SOME EFFECTS OF AMMONIUM THIOCYANATE ON METABOLIC PROCESSES IN THE COTTON PLANT

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CHAPTER I

INTRODUCTION

Weeds are important to almost everyone. Weed control is one of the most expensive steps in crop production. Weeds may poison or seriously slow down weight gains of livestock. Ammonium thiocyanate has been known for many years to be a plant poison, yet it has not been applied for weed eradication. Suggestion of the use of this compound was made by Dr. Paul Peterson of the Kopper's Research Laboratory.

The science of weed control has advanced greatly since 1942. In many cases new chemical methods are far superior to older practices in terms of control and are less costly. In the general field of plant control numerous new chemicals, combinations of materials and additives have been investigated for improved herbicidal activity. An important development has been the use of ammonium thiocyanate as an additive with amitrole. The addition of this compound applied at a similar dose to the amitrole greatly improves the effect of the latter on quackgrass. It is claimed that ammonium thiocyanate may also enhance the activity of amitrole on stoloniferous species of Agrostis and certain other plants (2, 6).

Due to the possibility of ammonium thiocyanate improvement of herbicidal activity for effective and economical plant control, more information is needed on the herbicidal behaviour of ammonium thiocyanate. This study was designed to determine the effect of ammonium thiocyanate

on some of the basic metabolic activities of plant tissue. The starch content, amylase activity, photosynthesis, respiration, Hill reaction, and incorporation of glycine-^{14}C into amino acids, organic acids, and sugar fractions in cotton leaves as affected by ammonium thiocyanate were studied.

CHAPTER II

REVIEW OF LITERATURE

Ammonium thiocyanate (NH_4SCN) was shown to possess herbicidal properties when Harvey (19) found that on a square rod which received 10 pounds of ammonium thiocyanate there were no weeds or crop plants growing after 12 weeks. There was sterilization of the soil for at least 4 months. If only 2 pounds were applied, the weeds were mostly killed, yet the soil was not sterile for more than 2 to 4 weeks. NH_4SCN when sprayed onto weed leaves, produced death and discoloration within 2 days. The discoloration of leaves of Canada thistle was noted within 2 hours after applying. Ammonium thiocyanate sprays destroyed annual weeds in cereal crops in later experiments by Singh and Das (33). Landen (21) found that the activity of catalase was reduced about 65% with 0.1 M NH_4SCN in cabbage tissue preparations. According to Harvey (18) NH_4SCN acted as a protoplasmic poison. He stated that protoplasmic poisons may check the activity of certain enzymes, coagulate proteins, or combine with some of the cell constituents. But Landen (21) suggested the affinity of NH_4SCN for iron may, in part at least, account for its toxicity.

The germination and respiration rates in potato tubers were reduced at a concentration of 2% NH_4SCN as shown by Ranjau and Kaur (30). Novikov and Barannikova (26) showed that at the optimum temperature for plant growth 10^{-4} M NH_4SCN stimulated root formation on the stems of

bean and geranium by 500%. But at lower temperatures, the same dose killed the plants.

A number of investigators have shown that NH_4SCN enhanced the translocation of amitrole in plants. Donnalley (12) suggested that NH_4SCN increased amitrole toxicity by increasing the translocation of the phytocide. Crafts and Robins (10) showed by autoradiography that a greater amount of ^{14}C amitrole was distributed throughout the plant when amitrole was combined with NH_4SCN than when amitrole was applied alone. Carder (7) reported that amitrole-NH_4SCN was 20 to 30% more effective in couchgrass control than amitrole alone. Using ^{14}C labeled materials, Donnalley and Ries (13) studied the absorption and translocation of ^{14}C-amitrole after applications to foliage by determining the radioactivity in the wash solution and plant extracts. Regardless of the time of application, the addition of NH_4SCN did not alter the amount of ^{14}C-amitrole absorbed. But 5000 ppm of NH_4SCN greatly increased the amount of ^{14}C translocation. They proposed that this increase in translocation may account for the greater herbicidal effectiveness of the mixture of amitrole and NH_4SCN. Forde (15) showed by gross autoradiography that translocation of ^{14}C-labeled amitrole from the leaf of quackgrass was retarded over 12 hours by NH_4SCN applied as a spray or as a spot. While considerable export of ^{14}C from the treated leaf occurred during 12, 24, and 96 hours after ^{14}C-amitrole was applied, ^{14}C-NH_4SCN used as a tracer was largely restricted to the treated leaf over the first 24 hours and showed only slight export over 96 hours. He concluded that the synergistic effect of NH_4SCN on amitrole is exerted in the treated areas and not at the ultimate site of amitrole action.

Studies on the effect of NH_4SCN on the activity of herbicides

other than amitrole have included 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Robison (31) reported on some effects of ammonium thiocyanate in combination with 2,4,5-T on the defoliation and kill of mesquite. The largest differences between 2,4,5-T and 2,4,5-T/NH_4SCN combinations were found in the presence of more top kill or trees with complete defoliation and less basal sprout growth. However, no consistent difference in total plant kill were attributed directly to the addition of NH_4SCN. Basler et al. (1) investigated the effects of ammonium tiocyanate on the penetration and translocation of 2,4,5-T in cotton, winged elm, and blackjack oak. They found the higher concentration of 2500 and 5000 ppm NH_4SCN increased the translocation of 2,4,5-T-1-^{14}C to both the lower and upper stem segments of cotton seedlings but lower concentrations did not increase translocation. Various concentrations of NH_4SCN did not affect the penetration of 2,4,5-T-1-^{14}C or the translocation of 2,4,5-T-1-^{14}C to the stems or untreated leaves of blackjack oak. The penetration of 2,4,5-T-1-^{14}C in winged elm was not affected by NH_4SCN. The translocation from the treated leaf to the lower stem and leaves was significantly increased by NH_4SCN treatment and translocation to the upper stems and leaves also appears to be enhanced in winged elm. However, there was no increase in translocation to root tissue of winged elm after NH_4SCN treatment. Field studies by Elwell (14) showed that NH_4SCN enhanced the effectiveness of 2,4,5-T in the defoliation of winged elm.

CHAPTER III

MATERIALS AND METHODS

The test plants were cotton (Gossypium hirsutum var., Acala 44) growing either as mature plants in a greenhouse or as seedlings in a growth chamber. Determinations of the effects of NH_4SCN on starch content, respiration, photosynthesis, Hill reaction, and incorporation of glycine-^{14}C into amino acids, organic acids, and sugar fractions were made.

A. Determination of starch content

Greenhouse-grown mature cotton leaves were used. Each cotton leaf was cut into half by separating at the midrib. Each half leaf was treated either with zero, 2000 ppm, or 5000 ppm of NH_4SCN in a 20-cm petri-dish for 24 or 48 hours in continuous fluorescent light of 2500 ft-c intensity at 30° C.

At the end of the culture period, each half leaf was washed free of chlorophyll by boiling in 80% ethanol. The starch content in each half leaf was visualized qualitatively by difference in color after treatment with KI-I solution according to methods similar to those described by Meyer and Anderson (23).

B. Determination of amylase activity

Seven 10-day old cotton cotyledons grown in a growth chamber were used for each sample. Cotton cotyledons were cultured in 10-cm petri-

dishes containing 25 ml of zero and 2000 ppm of NH_4SCN in continuous fluorescent light of 2500 ft-c at 30° C. for 24 and 48 hours.

After each treatment period, the cotton cotyledons were washed in distilled water 6 times and ground in 5 ml 0.02 M phosphate buffer at pH 6.9 in a glass hand homogenizer. The homogenate was centrifuged at 27,750 x G for 30 minutes and the supernatant was saved for amylase activity determinations. The procedure for the determination of amylase activity was similar to methods outlined by Bernfeld (4). The sugar level found in the reaction mixtures at 0 time were used as measurements of the effects of NH_4SCN on the sugar level of the tissue. The reaction mixture consisted of the sugar level of the samples. These were removed for analysis at 15 minute intervals for a one hour period.

C. Determination of Photosynthesis and Respiration Rates

Ten 15 mm cork borer punches taken from mature cotton leaves growing in the greenhouse were used for each sample. The leaf discs were cultured in 20 ml of zero and 2000 ppm NH_4SCN in continuous fluorescent light of 2500 ft-c for 4 and 17 hour culture periods at 30° C. in a growth chamber. The determination of photosynthesis and respiration were made by means of manometric methods described by Umbreit and Burris (35). The ten leaf discs were placed in the outer compartment of the vessel and 0.5 ml of Pardee's buffer (28) was placed in the center well with a fan of filter paper to facilitate gas exchange and 0.5 ml of distilled water was added in the side-arm to maintain high humidity. The Pardee's buffer was adjusted to provide about 1 percent CO_2 in the atmosphere of the vessel. Photosynthesis was determined in light provided by a bank of 50 watt bulbs.

D. Determination of Hill-Reaction

1. Isolation of Chloroplasts

Ten grams of cotton cotyledons were ground in a chilled mortar with 30 ml ice-cold sucrose-tris buffer consisting of 135 g sucrose, 3.0g tris(hydroxymethyl)aminomethane, and 1.0 g NaCl per liter and HCl to adjust the pH to 7.95. The homogenate was filtered through glass wool into ice-encased centrifuge tubes and the mortar was rinsed with an additional 10 ml of cold buffer. After centrifugation for 3 minutes at 4,000 x G the supernatant was discarded and the chloroplasts were re-suspended in 30 ml of cold buffer and again centrifuged for 2 minutes at 4,000 x G. The chloroplasts were then suspended in 2 ml sucrose-tris buffer and filtered through glass wool again. The final dense suspension was stored in an ice bath and kept in dim light by using an aluminum foil to wrap the tube. Chlorophyll determination, based on the work of Mackinney (22), was made by determinations of absorption of light by aqueous acetone (80%) extracts of chlorophyll from the chloroplast preparations.

2. Determination of $K_3Fe(CN)_6$ Reduction

A 0.1 ml portion of the chloroplast suspension was placed in a 10 ml reaction mixture containing 24 micromoles of methylamine-HCl, 2.0 micromoles of $K_3Fe(CN)_6$, 2.0 ml sucrose-tris buffer and zero or 2000 ppm of NH_4SCN. The reaction temperature was held at about 15° C. by rapidly circulating tap water through a beaker in which the test tube was immersed. The light for photosynthesis was provided by a high intensity 300 watt incandescent flood light held about 6 inches from the surface of the reaction tube. After reaction time intervals of about 2 minutes, samples were removed and the reaction was stopped

by adding 0.5 ml of 25% trichloroacetic acid. The samples were centrifuged at 7,900 x G for 10 minutes and determinations of the reduction of $K_3Fe(CN)_6$ were made spectroscopically by light absorption changes at 420 mμ. These methods are similar to those described by Good (16).

E. Incorporation of Glycine-^{14}C into Amino Acids, Organic Acids, and Sugar Fractions in Cotton Leaf Discs

Twenty 15 mm leaf discs from greenhouse-grown mature cotton leaves were used in each sample. The leaf discs were placed in a flask containing glycine-^{14}C feeding solution and placed in a growth chamber under continuous illumination of 2500 ft-c and 30° C. Duplicate samples contained 10 microcuries of uniform labeled glycine-^{14}C (specific activity 116.0 millicurie/millimole) in 20 ml of Hoagland's nutrient solution at pH 5.5 containing zero or 2000 ppm NH_4SCN. The culture period was 4 hours.

At the end of culture period, the discs immediately were washed 5 times with distilled water and quickly dropped into 30 ml of boiling 85% ethanol. The tissues were then refluxed for 1 hour each, twice with 80%, and finally with 40% ethanol. The extracts were combined and evaporated in vacuo to dryness. Twenty ml of water and 50 ml of chloroform were added to the residues and after thorough agitation the chloroform layer along with dissolved pigments was removed. The water layer which was free from chloroform was then dried in vacuo and redissolved in 10 ml of 40% ethanol and 0.1 ml was taken for the determination of radioactivity in a liquid scintillation counter.

The alcoholic extract, less chloroform soluble substances, was further fractionated by means of an ion-exchange resin method outline by Wang (36) into sugar, amino acids, and organic acid fractions. After

the evaporation of these fractions to dryness in vacuo, the residues were redissolved in 40% ethanol, each was made up to a desired volume (2ml) and 0.1 ml were taken for the determination of radioactivity.

CHAPTER IV

RESULTS

A. Effect of NH_4SCN on Starch Content

The original experiments in these studies dealt with the effects of NH_4SCN on the starch content of cotton leaf tissue as determined by the well-known iodine reaction of starch, in which starch yields an intense blue complex in the presence of iodine. Cotton was chosen for the experimental plant because previous studies (1) had shown that NH_4SCN effects a large increase in 2,4,5-T translocation in this plant. Figures 1 and 2 show that the color of the cotton leaves treated with NH_4SCN were lighter than the untreated leaves. The leaves treated with ammonium thiocyanate for 48 hours at either 2000 ppm or 5000 ppm had the lightest color after staining for starch showing that cotton leaves were decreased in starch content by NH_4SCN treatment. Increasing the concentration of NH_4SCN or increasing the treatment period increased the depletion of starch.

B. Effect of NH_4SCN on Amylase Activity

The decrease in starch content of leaf tissue after NH_4SCN treatment possibly could be brought about by effects on a number of processes, i.e. increased amylase and respiratory activity and decreased photosynthetic activity. The amylase activity of treated and untreated tissue was analyzed first in effort to determine the cause of the decreased starch in treated tissue. These results are shown in Table 1.

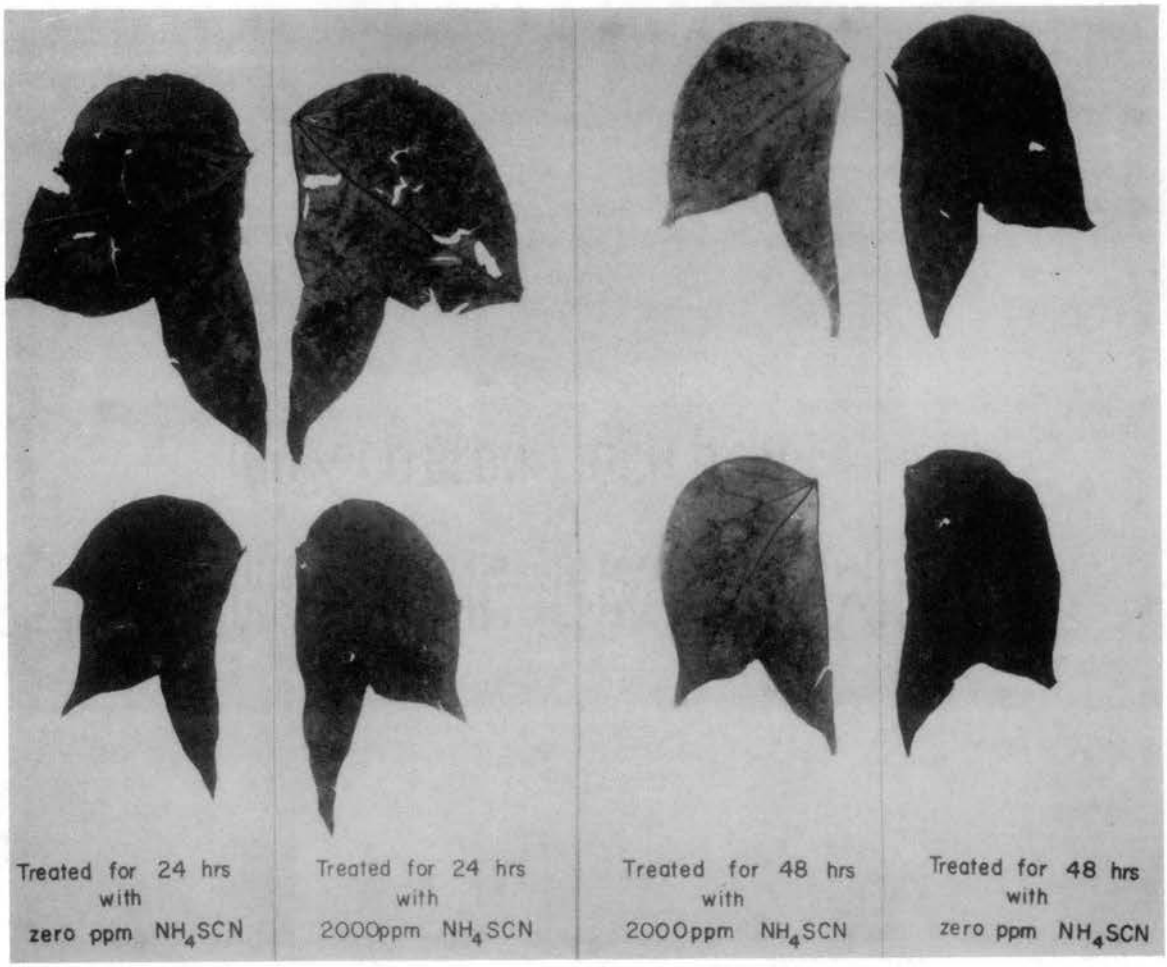

Figure 1. Effect of 2000 ppm NH_4SCN on Starch Content in Cotton Leaves

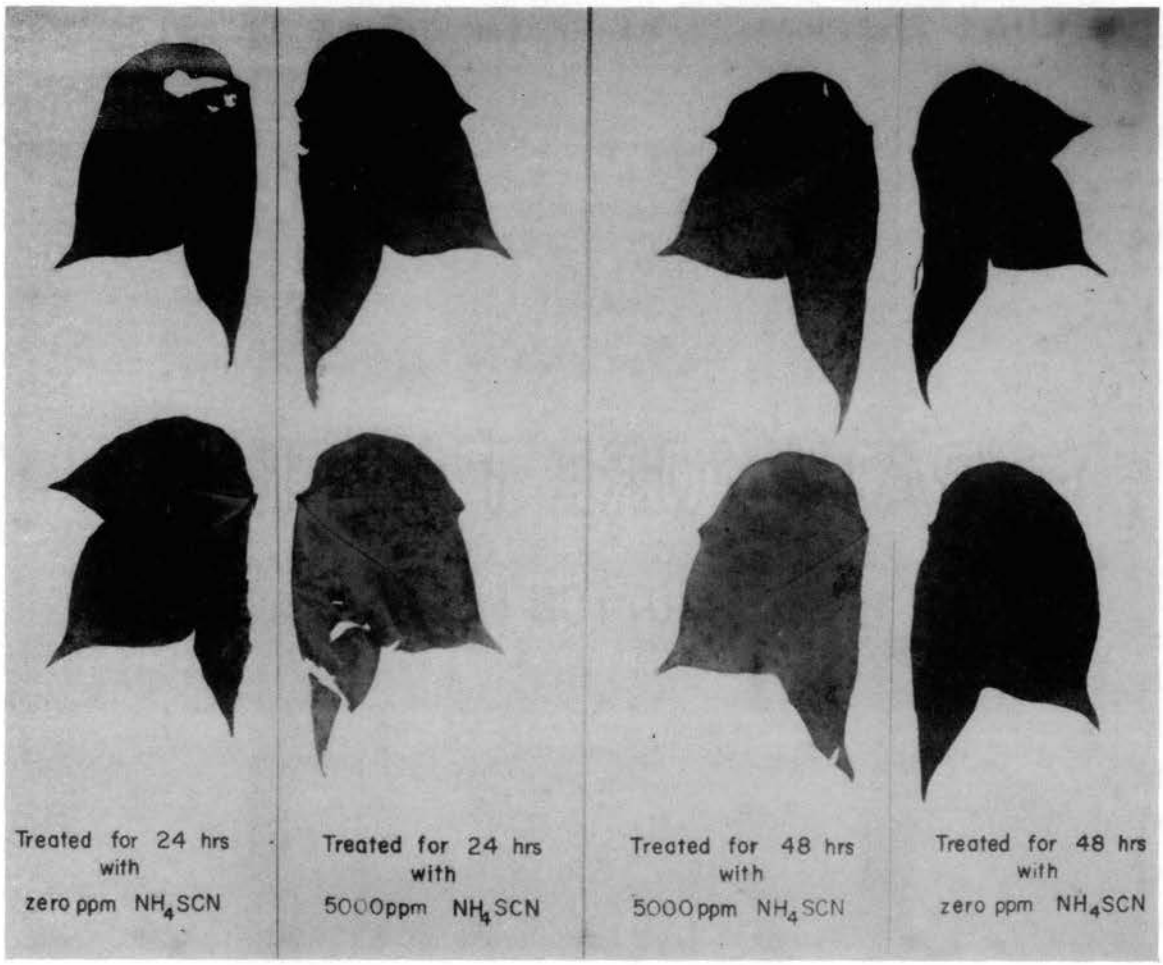

Figure 2. Effect of 5000 ppm NH_4SCN on Starch Content in Cotton Leaves

The amylase activity increased in treated tissue both at 24 and 48 hours of treatment. Thus, NH_4SCN may by produce initial effects on tissue by increasing the synthesis of amylase. However, as shown in Table 2, the sugar levels of the tissue decreased considerably at 24 and 48 hours after treatment showing that free sugars are not accumulating as a result of increased amylase activity. It appears more probable that amylase synthesis may be enhanced by low sugar levels in the tissue. The low sugar levels might in turn be produced by increased rates of respiration and/or decreased photosynthetic rates.

C. Effect of NH_4SCN on Photosynthesis and Respiration

Tables 3 and 4 show the effect of 2000 ppm NH_4SCN on photosynthesis and the respiration in cotton leaf discs. The average O_2 release by photosynthesis is 511 microliter per hour after 4 hours treatment with 2000 ppm of NH_4SCN and 237 microliter per hour of O_2 release after 17 hours treatment with 2000 ppm of NH_4SCN. The O_2 release by photosynthesis in the control is 1001 microliter per hour for the 4 hour culture period, and 1064 microliter per hour for 17 hours culture period. Photosynthesis after treatment with NH_4SCN, was inhibited by 47% at 4 hours, and 76% for the 17 hour treatment period when compared with the control.

The O_2 consumed by respiration was 190 microliter per hour in the control for the 4 hour culture period and 253 microliter per hour for the 17 hour culture period. The O_2 consumed by the treatment with 2000 ppm of NH_4SCN is 295 microliter per hour for the 4 hour treatment and 366 microliter per hour for the 17 hour treatment period.

Respiration after treatment with 2000 ppm NH_4SCN was increased about 56% for 4 hour treatment period and 38% for the 17 hour treatment

TABLE I

EFFECT OF 2000 ppm NH_4SCN ON AMYLASE ACTIVITY OF COTTON COTYLEDONS AFTER 24 AND 48 HOUR OF TREATMENT

	Amylase Activity	
	mg. glucose produced per hr. per g. fresh weight	
	Control	2000 ppm NH_4SCN
24 hr. treatment		
Expt. 1	1.00	1.61
48 hr. treatment		
Expt. 1	1.02	1.38
Expt. 2	0.94	2.03

TABLE II

EFFECT OF 2000 ppm NH_4SCN ON SUGAR LEVEL IN COTTON COTYLEDONS AFTER 24 AND 48 HOUR TREATMENT

	mg. sugar per g. fresh weight	
	Control	2000 ppm NH_4SCN
24 hr. treatment		
Expt. 1	0.39	0.22
48 hr. treatment		
Expt. 1	0.64	0.24
Expt. 2	0.77	0.08

TABLE III

EFFECT OF NH_4SCN ON PHOTOSYNTHESIS IN COTTON LEAF DISCS AFTER 4 AND 17 HOUR TREATMENT

	μl O_2 released per hour per 10 discs		
	Control	2000 ppm NH_4SCN	% inhibition
4 hour treatment			
Expt 1	846	492	42
Expt 2	944	516	45
Expt 3	1212	546	55
Average	1001	511	47
17 hour treatment			
Expt 1	1092	186	83
Expt 2	948	288	70
Expt 3	1152	---	--
Average	1064	237	76

TABLE IV

EFFECT OF NH_4SCN ON RESPIRATION IN COTTON LEAF DISCS AFTER 4 AND 17 HOUR TREATMENT

	μl O_2 uptake per hour per 10 discs		
	Control	2000 ppm NH_4SCN	% increase
4 hour treatment			
Expt 1	174	285	64
Expt 2	186	294	58
Expt 3	210	307	46
Average	190	295	56
17 hour treatment			
Expt 1	270	350	30
Expt 2	250	362	45
Expt 3	240	386	38
Average	253	366	38

period. The results show that ammonium thiocyanate decreased photosynthesis, and on the other hand, increased the respiration rates in cotton leaf discs.

D. Effect of NH_4SCN on Hill Reaction

The previous experiments on photosynthetic O_2 release showed that 2000 ppm of NH_4SCN greatly reduced the photosynthetic rate in cotton leaf discs. The Hill reaction, the photolysis of water, is one of the initial steps in photosynthesis. The experimental data shown in Table 5 indicate that the Hill reaction was not altered by NH_4SCN treatment and, thus, the inhibition of photosynthesis was due to effects on systems other than the Hill reaction.

TABLE V

EFFECT OF 2000 ppm NH_4SCN ON THE HILL REACTION IN COTTON COTYLEDON CHLOROPLASTS

Experiment Number	O. D. change at 420 mμ per minute per mg. chlorophyll	
	0 ppm NH_4SCN	2000 ppm NH_4SCN
1	0.324	0.297
2	0.350	0.378
3	0.220	0.135

E. Effect of NH_4SCN on the Incorporation of Glycine-^{14}C into Amino Acids, Organic Acids, and Sugar in Cotton Leaf Discs

An observation made by Carter (8) indicated that either serine or phosphoserine might be low in NH_4SCN treated plants. His studies showed

that the formation of 3-amino-1,2,4-triazolylalanine from 3-amino-1,2,4-triazole did not occur in large amounts in NH_4SCN treated plants indicating a low level of the substrate serine in the treated plant. The early products of photosynthetic carbon fixation flow through glycine to serine and then to sugars and starch as indicated by recent studies by Ongun and Stocking (27) and earlier studies by Jimenez, Baldwin, and Tolbert (20) and Wang and Waygood (37). Since the work of Carter (8) indicated that the serine level was low in NH_4SCN treated tissue, the block in photosynthetic carbon assimilation appeared to be at some step prior to serine synthesis. These observations led to the present study on the effects of NH_4SCN on the conversion of glycine to sugars and organic acids.

The data presented in Table 6 show that the incorporation of glycine-^{14}C into organic acids was not greatly different between the NH_4SCN treated and untreated leaf discs. However, the incorporation of glycine-^{14}C into the sugar fraction was decreased by treatment with 2000 ppm of NH_4SCN indicating that NH_4SCN treatment prevented the flow of ^{14}C from glycine to serine and then to the neutral sugar fraction, and this is also reflected in the retension of more label in the amino acid fraction of treated leaf discs. The absorption or uptake of glycine-^{14}C by the cotton leaf discs was also decreased by about one-third by treatment with NH_4SCN as indicated by the ^{14}C level in the total alcoholic extract (Table 6).

TABLE VI

THE EFFECT OF NH_4SCN ON THE INCORPORATION OF GLYCINE-^{14}C INTO AMINO ACIDS, ORGANIC ACIDS, AND NEUTRAL SUGAR POOLS IN COTTON LEAF DISCS AFTER 4 HOUR CULTURE PERIOD

Treatment	Alcoholic Extract (c.p.m.)	^{14}C as % of total alcoholic extract		
		Amino Acids	Organic Acids	Neutral Sugar
Control				
Experiment 1				
Sample 1	1,067,200	32.1	25.1	17.8
Sample 2	1,056,680	25.9	27.4	18.3
Experiment 2				
Sample 1	1,567,519	29.9	16.03	21.83
Sample 2	1,619,452	29.4	17.53	21.25
2000 ppm NH_4SCN				
Experiment 1				
Sample 1	678,305	42.5	30.1	9.5
Sample 2	713,915	35.2	26.0	11.7
Experiment 2				
Sample 1	941,528	45.8	17.35	12.26
Sample 2	1,030,659	33.2	17.34	11.69

CHAPTER V

DISCUSSION

A number of reports have shown that ammonium thiocyanate enhanced the translocation of amitrole and 2,4,5-T in plants. There are several different views relative to the mechanism of the NH_4SCN increased translocation. The enchancement of amitrole toxicity by ammonium thiocyanate was attributed to increased translocation by Donnalley (11). Carter (8) in studies on the metabolic activity of amitrole suggests that 3-amino-1,2,4,-triazolylalanine was produced from 3-amino-1,2,4-triazole and serine or phosphoserine. The presence of ammonium thiocyanate considerably reduced the formation of 3-amino-1,2,4- triazolylalanine from amitrole by bean plants. Perhaps increased transport of amitrole by NH_4SCN treatment is due to a decreased formation of 3-amino-1,2,4-triazolylalanine which may be less mobile than amitrole. Crafts (9) suggested that NH_4SCN may be active in promoting translocation by reducing contact injury which could possibly restrict the intake of amitrole.

Mitchell and Whitehead (24) showed that the application of a number of different growth-regulating compounds increased the rate of starch digestion in young immature leaves. Mitchell and Brown (25) in studies on beans observed that 2,4-dichlorophenoxyacetic acid (2,4-D) was not readily translocated from leaves in which the sugar content was relatively low. It was evident that 2,4-D translocation from the leaves into other parts of plant was associated with the translocation of

organic food materials from the leaves into other parts of the plant. In the present studies we found that NH_4SCN decreased the starch content in the treated leaves, either by treatment with 2000 ppm or 5000 ppm of NH_4SCN for 24 or 48 hours. However, the sugar level was also low after treatment and it is not certain that the enhancement of translocation by NH_4SCN was due to increased breakdown of starch to glucose in the treated area.

The principal reserve carbohydrate of the higher plant is starch. Starch may be degraded by either of two processes, the phosphorolytic cleavage by starch phosphorylase or hydrolytic cleavage by amylase. The amylase activity studied in the present experiment was found to be increased by NH_4SCN. However, sugar did not appear to accumulate as a result of increased amylase enzyme levels in the tissue and it appears likely that the increased enzyme level was not due directly to NH_4SCN treatment. It appears more likely that the low sugar level, brought about by increased respiration and decreased photosynthesis, was responsible for the increased synthesis of amylase.

Ranjau and Kaur (30) observed a reduced respiration rate in potato tubers after treatment with a concentration of 2% of NH_4SCN. In the present studies it was found that the respiration rate increased about 54% after a 4 hour treatment, and 44% after a 17 hour treatment at a concentration of 2000 ppm (0.2%) of NH_4SCN. Many of the plant organic chemical components depend directly or indirectly on photosynthates as substrates and energy for synthesis. Decreased photosynthetic rates and available photosynthate must, therefore, have far reaching effects on the growth and well being of the plant. The data showed that the photosynthetic rate was reduced about 50% after 4 hours treatment with

2000 ppm of NH_4SCN and about 75% after treatment with NH_4SCN for 17 hours. This greatly reduced photosynthetic rate by NH_4SCN may be one of the basic effects of the herbicide on the plant. The increased respiration rates and reduced photosynthetic rate after NH_4SCN treatment both appear to relate to the decreased carbohydrate content in treated cotton leaves. The Hill reaction, involving the photolysis of water, is one of the major reactions in photosynthesis. The Hill reaction in the present experiment was not affected by NH_4SCN. The mechanism of the inhibition of photosynthesis by NH_4SCN must lie in other areas and needs further studies.

Donnalley and Ries (13) found that NH_4SCN, whether applied before, after, or in combination with ^{14}C-amitrole, did not significantly alter the amount of amitrole absorbed in quackgrass. Basler et al. (1) found NH_4SCN did not affect the penetration of 2,4,5-T-1-^{14}C in winged elm and blackjack oak seedlings. However NH_4SCN decreased the absorption of glycine-^{14}C in cotton in the present studies. The count of the radioactivity in the alcoholic extract of the control are 1,067,200 and 1,056,680 c.p.m., they are 678,305 and 713,915 c.p.m. after the treatment. The absorption of glycine-^{14}C was decreased about 35% when cotton leaf discs were treated with 2000 ppm of NH_4SCN. The experiments in the present study differ from those of Donnalley and Ries (13) and Basler (1) et al. in that the leaf discs were floated in aqueous solutions and the NH_4SCN was not applied as a foliar spray which may account for the differences in response to NH_4SCN in the process of absorption.

Benson and Calvin (3) and Wilson and Calvin (38) first showed glycine and glycolate to be fairly early and abundant products of photosynthetic carbon fixation and Tolbert (34) showed that at 0.01% CO_2

sedum leaves incorporated large percentages of ^{14}C in glycolate, glycine, and serine during photosynthesis. The work of Jimenez, Baldwin, and Tolbert (20) and Wang and Waygood (37) showed that the glycolate pathway, involving glycolate, glyoxylate, glycine, and serine is a part of a pathway leading to carbohydrate synthesis. Ongun and Stocking (27) showed that most of carbon fixed in photosynthesis flowed through glycine and serine prior to sugar and starch synthesis in tobacco leaves. In these experiments, the label accumulated in glycine, serine, and alanine after photosynthesis in leaf tissue did not appear in sugars and starch if the tissue was removed to the dark. Apparently light or a photosynthetic product was necessary for the conversion of the amino acids to sugars and starch. If a photosynthetic product is essential, apparently any agent which blocks photosynthesis would block this conversion. In the present study we found that NH_4SCN blocked the conversion of glycine to sugars. It is possible that NH_4SCN acts directly by blocking the conversion of glycine to serine. Serine appears to be low in NH_4SCN treated tissue as indicated by the work of Carter (8). However, it appears more likely that NH_4SCN blocks an earlier step in photosynthesis which prevents the flow of photosynthate through the glycolate pathway in a manner similar to the effects of darkness as shown by Ongun and Stocking (27). An interesting feature is that NH_4SCN inhibits the conversion of glycine to sugars by about the same percentage that the photosynthetic evolution of oxygen is inhibited.

The present studies do not lead to a complete understanding of the mechanisms whereby NH_4SCN might enhance transport of herbicides in plants but certain possibilities are evident. An increased amylase activity might result in enhanced carbohydrate transport with

concomitant herbicide movement. Rohrbaugh and Rice (32) showed that sugars applied to destarched bean leaves would enhance 2,4-D movement. The increased respiration might also furnish additional energy for transport. Dalrymple and Basler (11) showed that translocation rates of 2,4,5-T in blackjack oak parallel respiration rates. Harel and Reinhold (17) conducted experiments which indicated that the movement of substances from the mesophyll cells into phloem conducting cells is energy dependent even though downward movement in the phloem was not inhibited but was stimulated by the oxidative phosphorylation uncoupler dinitrophenol.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The effects of NH_4SCN on some of the basic metabolic processes in plant tissues were studied. It was hoped that these studies might lead to clarification of the process whereby NH_4SCN enhances translocation of some herbicides in some plants. Since NH_4SCN has been shown to enhance translocation of 2,4,5-T in cotton seedlings fairly extensively, cotton plants were chosen for these studies.

The initial studies showed that NH_4SCN decreased starch content in leaf tissue indicating either an enhancement of catabolism or inhibition of synthesis of the carbohydrate reserves of the leaf tissue. Other studies were designed to determine the effects of NH_4SCN on the breakdown of starch and utilization in respiration and synthesis of carbohydrates in the process of photosynthesis.

The studies showed that NH_4SCN treatment increased the amylase enzyme level in cotyledon tissue. There was a concomitant decrease in sugar levels in the treated tissue indicating that the possible increased amylase activity was not resulting in increased free sugars upon starch breakdown. The low sugar levels in treated tissue may have resulted because of two effects of NH_4SCN on the leaf tissue. There were increased respiration rates upon treatment indicating an increased carbohydrate utilization and, also, NH_4SCN inhibited photosynthesis as indicated by oxygen release.

The inhibition of photosynthesis was not related to an effect of NH_4SCN on the Hill reaction. There was an effective blockage of the flow of photosynthetically fixed carbon through the glycolate pathway and glycine to the carbohydrate fraction as indicated by an inhibition of the conversion of radioactive glycine-^{14}C into the neutral sugars after NH_4SCN treatment. It appears that this blockage would be an effective mechanism for NH_4SCN mediated carbohydrate decreases.

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APPENDIX

TABLE VII

DATA FOR GLUCOSE STANDARD CURVE

Table Number	mg. glucose	O. D. at 540 mμ
1	0	35
2	0.4	130
3	0.8	239
4	1.2	325
5	1.6	435
6	2.0	532

*The concentration of glucose solution is 2mg./ml.

TABLE VIII

ANALYSIS OF AMYLASE ACTIVITY OF COTTON COTYLEDONS AFTER 24 HOUR TREATMENT PERIOD WITH 2000 ppm NH_4SCN

Time (minutes)	mg. of glucose release/gm. fresh weight		
	Control		2000 ppm NH_4SCN
	Expt 1	Expt 2	Expt 1
0	0.392	0.424	0.220
15	0.664	0.606	0.630
30	0.854	0.969	1.008
40	1.087	1.151	1.291
60	1.388	1.515	1.764

TABLE IX

ANALYSIS OF AMYLASE ACTIVITY OF COTTON COTYLEDONS AFTER 48 HOUR TREATMENT PERIOD WITH 2000 ppm NH_4SCN

Time (minutes)	mg. of glucose release/gm. fresh weight			
	Control		2000 ppm NH_4SCN	
	Expt 1	Expt 2	Expt 1	Expt 2
0	0.635	0.774	0.244	0.080
15	0.888	0.967	0.518	0.709
30	1.143	1.258	0.914	1.161
40	1.555	1.355	1.219	1.419
60	1.651	1.742	1.646	2.032

TABLE X

OXYGEN RELEASE BY PHOTOSYNTHESIS OF COTTON LEAF DISCS AFTER 4 HOUR TREATMENT WITH NH_4SCN

	2000 ppm NH_4SCN			Control		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0	0	0	0	0	0	0
3	21.6	27	37.5	46.25	62.86	44.42
6	48.26	57.5	56	93	121.2	82.18
9	70.8	75.5	84.5	136.57	185.5	126.5
12	99.71	99.5	104	186		167.44

TABLE XI

OXYGEN RELEASE BY PHOTOSYNTHESIS OF COTTON LEAF DISCS AFTER 17 HOUR TREATMENT WITH NH_4SCN

	2000 ppm NH_4SCN			Control		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0	0	0	0	0	0	0
3	8.88	12.2	14.5	44.78	54.92	35.06
6	20.08	28.2	29.5	93.34	111.78	71.66
9	28.64	44.5	44	138.82	174.6	105.18
12	34.32	56	56	185.59	231.26	144.86

TABLE XII

OXYGEN UPTAKE BY RESPIRATION OF COTTON LEAF DISCS AFTER 4 HOUR TREATMENT WITH NH_4SCN

	2000 ppm NH_4SCN			Control		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0	0	0	0	0	0	0
5	24.32	30	30	16.39	20.02	16.94
10	47.12	51	51	29.80	35.42	27.72
15	68.40	70.5	76.5	41.72	49.28	43.12
20	95.76	94.5	97.5	59.60	66.22	53.90
25	121.60	123	129	78.97	90.86	73.92
30	141.36	138.0	151.5	90.89	100.1	86.24
35	167.2	169.5	175.5	110.26	123.2	100.1
40	186.96	187.5	198	120.69	137.06	115.5

TABLE XIII

OXYGEN UPTAKE BY RESPIRATION OF COTTON LEAF DISCS AFTER 17 HOUR TREATMENT WITH NH_4SCN

	2000 ppm NH_4SCN			Control		
	Expt 1	Expt 2	Expt 3	Expt 1	Expt 2	Expt 3
0	0	0	0	0	0	0
5	33.44	31.5	33	20.86	20.02	26.18
10	60.80	60	63	38.74	38.5	44.66
15	91.2	93	97.5	62.58	61.6	69.3
20	118.56	118.5	126	81.95	77	87.78
25	148.96	151.5	159	104.3	100.1	113.96
30	176.32	181.5	189	125.16	120.12	135.52
35	205.2	207	217.5	146.02	137.06	154
40	229.52	238.5	247.5	165.39	157.08	178.64

TABLE XIV

DATA FOR THE EFFECT OF NH_4SCN ON HILL REACTION

Absorption at 420 mμ		Expt. No.	Reaction time (minute)				
			0	0.5	2	4	6
2000 ppm NH_4SCN		1	0.702	0.660	0.633	0.612	0.594
		2	0.695	0.678	0.628	0.634	0.580
		3	0.673	0.661	0.639	0.628	0.614
Control		1	0.738	0.637	0.632	0.607	0.578
		2	0.741	0.658	0.637	0.595	0.595
		3	0.683	0.638	0.627	0.632	0.606

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