:1:1:1, v/v), and four times its fresh weight with boiling isopropyl azeotrope. The residue was placed in a Soxhlet extractor for a 24 hour extraction with isopropyl azeotrope and then for another 24 hours with isopropanol. The fixing solutions and extracts were combined and evaporated to dryness in vacuo. The residue was dissolved in IBMW, the final volume (ml) being approximately three times the fresh weight in grams of the harvested plant material.

Scopolin (the 7-glucoside of scopoletin), chlorogenic acid (3-0-caffeoylquinic acid), band-510 (4-0-caffeoylquinic acid), and neochlorogenic acid (5-0-caffeoylquinic acid) were quantitated utilizing one dimensional descending paper chromatography. An aliquot of the sample extract was streaked along a 15 cm line on Whatman #1 paper (23 x 57 cm) previously washed with 5% methanol for 20 hours. The chromatograms were developed with KFW (methyl isobutyl ketone: formic acid: water, 14:3:2, v/v) in glass cabs for 20 hours. After drying four distinct fluorescent bands were observed under U. V. light.

The fluorescent bands were cut out and eluted with 5% methanol for 18 hours. The eluates were brought to known volume with 5% methanol and read photometrically against a blank which was eluted from paper run through identical procedures. Chlorogenic acids were read at a wavelength of 324 mμ on a Beckman DB-G spectrophotometer. Scopolin was read

with a Model 110 Turner fluorometer using pyrex curvettes and a high sensitivity attachment at an instrument setting of 1X. A primary filter #7-60 and a secondary filter #2A plus #48 (Kodak Wratten filter) were utilized.

Standard reference curves (Fig. 1, 2) were prepared by carrying known quantities of authentic compounds through the same chromatographic procedure. The standard reference curve for chlorogenic acid was utilized for the quantitation of all three isomers, since their molecular extinction coefficients and absorption spectra are assumed to be the same (Zucker, Nitsch and Nitsch 1965).

Recovery of chlorogenic acid was 57% based on purissimum grade compound from Fluka AG, Bucks, Switzerland. Scopolin synthesized in Dr. S. H. Wender's laboratory on this campus gave a recovery of 78%.

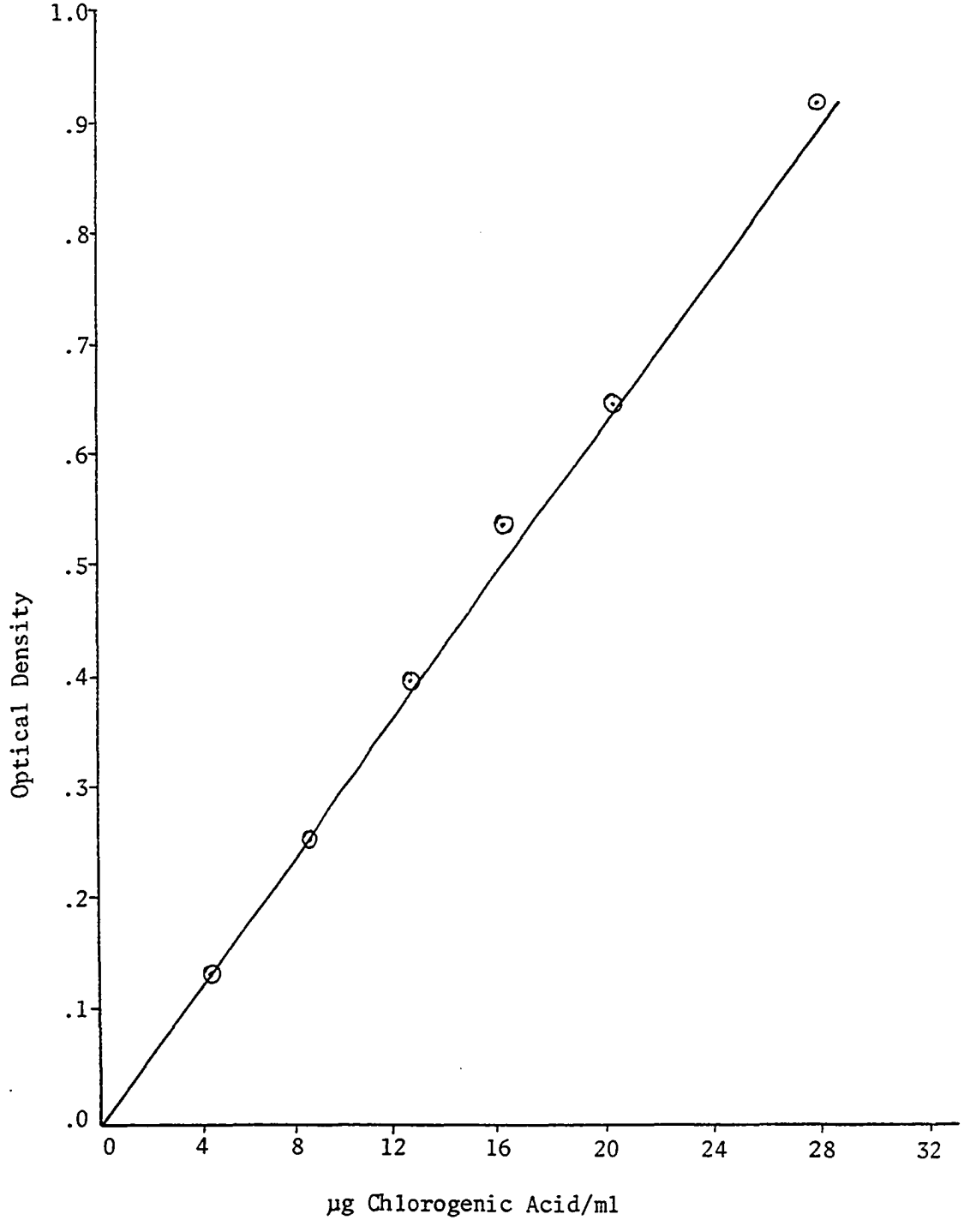

Fig. 1. Relationship of optical density and chlorogenic acid concentration read at 324 mµ on the Beckman DB-G spectrophotometer.

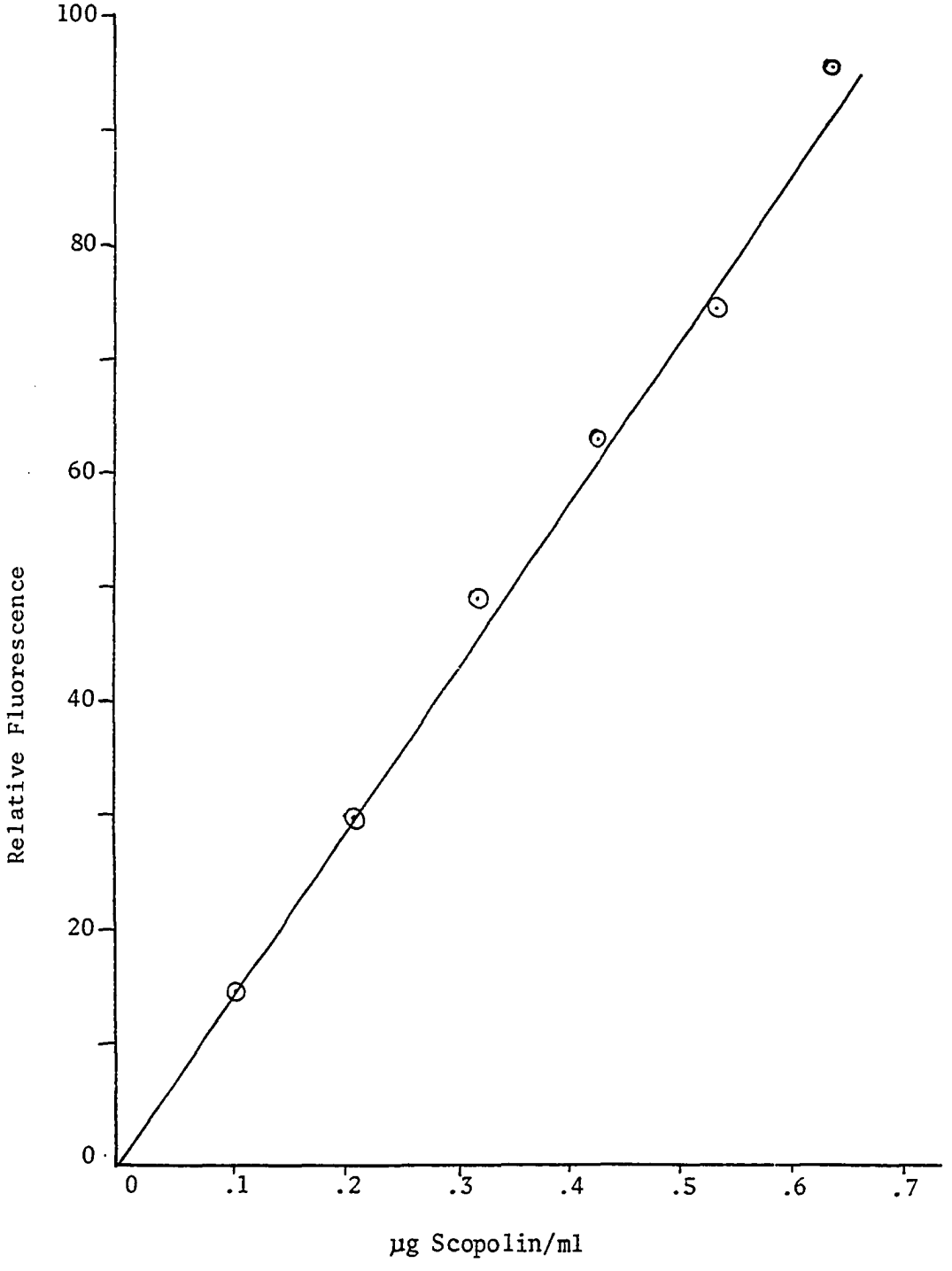

Fig. 2. Relationship of relative fluorescence and scopolin concentration on the Model 110 Turner fluorometer.

CHAPTER III

RESULTS

Concentrations of chlorogenic acid and neochlorogenic acid in control plants increased with age in leaves throughout the experimental period (Table 1, 3) and also in the roots and stems up to the third or fourth week but generally decreased after that. Band-510 increased with age in young control leaves and in old control leaves and roots up to the fourth week of treatment after which it decreased (Table 2). The amount was erratic from week to week in the stems and no trend was evident. The scopolin concentration in control plants increased with age in old leaves, remained rather stable in stems after the first week, and was not present in measurable amounts in young leaves and roots (Table 4).

Severe stunting and chlorosis of the more mature leaves were evident in nitrogen deficient plants after the first week of treatment. Young leaves of the nitrogen deficient plants remained green until the fourth week of treatment, yellowing (chlorosis) by the end of the fifth week. Little growth occurred in nitrogen deficient plants after the first week of treatment. A mottled chlorosis and slight stunting developed in potassium deficient plants after the first week of treatment. Neocrotic areas developed at the tips and margins of leaves and a "cupping under" was evident in potassium deficient plants after three

weeks of treatment. A slight chlorosis of the young leaves and stunting effect was evident in sulfur deficient plants after the fifth week of treatment.

Effects of Mineral Deficiencies on Chlorogenic Acids

Concentration of chlorogenic acid was significantly higher in the old potassium deficient leaves than in appropriate control leaves until the fourth week (Table 1). After a peak at the third week of treatment the concentration decreased until less chlorogenic acid was present than in old control leaves after five weeks. In nitrogen deficient plants the concentration of chlorogenic acid in old leaves was over ten times as high as in the appropriate controls each week (Table 1). The amount of chlorogenic acid in sulfur deficient old leaves was significantly higher than in appropriate control leaves during each week of treatment except the first (Table 1). Band-510 and neochlorogenic acid were generally significantly higher each week in sulfur and nitrogen deficient old leaves than in appropriate controls (Table 2, 3). In sulfur deficient old leaves band-510 and neochlorogenic acid increased in concentration with ageing of the plant (Table 2, 3). Potassium deficiency had little effect on the concentration of band-510 and neochlorogenic acid in old leaves during most weeks.

Chlorogenic acid concentration in young nitrogen deficient leaves remained stable after the third week of treatment and was significantly lower in concentration than corresponding control leaves during the second and fifth week of treatment (Table 1). Potassium and sulfur deficient young leaves generally had significantly higher concentrations of chlorogenic acid than the controls each week except the first

Table 1. Effect of mineral deficiencies on concentration of chlorogenic acid in sunflower. Each figure represents mean of 10 plants.

Treatment	µg chlorogenic acid/g fresh wt. Weeks of treatment				
	1	2	3	4	5
Control					
Old Leaves	132	235	317	255	727
Young Leaves	b	435	525	611	1275
Stems	137	129	283	295	249
Roots	35	85	109	372	208
Cotyledons	585	183	b	b	b
Potassium					
Old Leaves	403[a]	639[a]	1757[a]	1365[a]	576
Young Leaves	b	572[a]	1466[a]	899	1689[a]
Stems	500[a]	289[a]	360	517	763[a]
Roots	33	116	97	129[a]	85[a]
Cotyledons	653	b	b	b	b
Nitrogen					
Old Leaves	1541[a]	2931[a]	3321[a]	2624[a]	7492[a]
Young Leaves	b	b	783	721	789[a]
Stems	418[a]	1993[a]	1507[a]	1286[a]	2811[a]
Roots	27	351[a]	104	267	367[a]
Cotyledons	1643[a]	b	b	b	b

(Table 1. Continued)

Treatment	µg chlorogenic acid/g fresh wt. Weeks of treatment				
	1	2	3	4	5
Sulfur					
Old Leaves	196	1000[a]	879[a]	1374[a]	3259[a]
Young Leaves	b	1494[a]	2269[a]	1497[a]	3355[a]
Stems	353	401[a]	506[a]	520[a]	917[a]
Roots	15	215[a]	147	128[a]	297[a]
Cotyledons	447	b	b	b	b

[a] Amount differs significantly from appropriate control at 5% level or better.

[b] Below amounts determinable by procedure used.

Table 2. Effect of mineral deficiencies on concentration of 4-0-caffeoylquinic acid (band-510) in sunflower. Each figure represents mean of 10 plants.

	µg 4-0-caffeoylquinic acid/g fresh wt.				
	Weeks of treatment				
Treatment	1	2	3	4	5
Control					
Old Leaves	88	34	133	195	189
Young Leaves	b	244	237	222	379
Stems	40	48	51	33	52
Roots	b	24	29	160	48
Cotyledons	291	79	b	b	b
Potassium					
Old Leaves	89	92[a]	162	220	169
Young Leaves	b	247	187	219	171[a]
Stems	9	116	51	271[a]	350[a]
Roots	b	1[a]	28	41[a]	46
Cotyledons	b	b	b	b	b
Nitrogen					
Old Leaves	340[a]	788[a]	899[a]	398[a]	874[a]
Young Leaves	b	1[a]	75	510[a]	b [a]
Stems	15	219[a]	426[a]	400[a]	262[a]
Roots	b	39	1[a]	60[a]	42
Cotyledons	267	b	b	b	b

(Table 2. Continued)

Treatment	µg 4-O-caffeoylquinic acid/g fresh wt. Weeks of treatment 1	2	3	4	5
Sulfur					
Old Leaves	36[a]	116[a]	312[a]	343	562[a]
Young Leaves	b	91[a]	293	341[a]	543[a]
Stems	20	27	40	155[a]	141[a]
Roots	b	38	45	57[a]	69[a]
Cotyledons	b	b	b	b	b

[a] Amount differs significantly from appropriate control at 5% level or better.

[b] Below amounts determinable by procedure used.

Table 3. Effect of mineral deficiencies on concentration of neochlorogenic acid in sunflower. Each figure represents mean of 10 plants.

	µg neochlorogenic acid/g fresh wt.				
	Weeks of Treatment				
Treatment	1	2	3	4	5
Control					
Old Leaves	86	45	144	186	223
Young Leaves	b	36	39	70	83
Stems	32	56	67	62	82
Roots	b	27	53	62	47
Cotyledons	317	b	b	b	b
Potassium					
Old Leaves	33	96	106	211	87[a]
Young Leaves	b	26	214[a]	275[a]	141[a]
Stems	13	138	41	335[a]	345[a]
Roots	b	1[a]	12[a]	36[a]	47
Cotyledons	b [a]	b	b	b	b
Nitrogen					
Old Leaves	363[a]	658[a]	481[a]	186	518[a]
Young Leaves	b	1	1[a]	407[a]	84
Stems	64	287[a]	433[a]	326[a]	202[a]
Roots	b	119[a]	b [a]	56	81
Cotyledons	314	b	b	b	b

(Table 3. Continued)

Treatment	µg neochlorogenic acid/g fresh wt. Weeks of Treatment				
	1	2	3	4	5
Sulfur					
Old Leaves	42[a]	114[a]	219[a]	265	578[a]
Young Leaves	b	54	257[a]	259[a]	374[a]
Stems	47	35	11[a]	170[a]	134[a]
Roots	b	48	36[a]	63	98[a]
Cotyledons	b [a]	b	b	b	b

[a] Amount differs significantly from appropriate control at 5% level or better.

[b] Below amounts determinable by procedure used.

Table 4. Effect of mineral deficiencies on concentration of scopolin in sunflower. Each figure represents mean of 10 plants.

	µg scopolin/g fresh wt.				
	Weeks of treatment				
Treatment	1	2	3	4	5
Control					
Old Leaves	.1	.8	.8	2.7	7.2
Young Leaves	b	b	b	b	b
Stems	b	1.9	1.6	1.8	1.8
Roots	b	b	b	b	b
Cotyledons	b	b	b	b	b
Potassium					
Old Leaves	.1	.7	16.1[a]	31.4[a]	16.5[a]
Young Leaves	b	b	.2	1.3	1.1
Stems	b	.1	5.1[a]	17.3[a]	2.1
Roots	b	b	b	b	b
Cotyledons	b	b	b	b	b
Nitrogen					
Old Leaves	b	.4	.9	2.5	6.4
Young Leaves	b	b	b	b	b
Stems	b	b	b	b	b
Roots	b	b	b	b	b
Cotyledons	b	b	b	b	b

(Table 4. Continued)

Treatment	µg scopolin/g fresh wt. Weeks of treatment 1	2	3	4	5
Sulfur					
Old Leaves	b	2.9[a]	4.2[a]	3.7	7.7
Young Leaves	b	b	.3	b	b
Stems	b	6.7[a]	3.3	1.5	.6
Roots	b	b	b	b	1.8
Cotyledons	b	b	b	b	b

[a] Amount differs significantly from appropriate control at 5% level or better.

[b] Below amounts determinable by procedure used.

(Table 1). Band-510 and neochlorogenic acid concentration increased in sulfur deficient young leaves with increasing age of the plant and the concentrations were significantly higher generally than in appropriate controls after three weeks of treatment (Table 2, 3). The amount of neochlorogenic acid increased in potassium deficient young leaves up to the fourth week and then decreased (Table 3). The concentration was consistently significantly greater than in appropriate controls after three weeks of treatment. Potassium deficiency had little effect on the concentration of band-510 in young leaves except during the fifth week of treatment at which time the concentration was significantly reduced. The effects of nitrogen deficiency on the neochlorogenic acid and band-510 concentrations in young leaves were erratic (Table 2, 3).

The cotyledons dried up and abscissed by the end of the third week of treatment in all plants. Concentrations of chlorogenic acid in control, potassium and sulfur deficient cotyledons were generally similar (Table 1). A significantly higher concentration of chlorogenic acid occurred in the nitrogen deficient cotyledons after one week of treatment (Table 1). Band-510 and neochlorogenic acid occurred in rather high amounts in the control cotyledons during the first two weeks of the experiment and in nitrogen deficient cotyledons after one week of treatment. They were not present in measurable amounts in any other cases (Table 2, 3).

Chlorogenic acid increased in the stems of sulfur deficient plants with increasing duration of treatment (Table 1). The concentrations of chlorogenic acid in nitrogen, potassium and sulfur deficient stems were generally significantly higher each week than in appropriate controls (Table 1). Band-510 and neochlorogenic acid were significantly higher

in the stems in all deficiency experiments during the last two weeks of treatment than in corresponding controls (Table 2, 3). The stems of nitrogen deficient plants had significantly higher amounts of these compounds during the second and third week of treatment also.

Concentrations of chlorogenic acid, band-510 and neochlorogenic acid were generally higher each week in sulfur deficient roots and lower in potassium deficient roots than in appropriate controls (Table 1, 2, 3). The concentrations of these compounds were extremely variable from week to week in nitrogen deficient roots.

Effect of Mineral Deficiencies on Scopolin

Scopolin concentrations were generally not significantly different in nitrogen and sulfur deficient leaves than in appropriate controls (Table 4). There was a significant increase in sulfur deficient old leaves in the second and third weeks of treatment (Table 4). Potassium deficient leaves had significantly higher scopolin concentration during the last three weeks of treatment. In fact the young leaves of potassium deficient plants were the only young leaves with measurable amounts of scopolin. Scopolin in sulfur deficient stems decreased in concentration with age but the concentration was significantly different from the appropriate control concentration during only the second week of treatment (Table 4). The concentrations of scopolin in potassium deficient leaves differed significantly from appropriate controls only during the third and fourth week of treatment at which time they were higher (Table 4). Concentrations of scopolin were reduced in nitrogen deficient stems and were not present in amounts which were detectable with the methods employed. The concentrations of scopolin in roots and

cotyledons of controls and mineral deficient plants were below amounts determinable by procedures used.

Relationship of Phenolic Concentration to Appearance of Deficiency Symptoms

In general, pronounced changes in concentrations of the measured phenolics appeared at about the same time as marked deficiency symptoms except for sulfur deficiency. In the sulfur deficient plants, sizeable changes in phenolic concentrations occurred considerably before pronounced deficiency symptoms appeared.

CHAPTER IV

DISCUSSION

Effects of Age

The concentration of chlorogenic acid, 4-0-caffeoylquinic and neochlorogenic acid increased with age in the leaf tissue of Helianthus annuus. This agrees with the work of Koeppe et al. (1970). These findings differ from those observed in tobacco (Koeppe et al.1970, Armstrong 1968, Zucker and Ahrens 1958, Shiroya et al.1955), Coffea arabia (El-Hamidi and Wanner 1964), cotton (Morgan 1964) and in Theobroma cacao (Griffiths 1958).

Morgan (1964) reported that the amount of chlorogenic acid within a leaf may increase with age but remain lower in concentration than in a young leaf on the same plant. Zucker and Ahren (1958) suggested that this is related to the distance from the meristematic region. The results of this investigation support these findings because pronounced increases in chlorogenic acid concentration with age were indicated in the young leaves compared with concentrations in old leaf tissue.

Basipetal decreases in chlorogenic acid concentrations have been observed in several different plants including sunflower (Koeppe et al. 1970), peas (Galston 1957), Coffea arabica (El-Hamidi and Wanner 1964), cotton (Morgan 1964), tobacco (Koeppe et al. 1969, Armstrong 1968). Morgan (1964) found that the basipetal decrease in chlorogenic acid was

correlated with an increase in IAA oxidase activity in cotton. Basipetal increases in IAA oxidase activity have also been reported in pea stems (Galston and Dalberg 1954), in tobacco stems (Kerstetter and Keitt 1966) and are suggested to occur in tobacco and sunflower stems (Koeppe, Rohrbaugh and Wender 1969, Koeppe, Rohrbaugh, Rice and Wender 1970). Leopold (1964) reported that IAA concentrations generally decrease basipetally. Scott and Briggs (1960) reported a similar decrease in pea stems. The decreasing concentration with position of chlorogenic acid found in the sunflower plants of this experiment corresponds to the pattern observed by earlier workers.

It has generally been postulated that chlorogenic acid and other polyphenols have a synergistic effect on IAA activity, perhaps acting to suppress IAA oxidase (Rabin and Klein 1957, Tomaszewski and Thimann 1966, Taylor and Zucker 1966). Stafford (1965, 1967), Taylor (1968) and Taylor and Zucker (1966) suggested that ferulic and chlorogenic acids could be affected by age and may be involved in lignin synthesis.

Levy and Zucker (1960) found that randomly labeled glucose-C^{14} resulted in the formation of a chlorogenic acid molecule with activity in both caffeic and quinic acid. They suggested the occurrence of a cinnamyl-quinic-acid ester followed by hydroxylation to coumaryl ester acid and enzymatic conversion to chlorogenic acid. Steck (1967) suggested a biosynthetic pathway from phenylalanine involving caffeic, ferulic and glucosidoferulic acid into scopolin through an ester of ferulic glucoside. He also implied that scopolin may be produced as a metabolic end product when fiber production is inhibited. Gamborg (1967) found that quinic acid and caffeic acid served as a direct precursor of chlorogenic acid in potato cells. He postulated a metabolic pathway

involving the conversion of shikimic acid to chlorogenic acid through phenylalanine and the esterification of caffeic with quinic acid. He also suggested the incorporation of aromatic compounds into lignin synthesis. Taylor (1968) using $^{14}CO_2$ reported chlorogenic acid was converted into insoluble polymers and presumed these to be lignin. It is generally implied that the concentration of precursors may be altered by an increased synthesis of chlorogenic acid, scopolin, scopoletin or lignin. The rapid removal of these precursors from the pathway could alter the production of other phenolics.

Koeppe et al. (1970) suggested that scopolin accumulation in the leaves of tobacco may be a result of apical translocation. The increase in concentration of scopolin with age in the old leaves may be due in part to translocation from the stem, or as implied by Steck (1967) may result from an inhibition of fiber production.

Effect of Mineral Deficiencies

Chouteau and Loche (1965) reported that concentrations of chlorogenic acid in tobacco leaves increased with decreasing levels of nitrogen while in potassium deficient plants the concentration of chlorogenic acid decreased. Tso et al. (1967) studied four varieties of flue-cured and air-cured tobacco leaves at various levels of nitrogen fertilizations. They reported a direct relationship between the levels of nitrogen fertilization and concentration of chlorogenic acid and scopolin in three of the varieties with an inverse relationship in the fourth variety.

Armstrong (1968) found a pronounced increase in chlorogenic acid concentration in nitrogen deficient tobacco leaves and a decrease in

potassium deficient leaves only when exposed to less than 1300 ft c of light. He also reported an increase in scopolin concentration in nitrogen and potassium deficient tobacco leaves. The significant increases in concentration of chlorogenic acid in nitrogen deficient old leaves and significant decrease in potassium deficient old leaves by the end of the fifth week of treatment generally support the findings of Chouteau and Loche (1965) and Armstrong (1968). The increase in scopolin concentration with age in the nitrogen and potassium deficient old leaves supports the findings of Armstrong (1968).

The concentration in old leaves of chlorogenic acid was significantly higher in nitrogen deficient plants than in controls and was twice as high as in sulfur deficient plants. Also in nitrogen deficient plants one would expect the level of IAA to be low since both IAA and the enzyme system responsible for its synthesis contain nitrogen. Rabin and Klein (1957) and Henderson and Nitsch (1962) reported that chlorogenic acid has a synergistic effect on IAA or tryptophan synthesis. All chlorogenic acids inhibit IAA oxidase thus preventing breakdown of IAA (Sondheimer 1962). Thus a change in the content of chlorogenic acid and its isomers may influence the auxin level in the plant. Siegel et al. (1960) indicated that organic nitrogen compounds including IAA inhibit lignin synthesis. Thus the low concentrations of organic nitrogenous compounds in nitrogen deficient plants could stimulate lignin synthesis. Since it was observed that nitrogen deficient stems were most difficult to grind, this could be due to an increase in lignin. Taylor (1968) suggested also that chlorogenic acid may serve as a precursor of lignin and thus the large quantities of chlorogenic acids in some mineral deficient plants could stimulate lignification.

The significant increase in chlorogenic acid until the fourth week of treatment in potassium deficient old leaves with accompanying increases in scopolin concentrations, followed by a significant decrease in both by the fifth week, could be due to several factors. Chlorogenic acid may migrate out of the leaf, though active migration is not likely except in the precursor form (Taylor and Zucker 1966). There may be a decrease in synthesis and/or an accelerated destruction of chlorogenic acid, due to the activation of an enzyme system which metabolizes chlorogenic acid. A decrease in precursors could result in decreased chlorogenic acid concentrations in old leaves. Zucker et al. (1965) indicated that chlorogenic acid is easily metabolized and has a relatively rapid turnover rate in the living leaf. Since potassium is essential as an activator for enzymes involved in the synthesis of peptide bonds (Nason and McElroy 1963), increased concentrations of chlorogenic acid in potassium deficient plants may be attributed to lack of activation of enzymes which breakdown chlorogenic acid or enzymes which reduce nitrates. An imbalance of potassium also alters the uptake of nitrogen. The increase in scopolin concentration may be caused by an accumulation of caffeic or ferulic acid or as previously mentioned, translocation from the stem to the leaves, or may be an end product from the inhibition of fiber production.

The significant accumulation of chlorogenic acid with age in the sulfur deficient plant could be due to an induced nitrogen deficiency. McKee (1962) reported that before nitrates can be utilized by the plant in the synthesis of protein and other organic nitrogen compounds they must be reduced through a metabolic process. Eaton (1941) reported an

accumulation of nitrates and carbohydrates in sulfur deficient sunflower plants. He suggested that this was due to low nitrogen assimilation resulting from a low nitrate reductase content of the plant.

Increases of phenolic compounds in nitrogen deficient plants are considered important in the resistance of higher plants to pathogen infections. Kuć et al. (1956) reported that both chlorogenic acid and caffeic acid increased in potato tissues in response to inoculation with pathogens. Uritani and Muramatsu (1953) found that sweet potato produced more chlorogenic acid, scopoletin and other coumarins after inoculation with various pathogens. However, Johnson and Schall (1957) showed that although chlorogenic acid increased in tissue infected with a virus which causes "net necrosis", a similar increase was observed in uninfected cut slices, but the amounts of unidentified phenols differed. Uritani and Muramatsu (1953) found that scopoletin and certain other unidentified phenolic compounds from sweet potato have a much greater fungistatic effect than chlorogenic acid and suggested that they may play a role in resistance. Kiraly (1962) found that increased susceptibility was due to a decrease in phenol content, and reported that application of NH_4NO_3 at the rate of 280 lb/acre decreased the phenol content of healthy plants 60-70%, indicating that low levels of nitrogen fertilization enhanced wheat rust resistance. Kiraly and Farkas (1962) reported that rust-infected wheat tissue accumulated phenolic compounds and the initial speed of phenol accumulation was greater in the resistant combinations.

In addition to the physiological roles mentioned, scopolin, chlorogenic acid and its isomers and other phenols have been reported as having an important ecological role. Rice (1965) identified some of the

phenolic compounds important in ecological roles especially in the inhibition of nitrogen-fixing and nitrifying bacteria. Abdul-Wahab and Rice (1967) reported that chlorogenic acid and other phenolic compounds inhibited seed germination and seedling growth of most species found in the early stages of old-field succession. Wilson and Rice (1968) found that chlorogenic acid and isochlorogenic acid were present in all extracts of the various sunflower organs, and scopolin was present in the leaf leachate. They also reported that Helianthus annuus (native variety) was allelopathic to many of the early weeds including some of its own seedlings. Fields are abandoned in central Oklahoma because of low fertility and according to results of this investigation, this low fertility would accentuate production of phenolics by the mineral deficient inhibitor species. It is probable, therefore, that the increase in phenolics would increase the allelopathic effects of the inhibitor species in the first stage of succession and hasten the disappearance of these pioneer species.

CHAPTER V

SUMMARY

Concentrations of chlorogenic acid increased with age in control leaves of Helianthus annuus and also in stems up to the fourth week of experimentation. Scopolin concentrations increased with age in old leaves, remained stable in the stems during most of the experimental treatment, and was not present in measurable amounts in young leaves or in roots.

Chlorogenic acid concentrations were generally significantly higher in stems and leaves of mineral deficient plants than in controls. Except for nitrogen deficient plants, young leaves accumulated greater concentrations of chlorogenic acids than old leaves. Concentrations in roots were erratic and no definite trends occurred.

Band-510 and neochlorogenic acid were present in all plant tissues but not at all sampling times. Concentrations of both were generally higher in potassium deficient stems; nitrogen deficient old leaves and stems; and sulfur deficient old leaves, young leaves, and stems than in controls. In addition, neochlorogenic acid was generally higher in potassium deficient young leaves than in controls. Band-510 was usually lower in concentration in nitrogen deficient young leaves and roots, and neochlorogenic acid was generally lower in potassium deficient roots than in appropriate controls.

The concentrations of scopolin increased considerably in potassium deficient old leaves, young leaves and stems during the last three weeks of treatment over amounts in appropriate controls. In sulfur deficient stems the scopolin concentration decreased with age but was generally not significantly different from the control concentration. Nitrogen deficiency decreased the concentration of scopolin in stems but had no other effects.

In general, pronounced changes in concentrations of the measured phenolics appeared at about the same time as marked deficiency symptoms except for sulfur deficiency. In the sulfur deficient plants, sizable changes in phenolic concentrations occurred considerably before pronounced deficiency symptoms appeared.

The increased concentration of chlorogenic acids in sunflower with age and mineral deficiencies probably increases the allelopathic effects of that species in the first stage of old-field succession. Such fields are abandoned from cropping, of course, because they are so infertile that they are no longer profitable for cultivation.

LITERATURE CITED

Abdul-Wahab, A. S. and E. L. Rice. 1967. Plant inhibition by Johnson grass and its possible significance in old-field succession. Bull. Torrey Bot. Club 94: 486-497.

Armstrong, G. M. 1968. Effect of potassium, magnesium, and nitrogen deficiencies on the concentration of chlorogenic acid and scopolin in tobacco. Ph.D. Dissertation, Univ. of Okla. (Order No. 68-17, 584) 48 p. Univ. Microfilms. Ann Arbor, Mich. (Diss. Abstr. 29: 2311 B).

Best, R. J. 1944. Studies on fluorescence substances present in plants. II. Isolation of the substance in a pure state and its identification as 6-methoxy-7-hydroxy 1:2 benzopyrone. Aust. J. Exptl. Biol. Med. Sci. 22: 251-255.

Chouteau, J. and J. Loche. 1965. Incidence de la nutrition azotée de la plante de tabac sur l'accumulation des composés phénoliques dans les feuilles. C. R. Acad. Sci. 260: 4586-4588.

Dieterman, L. J., C-Y. Lin, L. M. Rohrbaugh, V. Thiesfeld and S. H. Wender. 1964. Identification and quantitative determination of scopolin and scopoletin in tobacco plants treated with 2,4-dichlorophenoxyacetic acid. Anal. Biochem. 9: 139-145.

Dieterman, L. J., C-Y. Lin, L. M. Rohrbaugh and S. H. Wender. 1964. Accumulation of ayapin and scopolin in sunflower plants treated with 2,4-dichlorophenoxyacetic acid. Arch. Biochem. Biophys. 106: 275-279.

Eaton, S. V. 1941. Influence of sulfur deficiency on metabolism of the sunflower. Bot. Gaz. 102: 536-556.

Einhellig, F. A., E. L. Rice, P. G. Risser, S. H. Wender. 1970. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. Bull. Torrey Bot. Club 97: 22-33.

El-Hamidi, A. and H. Wanner. 1964. The distribution pattern of chlorogenic acid and caffeine in Coffea arabica. Planta 61: 90-96.

Galston, A. W. 1957. Studies on indoleacetic acid oxidase and its inhibitor in light grown peas. Plant Physiol. 32 suppl. : xxi.

Galston, A. W. and L. Y. Dalberg. 1954. The adaptive formation and physiological significance of indoleacetic acid oxidase. Amer. J. Bot. 41: 373-380.

Gamborg, O. L. 1967. Aromatic metabolism in plants. V. The biosynthesis of chlorogenic acid and lignin in potato cell cultures. Can. J. Biochem. 45: 1451-1457.

Henderson, J. H. M. and J. P. Nitsch. 1962. Effects of certain phenolic acids on the elongation of Avena first internodes in the presence of auxins and tryptophan. Nature 195: 780-782.

Hoagland, D. R. and D. L. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. 347.

Johnson, G. and L. A. Schaal. 1952. Relations of chlorogenic acid to scab resistance in potatoes. Nature 115: 627-629.

Kerstetter, R. E. and G. W. Keitt, Jr. 1966. Direct assay of IAA decarboxylation rate in excised tobacco pith: relation to aging. Plant Physiol. 41: 903-904.

Kiraly, A. 1962. Phenol content in rust infected and nitrogen fertilized wheat leaves. (Abstr.) Phytopathology 52: 738.

Kiraly, Z. and G. L. Farkas. 1962. Relation between phenol content and stem rust resistance in wheat. Phytopathology 52: 657-664.

Koeppe, D. E., L. M. Rohrbaugh and S. H. Wender. 1969. The effects of varying U. V. intensities on the concentration of scopolin and caffeoylquinic acids in tobacco and sunflower. Phytochemistry. 8: 889-896.

Koeppe, D. E., L. M. Rohrbaugh, E. L. Rice and S. H. Wender. 1970. Tissue age and caffeoylquinic acid concentration in sunflower. Phytochemistry 9: 297-301.

Kuć, J., R. E. Henge, A. J. Ullstrup and F. W. Quackenbush. 1956. Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with Helminthosporium corbonium. J. Amer. Chem. Soc. 78: 3123-3125.

Leopold, A. C. 1964. Plant growth and development. McGraw-Hill Book Co., New York.

Levy, C. C. and M. Zucker. 1960. Cinnamyl and p-coumaryl esters as intermediates in the biosynthesis of chlorogenic acid. J. Biol. Chem. 235: 2418-2425.

Loche, J. and J. Chouteau. 1963. Incidences des carences en Ca, Mg, ou P sur l'accumulation des polyphénols dans la feuille de tabac. C. R. Acad. Agr. 16 octobre 1017-1026.

McKee, H. S. 1962. Nitrogen metabolism in plants. Clarendon Press, Oxford.

Morgan, P. W. 1964. Distribution of indoleacetic acid oxidase and inhibitors in light-grown cotton. Plant Physiol. 39: 741-746.

Nason, A. C. and W. D. McElroy. 1963. Modes of action of the essential mineral elements. In Plant Physiology (F. C. Steward, ed), vol. 3, p. 451-536, Academic Press, New York.

Rice, E. L. 1965. Inhibition of nitrogen fixing and nitrifying bacteria by seed plants. II. Characterization and identification of inhibitors. Physiol. Plant 18: 255-268.

Scott, T. K. and W. R. Briggs. 1960. Auxin relationships in Alaska pea. Amer. J. Bot. 47: 492-499.

Shiroya, M., T. Shiroya and S. Hattori. 1955. Studies on the browning and blackening of plant tissue. IV. Chlorogenic acid in the leaves of Nicotiana tabacum. Physiol. Plant 8: 594-605.

Siegel, S., P. Frost and F. Porto. 1960. Effects of indoleacetic acid and other oxidation regulators on in vitro peroxidation and experimental conversion of eugenol to lignin. Plant Physiol. 35: 163-167.

Sondheimer, E. 1962. The chlorogenic acids and related compounds. In Plant Phenolics and Their Industrial Significance (V. C. Runeckles, ed), p. 15-37. Symp. Plant Phenolics Group of North America. Imperial Tobacco Co. of Canada, Montreal, Quebec.

Sondheimer, E. 1964. Chlorogenic acids and related depsides. Bot. Rev. 30: 667-712.

Stafford, H. A. 1965. Factors controlling the synthesis of natural and induced lignins in Phleum and Elodea. Plant Physiol. 40: 884-851.

Stafford, H. A. 1967. Biosynthesis of phenolic compounds in the first internodes of Sorghum: Lignin and related products. Plant Physiol. 42: 450-455.

Steck, W. 1967. Biosynthesis pathway from caffeic acid to scopolin in tobacco leaves. Can. J. Biochem. 45: 1995-2003.

Taylor, A. O. 1968. The distribution and turnover rate of soluble and insoluble caffeoyl esters in Xanthium. Phytochemistry 7: 63-71.

Taylor, A. O. and M. Zucker, 1966. Turnover and metabolism of chlorogenic acid in Xanthium leaves and potato tubers. Plant Physiol. 41: 1350-1359.

Tomaszewski, M. and K. V. Thimann. 1966. Interactions of phenolic acids, metallic ions and chelating agents on auxin-induced growth. Plant Physiol. 41: 1443-1454.

Tso, T. C., T. P. Sorokin, M. E. Engelhaupt, R. A. Anderson, C. E. Bortner, J. F. Chaplin, J. D. Miles, B. C. Nichols, L. Shaw and O. E. Street. 1967. Nitrogenous and phenolic compounds of Nicotiana plants. I. Field and greenhouse grown plants. Tobacco Sci. XI: 133-136.

Uritani, I. T. and K. Muramatsu. 1953. Pathological chemistry of black rotted sweet potato 4. Isolation and identification of polyphenols from injured sweet potato. J. Agr. Chem. Soc. Japan. 27: 24-29.

Watanabe, R., W. Chorney, J. Skok and S. H. Wender. 1964. Effect of boron deficiency on polyphenol production in the sunflower. Phytochemistry 3: 391-393.

Watanabe, R., W. J. McIlrath, J. Skok, W. Chorney and S. H. Wender. 1961. Accumulation of scopoletin glucoside in boron-deficient tobacco leaves. Arch. Biochem. Biophys. 94: 241-243.

Wilson, J. L., R. J. Dunlap and S. H. Wender. 1968. Quantitative determination of chlorogenic acid in plant tissue by combined polyvinylpyrrolidase column and gas chromatography. J. Chromatography 35: 329-335.

Wilson, R. E., and E. L. Rice. 1968. Allelopathy as expressed by Helianthus annuus and its role in old-field succession. Bull. Torrey Bot. Club 95: 432-448.

Winkler, B. C. 1967. Quantitative analysis of coumarins by thin layer chromatography, related chromatographic studies, and the partial identification of a scopoletin glycoside present in tobacco tissue culture. Ph.D. Dissertation. Univ. Okla. (Order No. 68-729) 106 p. Univ. Microfilms. Ann Arbor, Mich. (Diss. Abstr. 28: 2722B).

Winkler, B. D., J. W. Mizelle, L. M. Rohrbaugh and S. H. Wender. 1969. Quantitative analysis of scopolin and scopoletin in tobacco plants treated with maleic hydrazide. Tobacco Sci. 13: 19-20.

Zucker, M. and J. F. Ahrens. 1958. Quantitative assay of chlorogenic acid and its pattern of distribution within tobacco leaves. Plant Physiol. 33: 246-249.

Zucker, M., C. Nitsch and J. P. Nitsch. 1965. The induction of flowering in Nicotiana. II. Photoperiodic alteration of the chlorogenic acid concentration. Amer. J. Bot. 52: 271-277.