

THE EFFECT OF VARIOUS CHEMICAL TREATMENTS
ON PEANUT POD ROT AND FREE ARGININE
CONTENT OF PEANUTS (ARACHIS
HYPOGAEA L.)

By
DAVID LEE NOWLIN
"
Bachelor of Science in Agriculture
Oklahoma State University
Stillwater, Oklahoma

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Thesis Approved:

Ray S. Sturgeon
Thesis Adviser
Kenneth E. Conway
Robert D. Morrison
Harmon A. Melton
E. L. Sing
Norman A. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

The most serious disease facing the peanut industry in Oklahoma is "pod rot," caused by a complex of organisms. Pythium myriotylum Dreschsler, Rhizoctonia solani Kuhn (Thanatephorus cucumeris [Frank] Donk) and Fusarium spp. have been identified as possible pathogens of the peanut pod rot disease complex (7, 8, 9, 10, 11, 13, 27, 31). This disease can reduce the average Oklahoma producer's yield and profit by more than 10% (31). Due to weather conditions favorable for the pod rot disease complex, 1977 was a damaging year, with peanut producers losing an estimated \$7,708,680 (29). Severe pod rot disease damage in Oklahoma is expected to continue because the causal agents are not fully known and no practical controls are available to peanut producers. The lack of above-ground symptoms in the pod rot disease complex makes it impossible to assess damages prior to harvest. A system is needed to detect the peanut pod rot disease (PPRD) prior to digging.

In 1972, Young et al. (38) investigated the effect of leafspot disease control on the newly developed Arginine Maturity Index (AMI) (35) of peanuts, because of the apparent delay of maturity when leafspot was controlled. They theorized that stress on plants, created by leaf loss (defoliation), caused the plants to mature at an early date.

The purpose of this investigation was to determine the effect of various chemical treatments on peanut pod rot disease severity and free arginine content of peanut fruit.

CHAPTER II

LITERATURE REVIEW

The Peanut

The peanut, Arachis hypogaea L., is a native South American legume (1). Johnson (19), in The Peanut Story, theorized that the peanut was gathered wild by South American people 10,000 years ago and supports his theory with a Peruvian ceramic vase which was excavated in ancient graves at Ancon, Pachacama (Peru) by archeologists. The vase was decorated with a design that resembled peanuts.

Controversy exists as to the range of the distribution of peanuts in America, prior to the arrival of explorers in the sixteenth century. Woodroof (33) reported that wild peanut species were abundantly distributed throughout most of South America. Higgins (15) reasoned that the peanut may have possibly grown wild in North America because there are indications that peanuts grew in Mexico and South America before the arrival of the Europeans. However, neither botanical records nor historical narratives are available to substantiate that the peanut was found by the early colonists (1).

In the sixteenth century, both Spaniards and Portugese explorers are thought to have carried peanuts to the East Indies. Later, the peanuts, grown in Africa, were used to feed the slaves being brought to the New World. In 1871, a Spanish cultivar was introduced into the United States to be grown commercially (19).

In 1921, George Washington Carver showed the importance of the peanut to the Ways and Means Committee of the House of Representatives by displaying numerous products he had developed (12). Today, the peanut is an important crop and a valuable food in every country where it is grown.

Measuring Free Arginine

Hoffpauir (16) was the first to publish a complete review of the chemical composition of the peanut. In 1958, Moore et al. (21), using ion-exchange chromatography, improved the procedure for analyzing amino acids. Their improved system made it possible for a complete amino acid analysis of a peptide or protein hydrolyzate in 24-28 hours, thus making the amino acid determination a quick and routine operation.

The most common means to quantitatively detect amino acids is by color reactions. Amino acids for which good color tests have been developed are: arginine, cysteine, histidine, proline, tryptophan, and tyrosine (6). Sakaguchi (26), in 1950, reported a new color reaction of arginine. He added oxine and hypobromite to arginine and produced a reddish-brown color which was stable enough to allow for a colorimetric measurement. Izumi (17,18), in 1964, reported the "New Sakaguchi Reaction" which had the following advantages: (a) the color produced by the Sakaguchi reaction was stabilized, (b) optimum conditions for the Sakaguchi reaction were easily maintained, and (c) a reliable standard curve could be produced.

In 1967, Newell (23) reported that with increasing maturity there was a decrease in the amino acid arginine, and an increase in peptide

II. Young and Mason (36) used the "New Sakaguchi Reaction" of Izumi (17,18) to quantitatively determine the level of free arginine in Oklahoma grown peanuts in order to predict seed maturity. In 1973, Young (35) adapted continuous flow equipment and found the precision and accuracy previously recorded by Young and Mason (36) was maintained. Using continuous flow equipment, Young's (35) method could analyze 30 to 50 samples per hour.

Hammons et al. (14) reported that free arginine was positively correlated to the percentage of "other kernels" or immature kernels, and that free arginine was negatively correlated to pod yield, sound mature kernels, total kernels, dry matter, and mature seed. Therefore, free arginine is believed to be highest in immature pods.

Peanut Pod Rot Disease

At present, pod rot disease of peanuts in Oklahoma is a complex mystery (29). Symptoms of the pod rot disease may appear at any stage of development of the peanut fruit. Light brown to dark lesions on the surface of the pod usually appear first. Rapid decay of the pod occurs and the entire pod may become brown to black. These symptoms vary widely among plants and within plants.

Wolf (32), reporting from Alabama, in 1914 was the first to report that Rhizoctonia solani Kuhn infects peanut fruit in the United States. He reported that there was no indication of the disease in the above-ground parts of plants, even when half of the fruit had been destroyed. A survey of the peanut diseases present in Alabama, Georgia, North Carolina, South Carolina, and Virginia was made in

1931 by Moore (22). Only one species of Fusarium was reported to cause peanut pods to rot; no mention of R. solani was made.

A survey of peanut diseases present in several states was taken in 1943 (4, 20, 25). Atkinson (4) isolated several species of Fusarium from fruit in various stages of rotting peanuts from fields in North Carolina and South Carolina. Rhoads (25) reported that R. solani was damaging fruit and lower stems on peanuts in Florida. Larsh (20) reported a Rhizoctonia root rot of peanuts in Oklahoma. The root rot was found in southern Oklahoma, with losses of less than 1% in all areas.

Prince (24), in 1944 isolated fungi from peanuts collected in South Carolina. He found several species of Fusarium, including Fusarium solani (Mart.) App. and Wr. emend Snyder and Hans., and reported finding Rhizoctonia solani; however, F. solani and R. solani were not reported as the more abundant fungal species found in peanut seeds.

Reports of substantial losses in yield and market quality, as a result of pod deterioration, came from Virginia in 1960 (10). In 1964, only four years later, Ashworth and Langley (3) reported this preharvest fruit rot had been observed in Virginia, North Carolina, Georgia, and Texas.

Garren (10), in Virginia, indicated Pythium myriotylum Drechsler as the prime pod rot pathogen and R. solani as an important pod rot pathogen which occurred sporadically. Garren (11) later found that R. solani could cause pod rot indistinguishable from that caused by P. myriotylum. He found that pod rot caused by R. solani developed much more slowly than that caused by P. myriotylum.

In 1975, Garcia and Mitchell (7, 8, 9) reported on the interactions of P. myriotylum, R. solani, F. solani, and Meloidogyne arenaria (Neal) Chitwood. They reported that pod rot disease was more severe when the soil was inoculated with P. myriotylum than when it was inoculated with F. solani, M. arenaria eggs, or the control. Also, they found that pod rot disease was more severe when pods were exposed to soil containing combinations of P. myriotylum with F. solani and M. arenaria than when pods were exposed to P. myriotylum alone (7). In 1975, they reported on the interactions of P. myriotylum, F. solani, and M. arenaria and on their effect on pre-emergence damping-off of peanuts. They again found the same synergistic results. R. solani was included in this investigation but did not produce the same synergistic effect with P. myriotylum that occurred with F. solani and M. arenaria (8). Garcia and Mitchell (9) reported again in December of 1975 that pods exposed to R. solani created an antagonistic effect to the development of rot in pods exposed to P. myriotylum. But this antagonistic effect was nullified if high populations of Macrophomina phaseolina (Maublanc) Ashby were present.

Sturgeon (31) reported that root knot (Meloidogyne hapla Chitwood), root lesion [Pratylenchus brachyurus (Godfrey) Filipjev and Stekhoven], and ring (Criconeoides spp.) nematodes seem to be involved in the pod rot disease complex in Oklahoma.

Shew and Beute (27) found mites (Caloglyphus spp.) to be associated with peanut pod rot caused by P. myriotylum. Ninety-eight percent of all mites tested were found to prefer P. myriotylum over five other fungi isolated from peanut fruit during food preference tests.

Pythium pod rot was reduced significantly in field and greenhouse tests of several acaricides and broad spectrum insecticides. Shew and Beute also found that the addition of soil-borne mites to field soil infested with P. myriotylum would significantly increase the incidence of peanut pod rot.

There are several other fungi capable of causing peanut pods to rot, either directly or indirectly. They are: Sclerotium rolfsii Saccardo, Sclerotinia sclerotiorum (Lib.) deBary varieties "minor" and "major" Purdy [Whetzelinia sclerotiorum (Lib.) Korf and Dumont], Aspergillus flavus (Link) Fries, Aspergillus niger van Tieghem, Diplodia gossypina Cooke, Cylindrocladium crotalariae (Loos) Bell and Sobers, Botrytis cinerea (Persoon) Fries, and Phymatotrichum omnivorum (Shear) Duggar. However, they all cause characteristic symptoms which distinguish them from the peanut pod rot complex.

CHAPTER III

METHODS AND MATERIALS

Pod Rot Disease Control Study

Six treatments replicated three times were selected from the 1978 Peanut Pod Rot Disease Test Plot located at the Caddo County Peanut Research Station near Fort Cobb, Oklahoma. They were: pentachloronitrobenzene and ethazole (Terraclor Super-X 10-2.5 G), methyl isothiocyanate and dichloropropene (Vorlex), bromomethane (Methyl-Bromide), carboxin (Vitavax 10G), ethazole, (Terrazole 35 W), and a non-treated control.

Chemical Treatments

A pentachloronitrobenzene and ethazole mixture (pentachloronitrobenzene and 5-ethoxy-e-trichloromethyl-1,2,4-thiadiazole) was applied at the rates of 2.24 kg. a.i. per ha. in an infurrow band at plant on June 12, 1978; 3.36 kg. a.i. per ha. in a 35.6 cm. band at midseason on July 19, 1978; and at 5.60 kg. a.i. per ha. in a 35.6 cm. band for the late season application on August 25, 1978. Carboxin 10 G (5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide) was applied at the rates of 2.24 kg. a.i. per ha. in an infurrow band at plant, 3.36 kg. a.i. per ha. in a 35.6 cm. band at midseason, and at 5.60 kg. a.i. per ha. in a 35.6 cm. band for the late season application. A soil fumigant, methyl isothiocyanate and dichloropropene mixture [methyl isothiocyanate

(20%) and chlorinated C₃ hydrocarbons (80%)] was applied on May 24, 1978, at the rate of 233.82 liters per ha. prior to planting. Bromomethane soil fumigant [bromo-methane (98%), chloropicrin (2%)] was applied on May 24, 1978, prior to planting at the rate of 46.76 liters per ha. Ethazole 35 W (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) was applied at the rates of 1.12 kg. a.i. per ha. as an infurrow spray with one 8003 fan nozzle per row at plant, and at 2.24 kg. a.i. per ha. as a basal spray with two 8003 fan nozzles per row at midseason and late season.

Granular infurrow band applications at plant were made in the seed furrow and banded in covering soil with a Gandy 901 Jr. applicator mounted on an International Harvester planter. Granular applications at midseason and late season were made through two seven-inch banders placed to distribute the fungicide at the base of the plant, covering the pegging zone. A Gandy Jr. applicator mounted on a tool bar was used to apply carboxin and a Lillston rolling cultivator was used to apply the pentachloronitrobenzene and ethazole mixture.

Soil fumigants were applied in liquid and gas forms into the seed beds. A gravity-flow applicator with two coulter per row was used to apply the liquid form of methyl isothiocyanate and dichloropropene mixture 15-20 cm. deep. The bromomethane gas was injected into the row beds and a latex soil sealer was sprayed over the bed to hold the fumigant in the seed bed. To offset the sterilization effect, Rhizoflo soil inoculant in the amount of 22.4 kg. per ha. was added to the bromomethane treated plots at plant. The yields and the rate of chemicals used are shown in Table I. Application dates and sampling dates are shown on the season calendar in Figure 1.

TABLE I

PEANUT POD ROT STATISTICAL REPORT: CADD O PEANUT
RESEARCH STATION, FT. COBB, 1978 (29)

Treatment	Yield* (kg. per ha.)
1. non-treated control	3751
2. pentachloronitrobenzene and ethazole mixture 10-2.5 G ¹	3707
3. methyl isothiocyanate and dichloropropene mixture; 233.82 liters per ha. ²	3707
4. bromomethane; 46.76 liters per ha. ³	3526
5. carboxin 10 G ⁴	3526
6. ethazole 35 W ⁵	3481

LSD .05	252

¹Pentachloronitrobenzene and ethazole 10-2.5 G (pentachloronitrobenzene and 5-ethoxy 3-trichloromethyl-1,2,4-thiadiazole) was applied at the rate of 2.24 kg. a.i. per ha. in an infurrow band at plant (6-12-78), 3.36 kg. a.i. per ha. was applied midseason (7-19-78), and 5.60 kg. a.i. per ha. was applied late season (8-25-78), both in a 35.6 cm. band.

²Methyl isothiocyanate and dichloropropene, a soil fumigant [methyl isothiocyanate (20%) and chlorinated C₃ hydrocarbons (80%)] was applied preplant (5-24-78) 15-20 cm. deep with two coulters per row.

³Bromomethane, a soil fumigant [bromomethane (98%) and chloropicrin (2%)] was injected preplant (5-24-78) into the top 25 cm. of the seed bed.

⁴Carboxin 10 G (5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide) 2.24 kg. a.i. per ha. was applied in an infurrow band at plant (6-12-78), 3.36 kg. a.i. per ha. applied midseason (7-19-78), and 5.60 kg. a.i. per ha. was applied late season (8-25-78), both in a 35.6 cm. band.

⁵Ethazole 35 W (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) was applied at the rate of 1.12 kg. a.i. per ha. as an infurrow spray at plant (6-12-78), and 2.24 kg. a.i. per ha. was applied midseason (7-19-78) and late season (8-25-78) as a basal spray.

*Mean yield for three replications.

May	24	5/24/78 Preplant Applications Made
	28	
	01	
	05	
	09	
June	13	6/12/78 Date Planted
	17	
	21	
	25	
	29	
July	03	
	07	
	11	7/19/78 Mid-Season Applications Made
	15	
	19	
August	23	
	27	
	31	
	04	
	08	
September	12	8/22/78 1st Sampling Date
	16	
	20	8/25/78 Late-Season Applications Made
	24	
	28	8/30/78 2nd Sampling Date
October	01	
	05	9/6/78 3rd Sampling Date
	09	
	13	9/11/78 4th Sampling Date
	17	
	21	9/18/78 5th Sampling Date
	25	
	29	9/24/78 6th Sampling Date
	03	
	07	9/29/78 7th Sampling Date
	11	
	15	
	19	10/17/78 Date Dug
	23	
	27	10/19/78 Date Harvested

Figure 1. Calendar for 1978 Peanut Pod Rot Study:
Caddo Research Station, Ft. Cobb,
Oklahoma

The plots were 3.66 meters wide (four rows) and 18.3 meters long. Treatments were applied to all four rows, with the inner two rows used for yield data, and the outer two rows used for sampling. The test was conducted on Tamnut peanut cultivar, which was irrigated.

Technique: Analysis of Free Arginine and
Peanut Pod Rot Disease Severity

Plants were dug at selected sampling points within the treated plots using a typical field spade. All plants were then washed with cold water to remove excess soil. All pods (Stages d, e, and f, Figure 2) were removed from the vine by hand picking and then stored at approximately -5°C. Each sample consisted of enough plants from each sampling site to provide at least one liter of pods. Samples were taken on August 22, August 30, September 6, September 11, September 18, September 24, and September 29 during the 1978 season.

The one liter samples were removed from the freezer and rated for disease severity. Light brown to black discoloration of pods were used as a measure of the presence of peanut pod rot disease. Hameed (13), Plant Pathologist with the Oklahoma State University Plant Disease Diagnostic Laboratory, isolated a Rhizoctonia sp. and Fusarium spp. from young pods collected at the Pod Rot Test Plot. The amount of sample discoloration was estimated visually on the basis of a 0 to 10 scale (0=no discoloration and 10=approximately 100% discoloration, Table II).

Samples were chopped in a Hobart food chopper for two minutes and two 30 gram subsamples were removed. One of the 30 gram subsamples

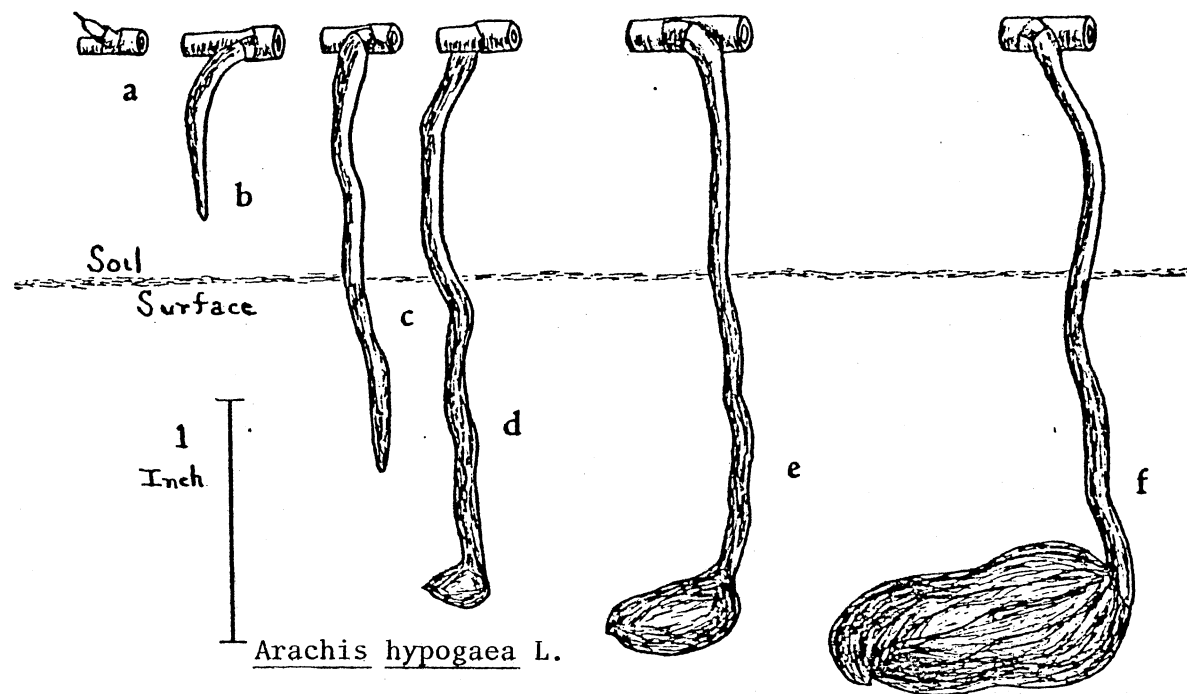


Figure 2. Successive Stages of Fruit Development in *Arachis hypogaea* L.
 a) Ovary at Time of Syngamy. b) Aerial Peg, 5-7 Days.
 c) Soil Penetration, 8-12 Days. d) The Beginning of Pod
 enlargement, 14-21 Days, e) Early Stage in Pod Development.
 f) Immature Fruit (28)

was dried for eight hours at 125°C in a forced heat oven and then reweighed to determine the percent dry weight. The second subsample was blended 30 seconds in a Waring Blendor with 200 ml of 2% trichloroacetic acid. The solution was then allowed to stand for a minimum of 10 minutes in order to avoid a milky filtrate, which could interfere with color readings (35). Approximately 75 ml was filtered through a Whatman #2 filter paper. A 20 ml cup was then partially filled with the filtrate and placed on the rotating sampler tray of the Technicon Autoanalyzer II. Samples were analyzed at the rate of 30 per hour (one sample was aspirated into the system every two minutes). Each sample required approximately 10 minutes to pass through the Technicon Autoanalyzer II system. All samples were processed on the same date.

TABLE II

POD ROT DISEASE SEVERITY RATING BASED ON A
MEASURE OF PERCENT SAMPLE DISCOLORATION

Pod Rot Disease Rating	Discoloration
0	None
1	Approx. 10%
2	Approx. 20%
3	Approx. 30%
4	Approx. 40%
5	Approx. 50%
6	Approx. 60%
7	Approx. 70%
8	Approx. 80%
9	Approx. 90%
10	Approx. 100%

All the samples were analyzed using the modified Sakaguchi reaction for arginine (17,18) in a continuous flow system (Technicon Autoanalyzer II) as reported by Young (35). The Technicon Autoanalyzer II system performed the analytical functions automatically (I. pumping, II. proportioning, III. mixing, IV. measuring, and V. recording). I. The Technicon Variable Speed Proportioning Pump module proportions the air segments or various liquids. The air segments separate the different samples to prevent unwanted contamination. II. The tubing size (used in the proportioning pump) determines the portion of air segments or various chemicals used in the analysis (Table III). III. The chemical reagents are mixed with the sample as they flow through vertical loops of glass mixing coils. IV. The Technicon Colorimeter measures the percent of light transmitted through the mixture as it moves through a flow cell. V. The Technicon Single-Channel Recorder receives the light readings from the colorimeter and records them as curvatures on a moving chart. The maximum height on the curve represents the value of maximum concentration for the sample.

The following analytical functions were made automatically in the Technicon Autoanalyzer II system. The sample was aspirated into the Technicon Autoanalyzer II system via an automatic sample probe. The sample probe was washed for 40 seconds between each sample with a wash solution (2 ml Brij/liter of deionized water). Two percent KOH (potassium hydroxide) and dry acetic anhydride, were added to the samples (samples were separated by air segments) and mixed as they moved through a 14-turn glass coil. A 1-naphthol solution (0.01% 0.00059 M in 10% aqueous potassium hydroxide) was added to the acetylated sample and mixed in a 12-turn glass coil. KObR (concentrated

potassium hypobromite [0.62 ml of bromine in 100 ml of 5% potassium hydroxide] which was stored at 4°C was diluted 10 times with 5% potassium hydroxide before using) was added to the sample to develop the color and was mixed by a 12-turn and a 5-turn glass coil. The tubing size determined the portion of air segments or various chemicals used in the analysis (Table III). The analytical functions made in the Technicon Autoanalyzer II system, in this study, included some modifications of Young's (35) method. A 10% KOH solution was used instead of a 20% solution. Stabilization of the color with KNO_2 was not necessary as was reported (35). The original acetylated sample was not subsampled for mixing with the 1-naphthol solution. This study used one 14-turn coil, two 12-turn coils, and one 5-turn coil. Young (35) originally used two 14-turn coils and one 4-turn coil.

TABLE III

PUMP TUBING SIZE USED IN THE TECHNICON VARIABLE SPEED PROPORTIONING PUMP IN THE ANALYSIS OF FREE ARGININE *

Substance	Pump Tubing Size (ml/min)
Sample	.05
10% KOH	1.00 and 1.00
Acetic anhydride	.05
1-Naphthol solution	.80
Air	1.60 and 1.00
KOBr solution	.80 and .60

*Personal communication; Dr. C. T. Young, Associate Professor, North Carolina State University, Raleigh, N.C. 27650.

The colorimetric response was measured at 510 nm using a flow cell (within the Technicon Colorimeter). Standard arginine solutions (1 g/liter with 10, 20, and 30 ml/100 ml dilutions) were used routinely to standardize the recorder (Technicon Single-Channel Recorder) response. In order to calculate the data, the percent transmittance (%T) was converted to optical density (O.D.) using the formula $O.D. = 2 - \log_{10} \%T$. The optical density value times 100, divided by the percent dry weight, equals the number of AMI units per sample. The data was statistically analyzed by the General Linear Models Procedure (Statistical Analysis Systems).

CHAPTER IV

RESULTS AND DISCUSSION

The data analyzed for this investigation had two variables: free arginine content and pod rot disease severity. The experiment was analyzed as a split plot design over time. The main plot treatments consisted of five chemical treatments and a non-treated control. The subplots were labeled "DAYS" in Table IV, with the first sampling date being August 22, 1978.

In order to correlate free arginine content and pod rot disease severity, a wider range of pod rot disease ratings (greater disease severity) were needed from within and among treatments and sampling dates. This type of disease severity occurred in preceding years; however, pod rot disease was not as severe in 1978. A control for pod rot and a better understanding of this disease is needed in order to run a meaningful correlation.

Free Arginine Content

The difference among replicates (labeled as "REPS," Table IV) of the free arginine data was significantly different (.05 level). Therefore, factors other than the chemical treatments and the day of sampling may have influenced the results of this study. These factors would include biological plant differences, variations in the soil environment, and pest populations.

TABLE IV
ANALYSIS OF VARIANCE

Source	df	Free Arginine		Pod Rot Disease Rating	
		MS	F Value	MS	F Value
REPS	2	1011	4.38*	2.44	.64 NS
TREAT	5	2795	11.13**	15.67	4.17*
REPS*TREAT (Error a)	10	251		3.76	
SUBUNIT ANALYSIS					
DAYS LINEAR	1	660476	848.94**	118.62	56.76**
DAYS QUADRATIC	1	8534	10.97**	20.58	9.84**
DAYS CUBIC	1	26343	33.86**	.93	.44 NS
DAYS QUARTIC	1	2660	3.42 NS	1.23	.59 NS
DAYS QUINTIC	1	97	.12 NS	5.09	2.43 NS
DAYS SEXTIC	1	930	1.19 NS	2.93	1.40 NS
TREAT*DAYS LINEAR	5	446	.57 NS	1.26	.60 NS
TREAT*DAYS QUADRATIC	5	2363	3.04*	4.34	2.08 NS
TREAT*DAYS CUBIC	5	763	.98 NS	1.35	.65 NS
TREAT*DAYS QUARTIC	5	1629	2.09 NS	.68	.33 NS
TREAT*DAYS QUINTIC	5	1814	2.33 NS	2.67	1.28 NS
TREAT*DAYS SEXTIC	5	393	.50 NS	1.35	.65 NS
REPS*DAYS IN TREAT (Error b)	72	778		2.09	
CV Error a		8%	90%
CV Error b14%	67%

*Significant at P=.05 level; **Significant at P=.01 level.

There was a highly significant difference (.01 level) among the treatments (labeled as "TREAT," Table IV). The LSD ($P=.01$ level) was used to compare the differences between the six treatments (Table V). These chemicals do control certain pests (2), which, in turn, may have an effect on the peanut plant. It is not known what influence the chemical treatments in this study have on the biochemical functions of the plants. The effect of the chemical treatments on the biochemical functions are difficult to determine and were not an objective of this study.

All chemical treatments showed a lower free arginine content than the non-treated plots as the season progressed (based on the mean values). This can be seen by examining the means in Table V. The LSD ($P=.01$ level) showed that the pentachloronitrobenzene (PCNB) and ethazole mixture and bromomethane treatments had a significantly lower free arginine content than the plots receiving no treatments. Newell (23) and Young (34) showed that free arginine content of the peanut fruit decreased with plant maturity. Hence, the free arginine response to the PCNB and ethazole mixture and bromomethane treatments may have been an indirect effect of their influence on plant maturity.

It was found that the free arginine content of peanut fruit decreases as the plants mature and this decrease is faster as the peanut plant approaches maturity. This faster decrease is shown by the highly significant (.01 level) "DAYS QUADRATIC" F value (10.97) in the AOV of free arginine (Table IV and Figure 3). This decrease in free arginine content of peanut fruit as the growing season progresses is probably due to the incorporation of arginine in protein synthesis (5). The free arginine response curves downward early in the growing season

TABLE V
MEANS FOR FREE ARGININE CONTENT (AMI VALUES)
FOR THE SEVEN SAMPLING DATES

Treatment	Sampling Dates							Mean
	8/22	8/30	9/6	9/11	9/18	9/24	9/29	
non-treated control	283.67*	279.67	278.00	236.33	171.33	137.33	113.00	214.19
ethazole	285.33	281.33	295.00	245.33	147.33	92.67	103.00	207.14
carboxin	296.67	326.33	204.00	203.67	160.67	110.33	112.00	201.95
methyl isothiocyanate and dichloropropene	323.33	284.33	228.67	199.00	151.33	96.00	120.33	200.45
bromomethane	291.67	268.67	223.00	213.67	131.67	111.00	92.33	190.28**
pentachloronitro- benzene and ethazole	265.33	270.33	250.00	204.33	105.33	86.00	94.00	182.19**

*Each data treatment mean is the average of three replicates. An automated colorimetric measurement procedure was used to measure free arginine; results are expressed as AMI values (34).

**Seasonal means significantly different from the check; LSD .01 = 15.49.

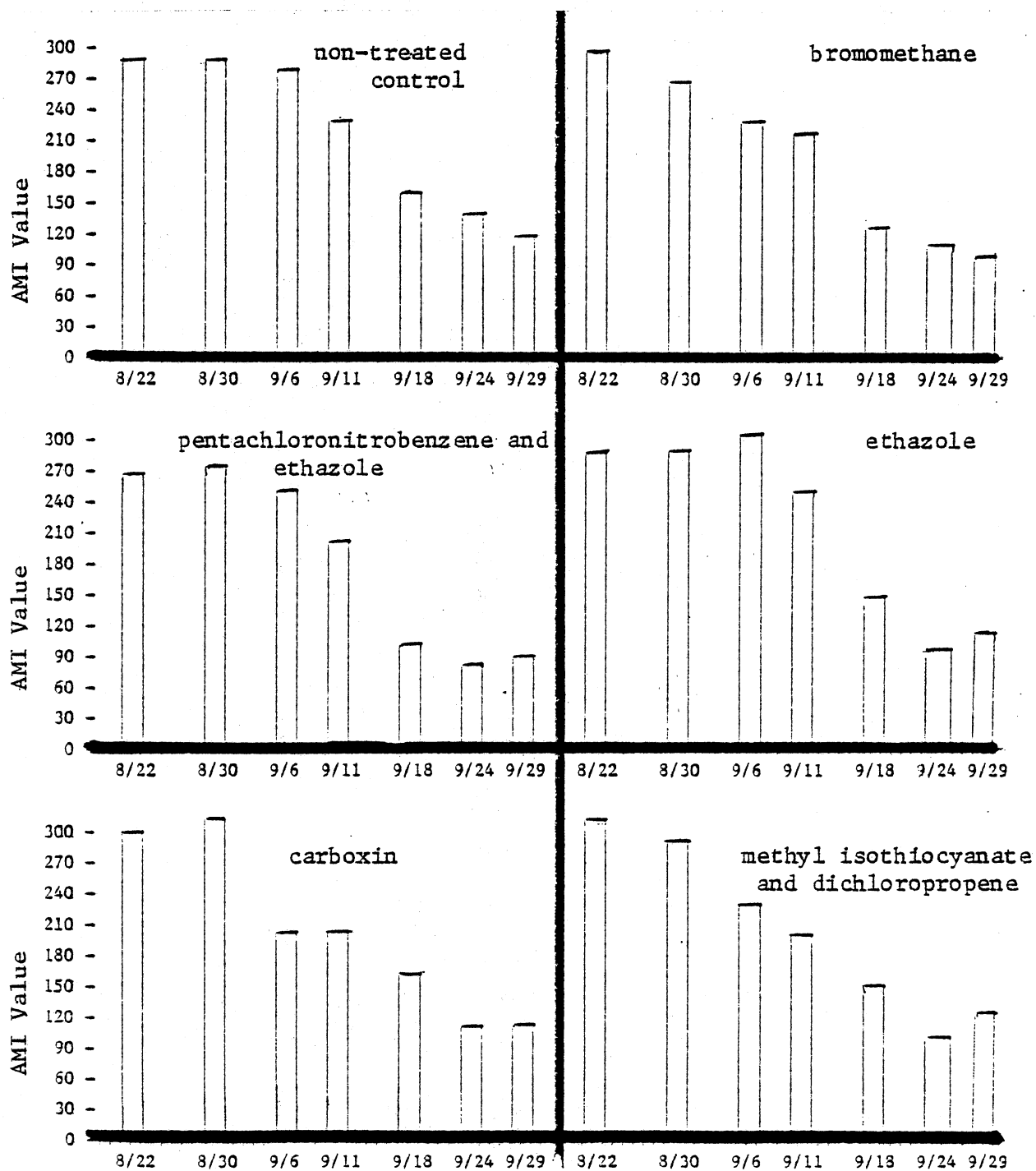


Figure 3. AMI Values Recorded from Six Treatments on Peanuts at Caddo Peanut Research Station, Ft. Cobb, Oklahoma, in 1978

and again upward late in the growing season. This is shown by the highly significant (.01 level) "DAYS CUBIC" F value (33.86) in the AOV of free arginine (Table IV). These results are consistent with previous reports (23,34). The increase of free arginine at the end of the growing season indicates an increase of immature peanut pods in the sample. The increase of immature peanuts may be related to the number of mature peanuts lost to peanut pod rot disease. This is supported by Young and Hammons (37) as they had suggested that the higher AMI values at the end of the growing season were due to the loss of the mature pods. This was clarified later by Hammons et al. (14) when they found that AMI values were positively correlated to the "percent other kernels" (percent immature kernels).

The rate of decline of free arginine as the season progressed was not the same for all treatments. This is shown by the significance (.05 level) of "TREAT*DAYS QUADRATIC" F value (3.04) in the AOV of free arginine (Table IV). This variation among treatments is believed to be due to the treatment effect on the soil fungal populations rather than a direct effect on the plant or free arginine.

The commercial AMI method was designed to predict the maturity date of a peanut field to gain optimum yields. The commercial method for predicting the optimum digging date (maturity) uses only two or three sampling dates to establish a curve. This study included enough sampling dates so the curvatures could be established by specific data and not predicted.

Pod Rot Disease

The random-like occurrence of plants infected with peanut pod rot

disease (PPRD), coupled with the lack of above-ground symptoms, makes early detection of this disease impossible. The test area had some visually obvious soil type and moisture differences; therefore, differences in PPRD severity due to location were expected. However, there was not a significant difference (.05 level) in the ratings of PPRD severity between replicates. This is indicated in the AOV, Table IV, of PPRD labeled as "REPS."

The application of the chemical treatments did not prevent peanut pod discoloration. However, the chemical treatments did significantly (.05 level) effect the amount of peanut pod discoloration (labeled as "TREAT," Table IV). The LSD ($P=.05$ level) was used to compare the differences due to the six treatments (Table VI). All chemical treatments showed an increase in PPRD severity. By using the LSD ($P=.05$ level), bromomethane, ethazole, and carboxin had significantly higher seasonal pod rot ratings than the non-treated plots. There were no obvious phytotoxic effects of the chemical treatments on the peanut plants. Since these chemicals have federal use labels for various agronomic crops, obtained from extensive evaluation, no harmful effects were expected. The direct effects these chemicals have on the production of free arginine is not known.

All of the chemicals used in this study have previously demonstrated control of various soil inhabiting organisms. Bromomethane and the methyl isothiocyanate and dichloropropene mixture (MITC) are soil fumigants and have a very broad spectrum of control. At high rates they are considered soil sterilants, thus controlling or reducing the populations of all soil inhabiting organisms. The fumigation of the soil with these chemicals may have reduced the populations of

TABLE VI
MEANS FOR PEANUT POD ROT DISEASE SEVERITY
RATINGS OF THE SEVEN SAMPLING DATES

	8/22	8/30	9/6	9/11	9/18	9/24	9/29	Mean
non-treated control	.33*	.67	.33	.67	1.33	.67	2.67	.95
methyl isothiocyanate and dichloropropene	.67	.33	1.00	.67	.33	3.67	3.00	1.38
pentachloronitrobenzene and ethazole	.67	.33	1.67	1.67	.00	3.67	3.00	2.00
bromomethane	.67	2.00	2.33	3.00	3.00	3.33	4.00	2.62**
carboxin	2.33	1.67	1.00	2.33	1.67	5.33	6.00	2.90**
ethazole	2.00	2.67	2.33	2.33	1.67	5.33	5.33	3.09**

*Each data treatment mean is an average of three replicates. Percent pod discoloration was used as a measure of peanut pod rot disease severity on a scale of 0-10 (0=no discoloration, 10=approximately 100% discoloration).

**Seasonal means significantly different from the non-treated control; LSD .05 = 1.16.

both pathogenic and nonpathogenic organisms and the increased disease severity was a result of the soil pathogens recovering at a faster rate with less competition.

Ethazole and carboxin are systemic fungicides. Ethazole tends to control diseases caused by pythiaceous fungi and carboxin is effective against damping-off diseases caused by Rhizoctonia (2). Although Rhizoctonia solani Kuhn and Pythium myriotylum Dreschler have both been identified as possible pathogens involved in the peanut pod rot disease complex, the use of ethazole and carboxin increased peanut pod rot disease severity. Ethazole and carboxin may have been effective against certain pathogens but allowed the other pathogens to be more damaging and cause greater PPRD severity.

An increase in PPRD was expected even in the absence of chemical treatments, and this is shown in the non-treated control (Figure 4). This increase in PPRD during the season may be due to an increase in inoculum under a more favorable environment for the pathogen as the season progressed, and an increase in host susceptibility as the plants reached maturity. The statistical analysis indicates an increase of disease severity as the season progressed by an increasing type of curvature, shown by the significant (.05 level) "DAYS QUADRATIC" F value (9.58) in the AOV of PPRD (Table IV). Therefore, the rate that PPRD severity increased was faster as the plants reached maturity. This type of trend was essentially the same for all treatments, as indicated in the AOV of PPRD (Table IV), in that "TREAT*DAYS LINEAR," "QUADRATIC," "CUBIC," "QUARTIC," "QUINTIC," and "SEXTIC" exhibited non-significant effects. Although this study involved two soil fumigants (bromomethane

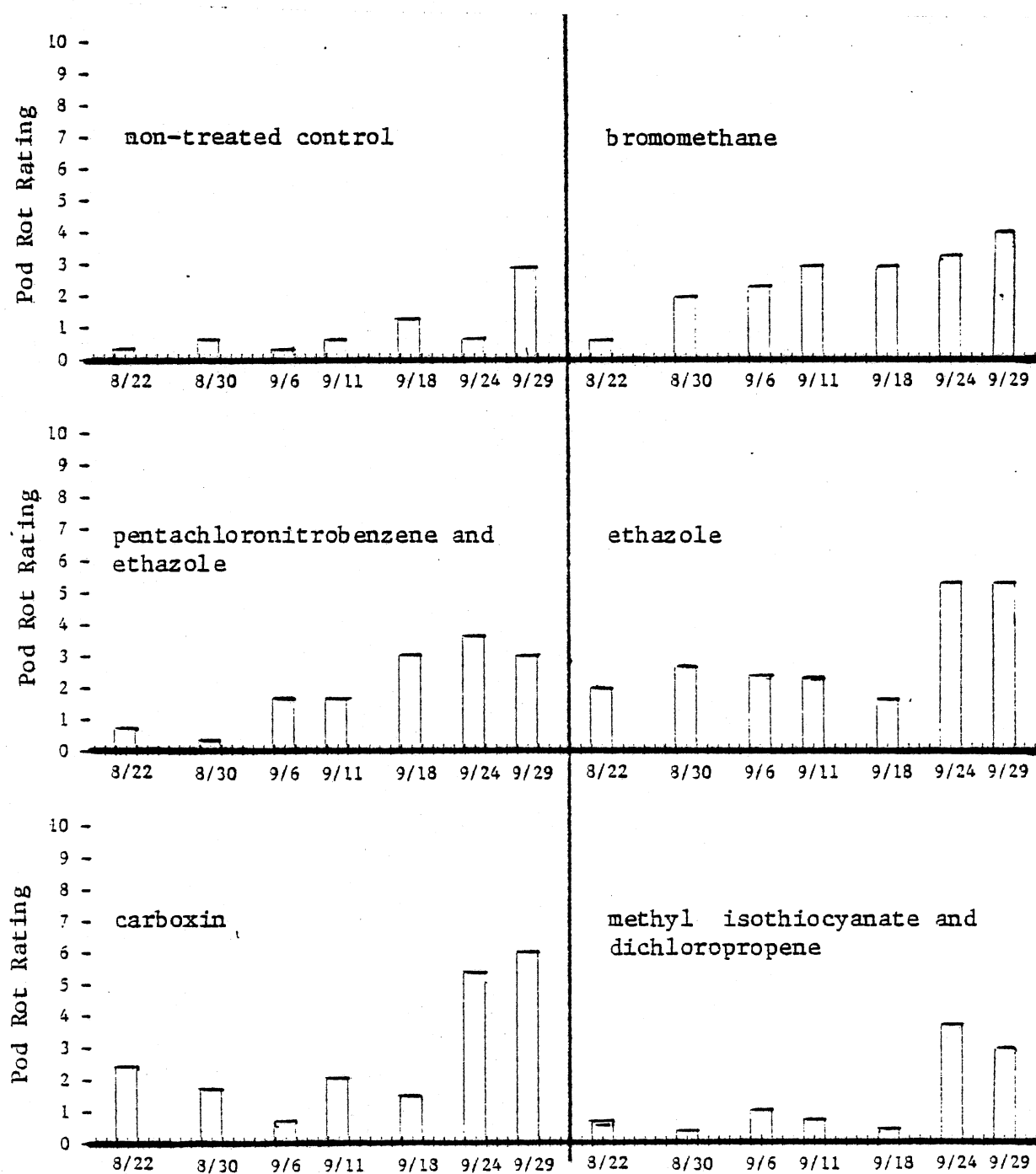


Figure 4. Pod Rot Ratings of Peanuts Grown at Caddo Peanut Research Station, Ft. Cobb, Oklahoma, in 1978 on Six Treatments

and MITC), two systemic fungicides (ethazole and carboxin), a combination of a soil fungicide and a systemic fungicide (pentachloronitrobenzene and ethazole), and a non-treated control, the same type of trend (the rate that PPRD severity increased was faster as the plants reached maturity) was expressed for all treatments. This indicates that the chemical treatments created a more favorable environment for the development of PPRD.

CHAPTER V

SUMMARY

1. Five chemical treatments and a non-treated control were used to study their effects on peanut pod rot disease severity and free arginine content of peanut fruit.

2. The free arginine content of peanut fruit, in samples collected, showed differences with respect to treatments.

3. All chemical treatments showed a lower free arginine content than the non-treated plots.

4. Bromomethane and a mixture of pentachloronitrobenzene and ethazole 10-25 G had significantly lower free arginine values than the non-treated plots.

5. Free arginine content of the peanut fruit decreased as the plants matured, as was found in previous reports (23, 34).

6. The rate that free arginine content decreased was faster as the plants reached maturity, and found not to be the same for all treatments.

7. The pod rot disease severity showed differences with respect to treatments.

8. All chemical treatments showed a higher pod rot disease severity than the non-treated plots.

9. Bromomethane, ethazole 35 W, and carboxin 10 G had significantly higher pod rot severity ratings than the non-treated plots.

10. Peanut pod rot disease severity increased as the fruit matured.

11. The rate that peanut pod rot disease severity increased was faster as the plants reached maturity, and was found to be essentially the same for all treatments.

12. A meaningful correlation of free arginine content and peanut pod rot disease severity could not be made due to a lack of a control for the disease, and a need for a wider range of disease readings with greater disease severity.

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VITA

David Lee Nowlin

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF VARIOUS CHEMICAL TREATMENTS ON PEANUT POD ROT
AND FREE ARGININE CONTENT OF PEANUTS (ARACHIS HYPOGAEA L.)

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Chickasha, Oklahoma, May 2, 1955, the son
of Alvin G. and Betty J. Nowlin.

Education: Attended Minco Grade School and Minco Junior High
School in Minco, Oklahoma; attended Fort Cobb High School in
Fort Cobb, Oklahoma, graduating in 1973; received the Bachelor
of Science in Agriculture degree from Oklahoma State University
in May, 1977; completed requirements for the Master of Science
degree at Oklahoma State University in December, 1980.

Professional Experience: Field Laborer, Caddo Peanut Research
Station, Fort Cobb, Oklahoma, in 1972 and 1973; Field Monitor
in the Oklahoma State University Multi-Crop Pest Management
Program conducted in Hughes County, Oklahoma, in 1974
and 1975; Caddo County Peanut Grower's Association Head
Field Scout in Oklahoma State University's Pest Management
Program conducted in Caddo County, Oklahoma, 1976; Field
Laborer, Extension Plant Pathology Research Project, 1977
and 1978; Multi-County Integrated Pest Management Specialist
for Oklahoma State University Extension Service in Blaine
and Caddo Counties in 1979.