ATRAZINE (2-CHLORO-4-ETHYLAMINO-6-ISOPROPYLAMINO-S-TRIAZINE) DRIFT STUDIES WITH HORTICULTURAL CROPS

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BY

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TABLE OF CONTENTS

TABLE OF CONTENTS cont'd.

LIST OF TABLES

LIST OF TABLES cont'd.

LIST OF FIGURES

ABSTRACT

The feasibility of diagnosing atrazine drift accurately with physical symptoms and tissue analysis was studied extensively with cucumber plants. Concentrations of atrazine ranging from 0.0005 to 1.0 lb active/A were sprayed on mature cucumber plants.

Although the physical symptoms resembled many other types of damage, the symptoms were sufficiently distinct to be used as secondary evidence along with tissue analyses. Physical symptoms appeared initially 2 or 3 days after the spraying as marginal and/or intervenal chlorosis. These chlorotic symptoms turned briefly to a bleached-white color on the leaves, which became necrotic within 5 days after spraying.

The H.S.P.A. Method, an ultraviolet spectrophotometric method, was evaluated and found suitable to detect the presence of atrazine in cucumber tissue at levels where physical symptoms were difficult to detect and essentially no damage occurred. Although the addition of alumina columns to the H.S.P.A. Method was not necessary with cucumber leaves under the test conditions, they were beneficial when snapbeans were analyzed. Alumina columns decreased interfering background and were not responsible for any loss of atrazine.

To determine the best time to sample, cucumber plots were sprayed with 0.1 lb active/A atrazine and harvested 12 hours, 1, 2, 4 and 7 days after the spraying. The samples were analyzed using the H.S.P.A. Method. The sampling time experiment showed a rapid decrease of atrazine from the time of spraying to 4 days after the spraying and very little decrease of atrazine from 4 to 7 days. For consistency samples should be taken 4 to 7 days after the suspected drift even though an earlier sampling would

give higher tissue readings. After 7 days the damaged leaves started to fall from the plant.

In all field experiments the sampling of only the most damaged leaves proved to be a very satisfactory procedure.

INTRODUCTION

The use of chemicals to control diseases, insects, and weeds in most agricultural industries is well known and accepted as necessary. However, the general public is becoming more and more aware of the other aspect of chemical control, namely the varied dangers of pesticides. The small farmer in Hawaii whose livelihood depends wholly on his few acres of diversified crops is certainly aware of the dangers of chemical drift. The sugar cane plantations' large scale use of chemical control of weeds is understood by most, but the anxiety of the small farmer in danger of herbicide drift must also be realized and understood. The possibility of future cases of doubt and eventual conflict is understandable. Herein lies the necessity for the fair and accurate determination of suspected herbicide drift. Both the individual plantations and the near-by farmers should know whether illegal drift is or is not occurring. This will have the long range effect of correcting drift (if occurring) and, on the other hand, decreasing the farmers' doubts and complaints when drift is properly controlled by the sugar cane plantations or any other party as verified by "proper" personnel using "proper" techniques.

Atrazine is one of the primary pre-emergent herbicides of the sugar cane industry in Hawaii. Since the aerial application of this herbicide is in common use by the sugar cane plantations, atrazine drift onto susceptible crops may possibly occur. Cases of suspected herbicide damage due to drift, whether real or not, will inevitably arise from near-by farmers growing diversified crops.

Obviously, the quickest and cheapest method of diagnosing herbicide damage is through visual symptoms. But even with the use of visual

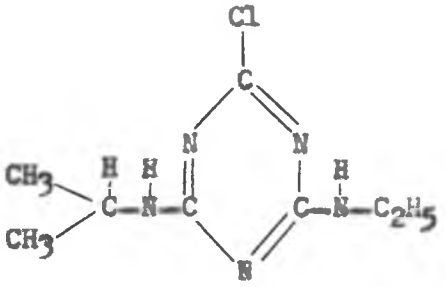
symptoms a suspected drift case may still be very difficult to diagnose. A second method of diagnosing a suspected drift case, which may prove to be more accurate and objective, is tissue analysis. The use of soil analysis is not usually practical because of the many complicated variables when working with the soil and the generally minute amounts of herbicide in a drift pattern. The use of sensitive indicator plants such as weed species growing near crop areas may be of some importance in diagnosing atrazine damage.

The main purpose of the studies was to accumulate information which might be helpful in diagnosing atrazine damage due to drift onto horticultural crops. Physical symptoms, sampling techniques, and various aspects of the tissue analysis were studied.

LITERATURE REVIEW

Physical and Chemical Properties of Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), which was discovered by Gysin and Knüsli, is described in patents owned by the Geigy Chemical Corporation. The chemical structure and formula are shown in Fig. 1. The chemically pure substance is a non-combustible, non-corrosive white crystal. Although only slightly soluble in water (70 ppm at 27°C) it is much more soluble in organic solvents. It has a melting point of 173 - 175°C and a pk value of 1.68 at 22°C. It is stable in neutral, slightly acidic or basic media, but hydrolyses at higher temperatures in a more acid or basic media to 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine, a compound with no herbicide properties. Atrazine will tend to sublime at higher temperatures (25).



$C_8H_{14}ClN_5$

Fig. 1. Atrazine Structure and Chemical Formula.

Residue Analysis for Atrazine

Knüsli (25) mentioned three methods of analyzing for atrazine residue: 1) a spectrophotometric method, 2) a colorimetric method, and 3) a bioassay method. In the spectrophotometric method atrazine was extracted from plant materials with chloroform, cleaned with a dry packed column of Woelm basic aluminum oxide, hydrolysed to the herbicidally inactive hydroxy form and the absorption of the hydroxy form read spectrophotometrically in the

spectrophotometrically in the ultraviolet range at 240 mu. The application of this method to soil residue was not always satisfactory. In the colorimetric method chlorotriazines were reacted with pyridine and alkali for color development. Although the color decreased rapidly, the method was still somewhat accurate and easy to use. This colorimetric method was tried only with soil residues and not with plant residues. For the bioassay method Knüsli referred to an unpublished work of Talbert (1960-1961) who recommended Clark soybeans as a suitable bioassay crop for atrazine. The suitable range in Mexican silt loam was 5 ppm and upward.

An ultraviolet spectrophotometric method very similar to the method mentioned by Knüsli (25) in the "Advances in Pest Control Research (Volume IV)" was used successfully by Hilton and Uyehara (24) to analyze sugar cane tissue for atrazine residue. In this method n-pentane was used as the extracting solvent and a centrifuge step was used to eliminate some of the pigments. Alumina columns were not found to be necessary.

Mattson et al. (28) compared the microcoulometric gas chromatograph method and the ultraviolet spectrophotometric method for the detection of various s-triazine residues in crop materials. The correlation between the two methods was very close. When soybean and wheat straw were spiked with atrazine in the range of 0.05 to 0.10 ppm the microcoulometric gas chromatograph method gave recoveries as high as 90%.

Abbott et al. (1) used thin-layer chromatography to separate and determine various triazine herbicides in soil and water. Clay soil spiked with atrazine in the range of 0.10 ppm gave a recovery of 72%.

Benfield et al. (6) applied a gas-liquid chromatograph method to detect triazine residues in soil and crops. They added a closely related

triazine before extraction as an internal standard.

Radke et al. (34) evaluated the pyridine-alkali and the pyridine-alkali-ethyl cyanoacetate colorimetric method for the determination of atrazine in soil residues. The pH of the system and the temperature of the atrazine-pyridine complex reaction with alkali influenced the color intensity. The pyridine-alkali-ethyl cyanoacetate colorimetric method was more sensitive than the pyridine-alkali method. Some of the advantages of the colorimetric methods mentioned were: 1) no interferences when applied to water extracts of soils, 2) less time-consuming than the ultraviolet spectrophotometric method (did not require hydrolysis of atrazine to hydroxyatrazine), and 3) no interference of the atrazine determination from hydroxyatrazine.

General Biological Properties of Atrazine

Atrazine can be used both as a pre-emergence and a post-emergence herbicide. It is used as a nonselective herbicide at high concentrations and as a selective herbicide at low concentrations with such crops as corn, sorghum, millet, vine, asparagus, sugar cane, and pineapple (25). Where rainfall is a limiting factor for simazine, atrazine can often replace simazine due to its higher water solubility (21). The Atrazine Technical Bulletin No. 63-1 (20) indicated that atrazine was toxic to a broader spectrum of weeds than simazine, but was less selective to crops. Atrazine damaged cereal grains, asparagus, soybeans, peanuts, potatoes and many vegetables (20). Atrazine has been shown to have a relatively low mammalian toxicity (acute oral LD_{50} of 3080 mg/kg with rats and 1750 mg/kg with mice) (25).

Absorption and Translocation of Atrazine

Simazine was shown by Sheets (37) and Davis et al. (14) to be absorbed by the roots, and translocated to the leaves where it accumulated. Similar results were found for atrazine by Davis et al. (13) and Wax et al. (42). Although Davis et al. (14) found almost no absorption or translocation of simazine from intact leaves, later work by Crafts (11) showed that simazine was absorbed and translocated via the apoplast within the treated leaf but was not translocated out of the treated leaf. Wax et al. (42) demonstrated that the uptake and translocation of root-fed atrazine increased with an increase in temperature or a decrease in relative humidity. The foliar absorption of atrazine has been shown by many workers (7, 9, 13, 42). Biswas (7) found both acropetal and basipetal movement of atrazine when atrazine was applied to peanut (Arachis hypogeae) foliage. Wax et al. (42) found a slightly greater foliar uptake and translocation at 80°F than at 60°F.

Metabolism of Atrazine

The degradation of simazine or atrazine in corn has been studied and confirmed by many workers (17, 29, 32). In 1962 a simazine-resistance factor was purified and described by Castelfranco et al. (10). The active ingredient was later isolated and identified as 2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine (22, 36). This cyclic hydroxamate was able to convert chlorotriazines to the hydroxy analogues in vitro and possibly in vivo (22). Montgomery et al. (30) indicated that this conversion appeared to be the primary reason for the high tolerance of corn to the chlorotriazines. Davis et al. (14) found that the extent of degradation of simazine in corn, cotton and cucumber was in agreement with the relative

susceptibilities of these plants. Freed (16) found a similar relationship with the breakdown of atrazine by corn, oats, alfalfa, and cucumber. Castelfranco et al. (10) showed that pyridine and hydroxylamine were also able to convert simazine to the 2-hydroxy analogue.

Although Funderburk et al. (17) found a reduction in the activity of catalase, peroxidase, phenol oxidase, ascorbic acid oxidase and glycolic acid oxidase in oats, soybeans, cotton, peanuts, Johnsongrass and corn with 1 lb/A atrazine, they were for the most part not able to correlate enzyme activity with sensitivity to atrazine. However, a slight correlation between increasing phenol oxidase activity with increasing resistance to atrazine was found.

Shimabukuro et al. (38) recovered 88 - 90% of the total activity in a chloroform-soluble fraction 48 hours after mature pea plants, Pisum sativum L., var Little Marvel, were fed ring-labeled atrazine-C^{14}. This chloroform fraction consisted of atrazine and one principal metabolic product as detected by thin-layer chromatographic analysis. The degraded metabolic product in the chloroform-soluble fraction turned out to be 2-chloro-4-amino-6-isopropylamino-s-triazine. No hydroxyatrazine could be detected in the water-soluble fraction.

Mode of Action of the Chlorotriazines

The mode of action of the triazines has not been fully worked out. Much evidence has been accumulated to indicate that triazines interfere with the photosynthetic mechanism (15, 19, 31, 44). By the addition of sucrose Gast (19) was able to counteract the starch synthesis inhibition of Coleus blumei Benth that was caused by simazine. Simazine was shown by Exer (15) to inhibit the photochemical reduction of diphospho-pyridine

nucleotide in isolated chloroplasts. In the light, CO_2 fixation and sucrose synthesis of red kidney bean, Phaseolus vulgaris L., were shown by Zweig et al. (44) to be inhibited by atrazine. Zweig et al. (44) also found that atrazine had no effect on nonphotosynthetic CO_2 fixation in the dark.

Ashton et al. (4, 2) postulated that other processes may be involved in the mode of action of the chlorotriazines in addition to the photosynthetic block. Ashton et al. (3) described various histological changes which occurred in the light but not in the dark when Phaseolus vulgaris was treated with atrazine. Similar results were found with red kidney bean plants by Ashton et al. (2). The effect of atrazine on sucrose-C^{14} and serine-C^{14} metabolism was also studied by Ashton et al. (4). Like the earlier work the results showed that atrazine not only blocked photosynthesis but also affected other metabolic processes independent of photosynthesis.

Herbicide Drift and Pesticide Laws

The food shortage problem is important to a rapidly growing world population. As farms increase in size more efficient use of land becomes essential. The chemical control of weeds and other pests over large land areas has been widely accepted as necessary, but the publicity and concern over the actual and potential dangers of pesticides are rapidly spreading. In the future the control over the types and uses of pesticides will probably tighten substantially.

Fuquay (18) stressed three areas necessary for a definitive study of drift problems. These three areas were: "... characteristics of the source, the diffusive properties of the applicator and its effects on the receptor."

The danger of generalisations based on cases lacking quantitative measurements was also pointed out.

To insure fairness to all those involved Marvin (27) pointed out the need for accurate diagnoses in investigations of claims against herbicides. The importance of honesty and an open mind by investigators in such cases was stressed. In his experience half of the claims, although honestly made, were still erroneous. Examples of many unusual cases were given.

Dale (12) discussed the U. S. Department of the Interior's position on the safe use of pesticides making it clear that the U. S. Department of the Interior is primarily concerned with safety. Dale admits that "...Labels are screened currently through an incomplete structure of factual information." The need for knowledge of the long-term effect of persistent applications of many common herbicides on humans, wildlife, and the total environment was also emphasized.

Of current interest is the use of invert emulsions and other thickening agents as an additive to control drift. Ogle (33) discussed the benefits of Norbak and Sprayberry (39) mentioned the use of Dacagin at the Washington State Weed Conference held on November 1-2, 1965.

The two major Federal pesticide laws are: 1) the Federal Insecticide (IF & R) Act of 1947 and 2) the Federal Food, Drug and Cosmetic Act of 1938, as amended by the Miller Pesticide Chemicals Amendment of 1954 (40).

The IF & R Act is a label registration law and is administered by the Department of Agriculture (Pesticide Regulations Division, Agricultural Research Service). Essentially, this law requires the manufacturer to show that the pesticide is effective and safe (40).

The Federal Food, Drug, and Cosmetic Act is administered by the

Department of Health, Education, and Welfare (Food and Drug Administration) which sets and enforces tolerances (40). This act may be of particular importance in drift cases since drift damage may occur on crops with a zero tolerance. In other words suspected crops containing small amounts of residue can be seized if transported across state lines (40).

Three laws of the State of Hawaii that are somewhat related to herbicide drift are: 1) Air Pollution Control Law, 2) Hawaii Food and Cosmetic Act and, 3) a law governing the sale and use of 2,4-D and related plant hormone herbicides. The first two laws come under the authority of the Hawaii Department of Health. The third law is governed by the Hawaii Department of Agriculture (23).

Detection of Atragine Drift in Hawaii

Obviously, the quickest and cheapest method of diagnosing herbicide damage is through visual symptoms. Work conducted during the summer of 1964 by Romanowski and Hilton (35) demonstrated the visual symptoms of atrazine, ametryne and diuron damage on Chinese cabbage (Nagaoka 60 days), cucumber (Burpee's Hybrid), papaya (various breeding lines), tomato (U. of H. Hybrid N-57) and snapbeans (Slendergreen). Various concentrations (0, 0.005, 0.05, 0.1 and 0.5 lb/A) of the herbicides were sprayed on the test crops at different stages of growth. Slight to moderate ratings of leaf burn symptoms occurred at the 0.05 and 0.1 lb/A concentrations, and most of the plants were severely damaged or dead at the 0.05 lb/A concentration. Symptoms appeared three to four days after spraying and became pronounced at six to eight days. With the exception of severe leaf burn and chlorosis persisting on the older leaves, most of the plants recovered fourteen to twenty-one days after spraying. The symptoms appeared first as water soaked

spots on the leaf surfaces and margins, developing later into marginal and intervenal chlorosis. Symptoms usually appeared only on the leaf surfaces of the exposed areas of the older leaves. Stunting was also a general symptom on most of the damaged plants.

A second method of diagnosing a suspected drift case, which may prove to be more accurate and objective, is tissue analysis. Romanowski and Hilton (35) showed that diuron tissue analysis, when interpreted properly by qualified personnel, can be used as an aid in diagnosing suspected diuron drift onto horticultural crops. An attempt to correlate atrazine and ametryne tissue analyses to visual observations was not as successful as for diuron.

MATERIALS AND METHODS

All field experiments were conducted on the Manoa campus of the University of Hawaii from June 1965 to May 1966. The cucumber plant 'Burpee's Hybrid' was the main test crop. Snapbean 'Slendergreen' and 'Bountiful' and tomato 'U. of H. Hybrid N-57' plants were also used in some of the experiments. Commerical atrazine (80% wettable powder) was sprayed on all field experiments at the rate of 40 gallons of spray mix per acre and at 30 pounds per square inch of pressure with the use of a fiberglass backsprayer. All spray-concentration values are assumed to be pounds active per acre throughout the thesis.

The field plots were 5 by 20 feet and furrow irrigated twice weekly. Insects and diseases were controlled with O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (Diazinon), S-(1,2-Dicarbethoxyethyl) O,O-dimethyl phosphorodithioate (Malathion), and Zinc ethylenebisdithiocarbamate (Dithane Z-78). Temperature and rainfall data, sampling technique, and the various analytical procedures are given in the Appendix.

Initial Screening for Atrazine Phytotoxicity

The main purpose of this experiment was to study the phytotoxicity of atrazine on cucumber 'Burpee's Hybrid', snapbean 'Slendergreen' and tomato 'U. of H. Hybrid N-57' plants. Physical symptoms were described and rated from one to five. The rating scale was as follows: 1) no burn, 2) slight burn and/or chlorosis, 3) moderate burn, 4) severe burn, and 5) dead. Besides the visual symptoms on the damaged plants the time between spraying and the appearance of visual symptoms, and the time before recovery were studied. If it could be shown that a definite and

consistent period of time elapsed between the spraying date and the appearance of visual symptoms, this period could be useful in diagnosing suspected atrazine drift. If the period of time for recovery was also definite and consistent it could be used in the same manner. Therefore, the time elements were also observed and recorded.

A randomized complete block experimental design with three replications was used in this experiment. The treatments for each of the three test crops were as follows:

Time of Spraying	Atrazine (lb active/A)
4 Weeks After Sowing or Transplanting	0.0
4 Weeks After Sowing or Transplanting	0.005
4 Weeks After Sowing or Transplanting	0.05
4 Weeks After Sowing or Transplanting	0.1
6 Weeks After Sowing or Transplanting	0.0
6 Weeks After Sowing or Transplanting	0.005
6 Weeks After Sowing or Transplanting	0.05
6 Weeks After Sowing or Transplanting	0.1

The experiment was not analyzed as a factorial arrangement of treatments because of the diversity of crops and environmental conditions between spray dates. The 4 and 6 week spray data were computed as separate randomized complete block experiments on a crop basis.

Two hundred gram (fresh weight) cucumber leaf tissue samples were taken to evaluate the use of tissue analysis in detecting atrazine drift. The samples consisted of the most damaged leaf tissues instead of a randomized sample because it was anticipated that the samples would contain

more atrazine and be more consistent. An ultraviolet spectrophotometric method, which was used successfully by Hilton and Uyehara (24) of the Hawaiian Sugar Planters Association to analyze sugar cane tissue for atrazine, was used to analyze the samples. This method is referred to in this thesis as the H.S.P.A. Method, and is given in detail in the Appendix. The method is very similar to that described by Knusli (25) in the "Advances in Pest Control Research (Volume IV)".

One hundred gram samples (fresh weight) of snapbean leaf tissues were also taken from the check and the 0.1 lb/A plots. In order to find out whether the undamaged leaves contained any detectable atrazine both the damaged and undamaged leaves were sampled separately from each plot. The H.S.P.A. Method mentioned above was used to analyze the samples.

Laboratory Studies

Standard Curves:

Atrazine standard curves following the H.S.P.A. Method were made with and without cucumber tissue. The detailed procedure is shown in the Appendix. The procedure was such that 0, 10, 20, 40, 50 and 60 ug of atrazine were contained in the final 10 ml of solution. Pure atrazine was mixed with chloroform to make up the stock solution (4 ug atrazine/ml of chloroform).

Two hundred gram cucumber tissue samples were harvested approximately 6 weeks after sowing and a standard curve determined. The atrazine standard curve without cucumber tissue was replicated four times and the curve with cucumber tissue three times.

Evaluation of Alumina Columns With the H.S.P.A. Method:

Part of the cleanup procedure in the ultraviolet spectrophotometric method mentioned by Knüsli (25) was the use of Woelm basic alumina (activity grade V) columns. The obvious advantage of using such columns was the elimination of some of the interfering background materials. A possible disadvantage of using the alumina columns was the partial loss of atrazine either in the wash solution or on the alumina columns. To evaluate the importance of using the alumina columns with the H.S.P.A. Method, standard curves with and without cucumber tissue were established in the presence and absence of alumina columns. In order to keep conditions as close as possible in both the alumina and no alumina treatments, a one-half aliquot from the solution spiked with atrazine was determined using the H.S.P.A. Method without alumina columns and the other one-half aliquot from the same solution was determined using the H.S.P.A. Method with alumina columns. The exact procedures are given in the Appendix. The standard curves without cucumber tissue were replicated four times and with cucumber tissue three times. A F-test was used to evaluate significant differences between the alumina and no alumina treatments.

Use of Alumina Columns in the Evaluation of Background Due to Snapbeans:

Preliminary studies indicated a high background due to snapbeans. In fact, the maximum absorption peak usually occurred around 237 mu rather than the desired hydroxyatrazine peak at 240 mu. This shifting of the maximum absorption peak was thought to be due to background extracted from the snapbeans. Therefore, five samples of snapbeans sprayed with atrazine at 0.1 lb/A were analyzed using the H.S.P.A. Method with and without alumina columns.

Evaluation of the Centrifuge Step in the H.S.P.A. Method:

An evaluation of the centrifuge step in the H.S.P.A. Method was initiated in an attempt to eliminate all unnecessary steps in the method. Standard curves with cucumber leaf tissues were established with and without the centrifuge step.

Chloroform Vs. Pentane Extraction:

Since atrazine is much more soluble in chloroform than pentane, a limited experiment was performed to try to evaluate the use of chloroform as an extracting solvent with cucumber tissues. Also, chloroform was used as the solvent in the method described by Knüsli (25). For this study each plot was sprayed with 0.1 lb/A atrazine, harvested, chopped, mixed and separated into two 100 gram samples. One 100 gram sample was analyzed using chloroform as the extracting solvent and the other 100 gram sample from the same plot was analyzed using pentane as the extracting solvent. The H.S.P.A. Method without the centrifuge step but with alumina columns was used to analyze both samples. The treatments were replicated four times.

Background of Check Cucumber Samples:

A true check tissue sample is hard to attain in drift cases for several reasons. Check samples must be sampled from fields other than the field in question. Naturally, environmental and cultural conditions may differ. Also, damaged samples are more necrotic than check samples. Therefore, the check tissue sample may not be a true representation of the background of the suspected sample. The solution to this problem may or may not be important depending on the type and consistency of the

background. Therefore, many check tissue samples were evaluated on the DK-2 spectrophotometer with water as the reference sample to evaluate the type and consistency of the backgrounds.

Validity of Diluting Samples Just Before Reading the Absorption:

When a sample is found to contain so much atrazine that a reading on the DK-2 spectrophotometer cannot be attained, a smaller sample is usually analyzed again or a smaller aliquot is taken from the extracted solution and analyzed. In either case it is time consuming. Therefore, the validity of diluting the final solution was checked.

Since the readings on the sampling time experiment turned out too high it was used conveniently for this study. The final solutions were diluted to one-tenth the concentration with water and read over. In order to check the validity of this dilution one-tenth aliquots were removed from the original extracted solutions and analyzed similarly. Supposedly, both readings should be approximately the same.

Final Field Phytotoxicity and Sampling Studies

Delayed Freezing of Field Samples:

Because field samples may not always be frozen immediately after sampling, an experiment was set up to study the effect of delaying the freezing of field samples. A randomized complete block experiment with three replications was used to study time intervals of 0, 3, 5, 7 and 14 days between sampling and freezing. A 200 gram sample was taken from each plot one week after spraying. The chopped samples were sealed in polyethylene bags and kept at 72°F for the desired time intervals before the respective samples were frozen. The samples were analyzed using the

H.S.P.A. Method without the centrifuge step.

Sampling Time:

Atrazine is not expected to remain completely unchanged both on and in cucumber leaves after it has been sprayed. Atrazine may be volatilized, washed off by rain, or altered on the leaf surfaces by normal environmental conditions to an inactive form. Once atrazine is in the leaf it can also be metabolized to an inactive form. It can be hypothesized that atrazine probably decreases with time both on and in cucumber leaves.

On the other hand, the sampling procedure used in this study could favor an increase of atrazine in the latter few days. This can be explained by the fact that atrazine symptoms do not appear until a few days after the spraying and the sampling procedure calls for the sampling of the most damaged leaves. In addition later samples would contain more leaves per unit sample (fresh weight basis) in that the damaged leaves are much lighter. A decrease of atrazine would therefore have to be high in order to affect the results. Moisture samples were not taken in this experiment.

A randomized complete block experimental design with three replications was used to determine the amount of atrazine found in the tissues at 0.5, 1, 2, 4 and 7 days after spraying. A 200 gram sample was taken from each plot (5 by 20 feet) that was sprayed with 0.1 lb/A atrazine and analyzed using the H.S.P.A. Method without the centrifuge step.

Correlation of Spray-Concentration to Tissue Analysis:

In order to evaluate the complete process as a whole from the sampling procedure to the final reading on the DK-2 spectrophotometer, an

experiment was set up to correlate the spray-concentration (lb/A atrazine) to the concentration (ppm) of atrazine found from tissue analyses. If the tissue analyses can accurately and consistently differentiate between the concentrations sprayed in the field, it can be used to diagnose atrazine drift onto cucumber plants.

Atrazine was sprayed on the leaf surfaces at 0, 0.005, 0.05, 0.1 and 0.2 lb/A and 200 gram samples were harvested from each plot one week after the spraying. A randomized complete block experimental design was used with five replications. Samples were frozen and analyzed later using the H.S.P.A. Method (without the centrifuge step) with and without alumina columns.

Lower Limit of Detection:

Although previous data indicated that atrazine physical symptoms were difficult to detect on cucumber plants at the 0.005 lb/A treatment, atrazine could still be detected by tissue analysis. Therefore, an even lower concentration (0.0005 lb/A) of atrazine was tested. A 200 gram sample was harvested from each of five plots which were sprayed with 0.0005 lb/A atrazine and later analyzed using the H.S.P.A. Method without the centrifuge step but with alumina columns.

Physical Symptoms on Cucumber Plants at 1.0 lb/A Atrazine:

Sufficient data of physical symptoms due to small concentrations of atrazine (0.0005 to 0.2 lb/A) were recorded from earlier experiments. A final experiment was conducted using atrazine spray-concentrations of 0, 0.1, and 1.0 lb/A to study the expression of physical symptoms due to a high concentration of atrazine. It was thought that the physical symptoms

might appear earlier with a heavier concentration (1.0 lb/A).

RESULTS AND DISCUSSION

Initial Screening for Atrazine Phytotoxicity

Plants were sprayed at 4 and 6 weeks after sowing and phytotoxicity symptoms recorded at given time intervals. Since the environmental conditions were so different on the two spraying dates, no attempt was made to analyze the effect of age on crop sensitivity to atrazine. Rather, the treatments from the 4 and 6 week spraying were analyzed separately as two different experiments.

4 Week Spraying:

A heavy downpour of rain (see Appendix) occurred almost immediately after the plots were sprayed; consequently, a substantial amount of atrazine was probably washed off the leaves.

No visible symptoms were detected on the tomato plants at any of the atrazine concentrations (Table 1). Slight damage was detected on cucumber and snapbean plants at the 0.005 lb/A concentration and moderate damage was noted at 0.05 and 0.1 lb/A (Fig. 2 and 3). On both of the affected crops the first symptoms were a marginal and/or intervenal chlorosis. Chlorotic symptoms began to appear on the second day after spray application and the symptoms usually turned necrotic within two days. In most cases the severity of the symptoms did not increase after the fourth day after the spraying. As new leaves grew and damaged leaves fell to the ground the symptoms produced by the atrazine gradually disappeared. The symptoms did not show up on any of the original undamaged tissues. This observation indicates that atrazine was not translocated from the damaged leaves.

Fig. 2. Chlorotic Symptoms of Atrazine Damage on Cucumber Plants.

Fig. 3. Bleached-Color on Cucumber Plants Due to Atrazine Damage.

The presence of atrazine in cucumber plants was detected using the H.S.P.A. Method (Table 2). All treatments were significantly different at the 1% level from all other treatments except between the check and the 0.005 lb/A treatment which were very close to significant at the 5% level (Table 2).

Although damaged and undamaged snapbean leaves were harvested from the 0.1 lb/A plots and analyzed chemically using the H.S.P.A. Method, the results were not completely dependable due to high interfering background of the snapbean leaf tissues. The maximum absorption peak was at approximately 237 mu instead of the expected 240 mu peak.

6 Week Spraying:

The expression of the symptoms on the cucumber and snapbean plants were essentially the same as for the 4 week spraying with the exception that the symptoms were more severe (Table 1 and 3).

All treatments with cucumber plants were analyzed chemically using the H.S.P.A. Method and were found to be significantly different from each other at the 1% level (Duncan's Multiple Range Test) except between the 0.005 and 0.05 lb/A treatments (Table 4). One of the replicates from the 0.05 lb/A treatment gave a very unusually low reading which was probably responsible for the lack of significance between the 0.005 lb/A and the 0.05 lb/A treatments. All of the plots were significantly different from the check at the 1% level of significance, which indicated that the presence of atrazine can be detected at very low spray-concentrations. More atrazine was detected chemically in cucumber tissues from the 6 week treatments (Table 4) as compared with the 4 week treatments (Table 2). This substantiated the slightly higher ratings of the physical symptoms from

Table 1. Average[a] subjective ratings[b] for leaf burn and chlorotic symptoms on plants from the 4 week spray application.

Crop	Treatment (lb active/A)	Days After Spraying 1	2	3	4	5	6 and 7
Cucumber	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	1.1	1.3	1.3	1.3	1.3
	0.05	1.0	2.0	2.7	2.7	2.7	2.7
	0.1	1.0	2.0	3.0	3.0	3.0	3.0
Snapbean	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	1.3	1.3	1.3	1.3	2.0
	0.05	1.0	2.0	2.3	2.3	2.3	3.0
	0.1	1.0	2.0	3.0	3.0	3.0	3.0
Tomato	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	1.0	1.0	1.0	1.0	1.0
	0.05	1.0	1.0	1.0	1.0	1.0	1.0
	0.1	1.0	1.0	1.0	1.0	1.0	1.0

[a] Each value represents an average of three replicates.
[b] Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 2. Cucumber leaf residue analysis for atrazine from plots sprayed 4 weeks after sowing and harvested 1 week after spraying (fresh weight basis).

Treatment (lb active/A)	ppm 1	2	3	Average[a]
0.0	0.0	0.0	0.0	0.0
0.005	0.037	0.079	0.055	0.057
0.05	0.105	0.215	0.169	0.163
0.1	0.195	0.323	0.278	0.265

[a] Means connected by a line are not statistically different at the 1% level (Duncan's Multiple Range Test).

Table 3. Average[a] subjective ratings[b] for leaf burn and chlorotic symptoms on plants from the 6 week spray application.

Crop	Treatment (lb active/A)	Days After Spraying 1	2	3	4	5	6 and 7
Cucumber	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	1.0	2.0	2.0	2.0	2.0
	0.05	1.0	1.7	2.3	3.0	3.0	3.0
	0.1	1.0	2.0	2.7	3.3	3.3	3.3
Snapbean	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	2.0	2.3	2.7	2.7	2.7
	0.05	1.0	2.3	2.7	3.0	3.0	3.0
	0.1	1.0	2.3	2.7	3.3	3.3	3.3
Tomato	0.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.005	1.0	1.0	1.0	1.5	1.5	1.5
	0.05	1.0	1.3	1.3	2.0	2.0	2.0
	0.1	1.0	1.7	1.7	2.0	2.0	2.0

[a] Each value represents an average of three replicates.
[b] Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 4. Cucumber leaf residue analysis for atrazine from the plots sprayed 6 weeks after sowing and harvested 1 week after spraying (fresh weight basis).

Treatment (lb active/A)	ppm 1	2	3	Average[a]
0.0	0.0	0.0	0.0	0.0
0.005	0.183	0.260	0.163	0.202
0.05	0.169	0.343	0.297	0.270
0.1	0.428	0.585	0.437	0.483

[a] Means connected by a line are not statistically different at the 1% level (Duncan's Multiple Range Test).

(see Appendix, pg)

the 6 week treatments. It should be noted that less rain fell immediately after the 6 week spray application (see Appendix).

Unlike the absence of physical symptoms on tomato plants after the 4 week spraying, physical symptoms appeared on the tomato plants shortly after the 6 week spraying. Slight symptoms appeared on a small per cent of the tomato leaves as marginal necrosis and/or small, intervenal, necrotic spots.

Tissue analyses for the 6 week snapbean tissue were not dependable due to the high interfering background of the snapbean leaf tissues.

Laboratory Studies

Laboratory studies were initiated to evaluate and possibly alter the H.S.P.A. Method for the purpose of analyzing horticulture crops for atrazine.

Standard Curves:

Atrazine standard curves were made with and without cucumber tissues using the H.S.P.A. Method. The exact procedures are given in the Appendix.

The standard curves with no cucumber tissues were linear and consistent (Table 5 and Fig. 4). One of the reasons for doing standard curves with cucumber tissue was to determine the amount of atrazine that was loss due to the addition of cucumber tissue. The per cent recoveries were low but consistent (Table 6 and Fig. 4). The per cent recoveries slowly increased from approximately 56% at the 0.1 ppm concentration to approximately 77% at the 0.5 and 0.6 ppm concentrations where the per cent recovery seemed to level off.

Table 5. Atrazine Standard Curve (No Crop), H.S.P.A. Method.

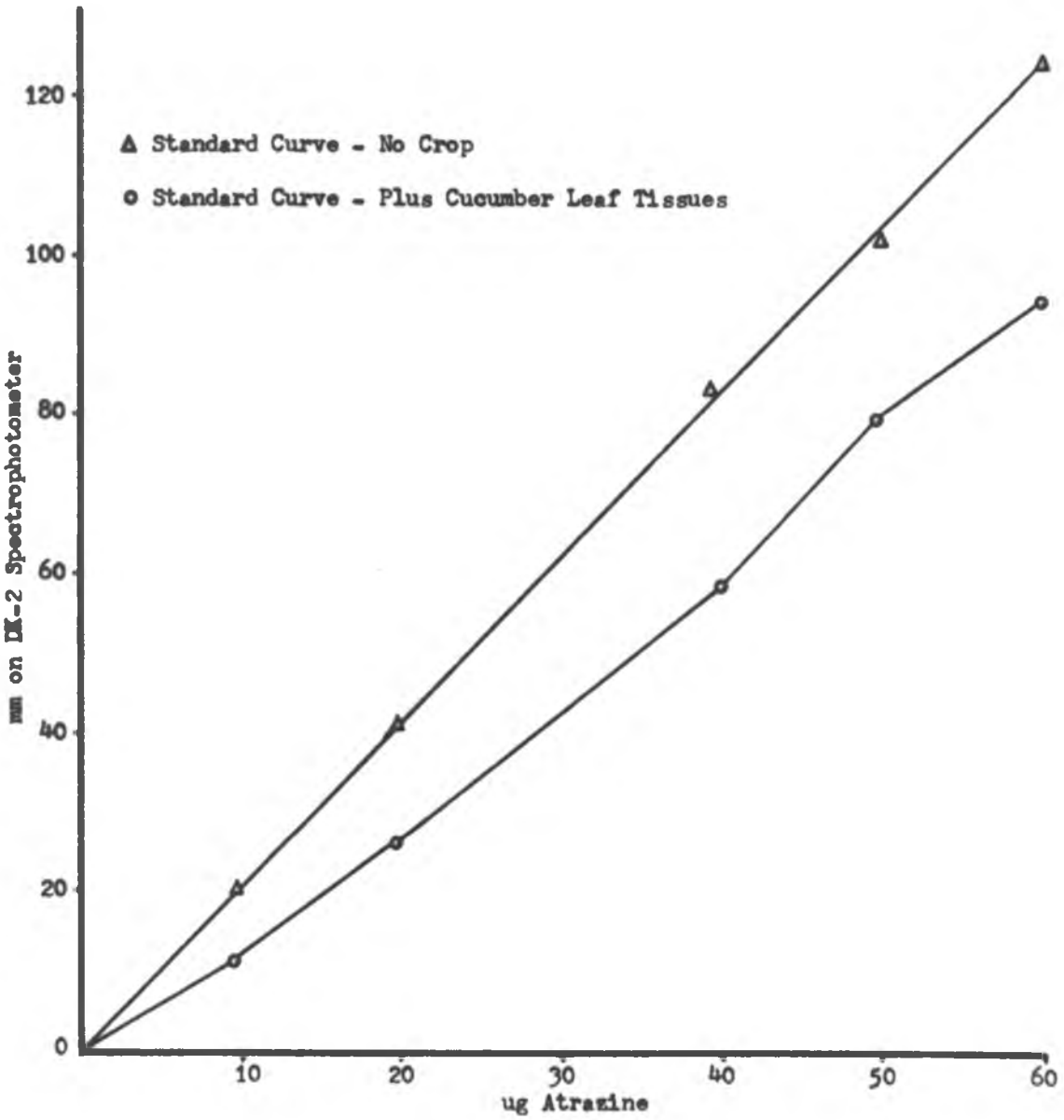
ug Atrazine/10 ml of Final Solution	mm on DK-2				Average
	1	2	3	4	
0	0	0	0	0	0.0
10	20	20	19	21	20.0
20	40	41	40	43	41.0
40	79	85	81	85	82.5
50	100	103	98	104	101.2
60	121	126	119	128	123.5

Table 6. Atrazine Standard Curve (With Cucumber Leaf Tissue), H.S.P.A. Method.

ug Atrazine/100 g of Tissue	mm on DK-2			Ave.	% Recovery[a]
	1	2	3		
0	0	0	0	0.0	0.0
10	12	12	10	11.3	56.5
20	26	27	26	26.3	64.1
40	58	58	57	57.7	69.9
50	81	81	77	79.7	78.8
60	95	--	93	94.0	76.1

[a] % Recovery is based on a comparison between the standard curve with no crop and the standard curve with cucumber leaf tissue.

Figure 4. Atrazine Standard Curves With and Without Cucumber Leaf Tissues, H.S.P.A. Method. The standard curves with no crop was replicated four times and the standard curves with cucumber leaf tissues three times.


120
100
80
60
40
20
0
mm on DK-2 Spectrophotometer
Standard Curve - No Crop
Standard Curve - Plus Cucumber Leaf Tissues
10
20
30
40
50
60
ug Atrazine

The concentrations of all cucumber samples were based on the atrazine standard curves with cucumber tissues. When the atrazine concentrations in undiluted cucumber samples fell within the 0.6 ppm concentration the values were read from the original standard curve with cucumber tissues, but when the atrazine concentrations in cucumber samples were higher than 0.6 ppm atrazine the final solutions were diluted and the concentrations read off a specially constructed standard curve based on a constant per cent recovery of 77% (Table 7).

Table 7. Atrazine Standard Curve With Cucumber Tissue Assuming 77% Recovery, H.S.P.A. Method.[a]

ug Atrazine/10 ml of Final Solution	77% Recovery (mm on DK-2)
0	0.0
10	15.4
20	31.6
40	63.5
50	77.9
60	95.1

[a]This table was for cucumber samples which had to be diluted because of high concentrations of atrazine.

An attempt will now be made to explain the low recoveries and the gradual increase of the recoveries as the concentration increased from 0.1 to 0.6 ppm. A substantial amount of atrazine was probably lost in the incomplete separation of the extracting solvent (pentane) and the cucumber tissues after the blending and mixing. No attempt was made to squeeze all of the extracting solvent out of the tissue. Any loss of atrazine in this way should have affected the per cent recoveries equally at all concentrations, in that, the same per cent of liquid should have

remained behind regardless of the concentration. However, this explanation does not account for the gradual increase in the per cent recoveries from 0.1 to 0.5 ppm. A possible reason for the gradual increase in the per cent recoveries with increase in the concentration from 0.1 to 0.5 ppm may have been a physical or chemical binding of atrazine to the cucumber tissues. It was presumed that all possible sites which could bind atrazine were saturated even at the lowest concentration with a relatively constant amount of atrazine. For example, if 2 ug of atrazine were absorbed, 20% of the 0.1 ppm concentration would have been lost compared to approximately 3% for the 0.6 ppm concentration.

Evaluation of Alumina Column With the H.S.P.A. Method:

In order to evaluate the use of alumina columns with the H.S.P.A. Method, standard curves with and without cucumber tissues were made with and without alumina columns. The detailed procedures are given in the Appendix.

The standard curves with no cucumber tissue indicated little or no decrease of atrazine due to the alumina columns (Table 8 and Fig. 5). The standard curves with cucumber tissue and alumina columns decreased the background but no decrease in the readings were noted (Table 9 and Fig. 6). Since the background due to cucumber tissue was low, the alumina columns were not necessary.

Use of Alumina Columns in the Evaluation of Background Due to Snapbeans:

Preliminary work with snapbean plants showed that the background due to snapbeans was high and tended to shift the maximum peak from 240 mu to approximately 237 mu. Therefore, an experiment was set up to evaluate

Table 8. Atrazine Standard Curves (No Crop) Using the H.S.P.A. Method With and Without Alumina Columns.

Treatment (ug Atrazine/10 ml of Final Solution)		mm on DK-2				Average
		1	2	3	4	
No Columns	0	0	0	0	0	0.0
	10	20	20	19	21	20.0
	20	40	41	40	43	41.0
	40	79	85	81	85	82.5
	50	100	103	98	104	101.2
	60	121	126	119	128	123.5
Alumina Columns	0	0	0	0	0	0.0
	10	19	20	18	20	19.2
	20	38	39	40	40	39.2
	40	78	83	75	83	79.8
	50	97	102	101	104	101.0
	60	116	125	113	124	119.5

Figure 5. Atrazine Standard Curves (No Crop) Using the H.S.P.A. Method With and Without Alumina Columns. Each point represents an average of four replications.

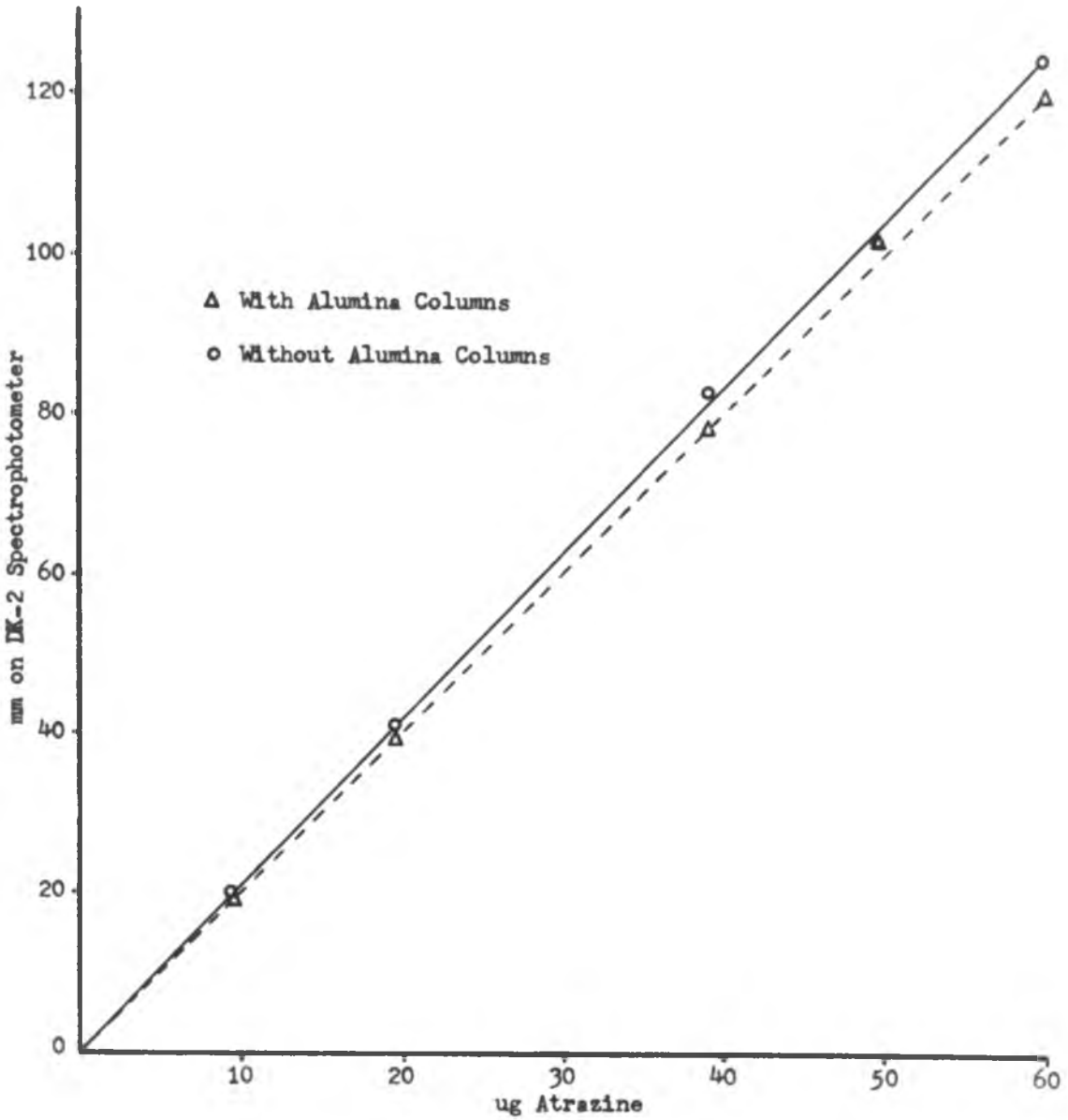
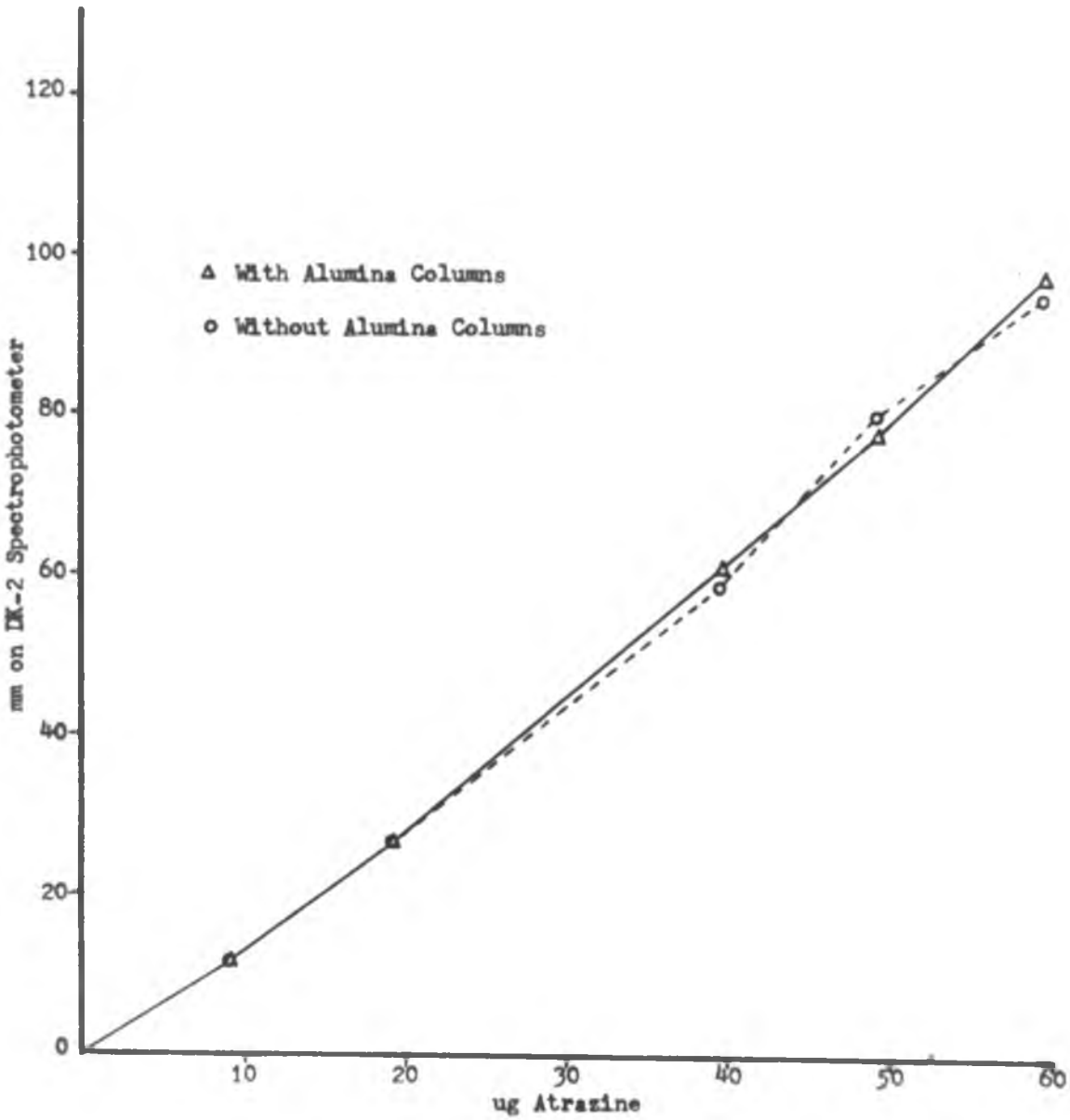

120
100
80
60
40
20
0
mm on DK-2 Spectrophotometer
Δ With Alumina Columns
o Without Alumina Columns
10
20
30
40
50
60
ug Atrazine

Table 9. Atrazine Standard Curves (With Cucumber Leaf Tissues) Using the H.S.P.A. Method With and Without Alumina Columns.

Treatment (ug Atrazine/100 g of Tissue)		mm on DK-2				
		1	2	3	Ave.	% Recovery[a]
No Columns	0	0	0	0	0.0	0.0
	10	12	12	10	11.3	56.5
	20	26	27	26	26.3	64.1
	40	58	58	57	57.7	69.9
	50	81	81	77	79.7	78.8
	60	95	--	93	94.0	76.1
Alumina Columns	0	0	0	0	0.0	0.0
	10	10	10	13	11.0	57.2
	20	24	27	27	26.0	66.3
	40	59	59	59	59.0	73.9
	50	80	75	77	77.3	76.5
	60	95	--	97	96.0	80.3

[a] % Recovery is based on a comparison between the standard curve with no crop and the standard curve with cucumber leaf tissue.

Figure 6. Atrazine Standard Curves (With Cucumber Leaf Tissues) Using the H.S.P.A. Method With and Without Alumina Columns. Each point represents an average of three replications.


120
100
80
60
40
20
0
mm on DK-2 Spectrophotometer
△ With Alumina Columns
o Without Alumina Columns
10
20
30
40
50
60
ug Atrazine

the usefulness of alumina columns in eliminating some of the interfering background. Four plots were sprayed with 0.1 lb/A atrazine and analyzed chemically with the H.S.P.A. Method with and without alumina columns. The results are expressed in mm recorded on the DK-2 spectrophotometer rather than in ppm because no standard curves were made for snapbeans (Table 10). The treatment with the alumina columns was statistically

Table 10. Atrazine Analysis of Snapbean Plants Using the H.S.P.A. Method With and Without Alumina Columns Including the Centrifuge Step.[a]

Treatment	mm on DK-2				Average[b]
	1	2	3	4	
No Column	50	53	36	26	41.25
Alumina Column	65	74	49	37	56.25

[a]All plots were sprayed with 0.1 lb/A atrazine. Values represent 100 gram samples that were diluted 10 times just before reading.
[b]The means are significantly different at the 1% level (F-test).

different from the treatment with no alumina column at the 1% level of significance. The alumina columns eliminated a substantial amount of background and tended to shift the maximum absorption peak back to 240 mu. Hence, it is strongly recommended that alumina columns be used with the H.S.P.A. Method when snapbean tissue is analyzed for atrazine.

Evaluation of the Centrifuge Step in the H.S.P.A. Method:

In order to evaluate the centrifuge step in the H.S.P.A. Method, standard curves with cucumber tissues were made using the H.S.P.A. Method without the centrifuge step and compared with previous standard curves determined similarly with the centrifuge step. Table 11 shows the results of the atrazine standard curves with cucumber tissues using the

Figure 7. Atrazine Standard Curves (With Cucumber Leaf Tissues) Using the E.S.P.A. Method With and Without the Centrifuge Step.

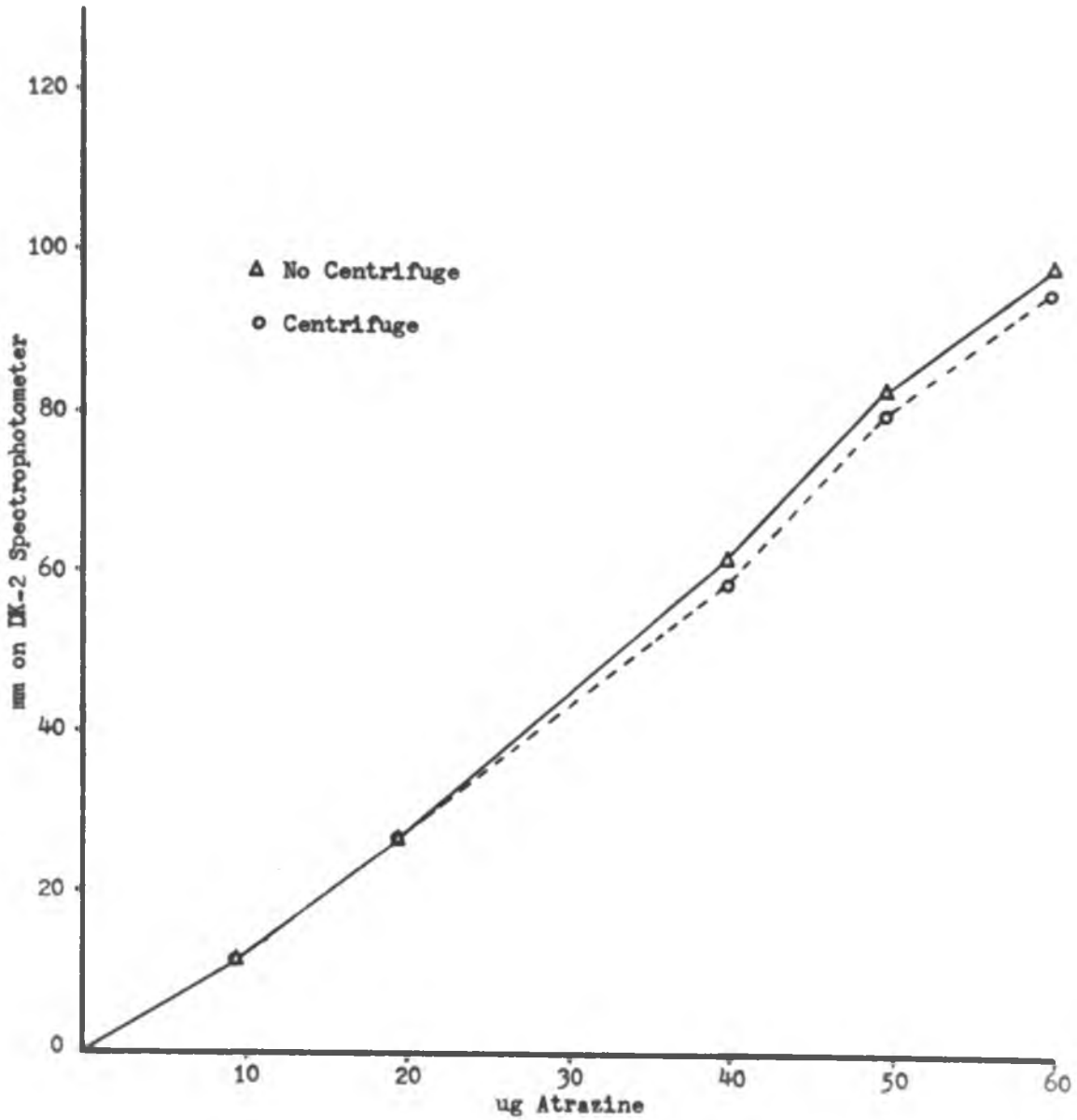

120
100
80
60
40
20
0
mm on DK-2 Spectrophotometer
No Centrifuge
Centrifuge
10
20
30
40
50
60
ug Atrazine

Table 11. H.S.P.A. Method With and Without the Centrifuge Step Using Atrazine Standard Curves.[a]

Treatment (ug Atrazine/100 g of Tissue)	Average mu Readings on DK-2	
	Centrifuge	No Centrifuge
0	0.0	0.0
10	11.3	11.5
20	26.3	27.0
40	57.7	60.5
50	79.7	81.5
60	94.0	97.0

[a]Data are an average of 3 replications.

H.S.P.A. Method without the centrifuge step. Fig. 7 shows the comparison of the H.S.P.A. Method with and without the centrifuge step. The data indicated that the centrifuge step was not necessary.

Chloroform Vs. Pentane Extraction:

Cucumber field plots were sprayed with 0.1 lb/A atrazine and the cucumber leaf tissue analyzed chemically using pentane and chloroform as extracting solvents. The H.S.P.A. Method was used with alumina columns which substituted for the centrifuge step. Although the data (Table 12)

Table 12. Chloroform Vs. Pentane Extraction.

Treatment	ppm				Average[a]
	1	2	3	4	
Pentane	2.52	2.07	2.52	2.65	2.44
Chloroform	2.53	2.47	3.50	3.16	2.92

[a]The values are significantly different at the 5% level (F-test).

indicated that chloroform tended to extract more atrazine, an F-test showed that the chloroform treatment was not statistically different

than the pentane treatment. With more replications chloroform could probably be shown to extract more atrazine than pentane at a significant level. Atrazine is much more soluble in chloroform than in pentane (25). One draw back with chloroform is that more interfering materials are extracted with chloroform than with pentane. However, with the use of alumina columns most of the interfering materials are eliminated.

Background of Check Cucumber Samples:

All check cucumber samples which were grown in areas safe from any possible atrazine drift gave either 0 or negative readings for atrazine (water used as a reference) when the H.S.P.A. Method was used. The values varied from 0 to -10 mm on the DK-2 spectrophotometer. The values were extracted from the various experiments. To be safe in drift cases, low readings should be read with water as the reference sample and all negative readings considered as 0 readings for atrazine, even though the readings might show positive results when check are used as reference samples. This precaution should be taken because of the variability of check cucumber samples. The above consideration becomes important when very small amounts of atrazine are involved.

Validity of Diluting Samples Just Before Reading the Absorption:

The fastest way of diluting samples when the concentration of atrazine is found to be too high for the DK-2 spectrophotometer, is to dilute the final solution. To justify this method of dilution it was compared with another common method of dilution frequently in use. This common method of dilution involves taking a smaller aliquot after the extraction and repeating the rest of the procedure. These two methods of dilution

gave essentially the same results (Table 13).

Table 13. Comparison Between Methods of Dilution. Data were taken from experiment on which the two methods of dilution were tried.

Method of Dilution	Treatments of Experiment[a] (mm on DK-2)				
	1	2	3	4	5
Dilution of Final Solution	171.7	109.0	34.3	12.3	7.0
Smaller Aliquot After Extraction	170.3	110.7	32.7	12.0	7.3

[a] Each value is an average of three replications.

Final Field Phytotoxicity and Sampling Studies

This last section contains some of the practical problems involved with the detection of atrazine drift and sampling procedures.

Delayed Freezing of Field Samples:

Cucumber samples from plots sprayed with 0.1 lb/A atrazine were frozen at time intervals from 0 to 14 days after harvesting and were analyzed chemically using the H.S.P.A. Method without the centrifuge step.

No dependable data resulted from this study due to very high interfering background which shifted the maximum absorption peak from 240 mu to approximately 237 mu. Alumina columns should have been used with the H.S.P.A. Method.

The samples that were frozen 3, 5, 7 and 14 days after the harvesting were foul smelling and in various stages of breakdown due to rotting organisms. Background due to cucumber tissue increased substantially with increasing delay in freezing.

Although the data were not suitable for accurate determinations some trends in the data are discussed. Atrazine did not seem to decrease much

from the 0 to 7 day delay in freezing. However, atrazine did decrease between the 7 and 14 day delay. It should be stated here that the shift cannot be positively attributed to interfering background, but could possibly be due to changes in the atrazine structure.

Sampling Time:

Cucumber leaf tissue samples were taken from field plots at time intervals from 12 hours to 7 days after they were sprayed with 0.1 lb/A atrazine and analyzed chemically using the H.S.P.A. Method without the centrifuge step.

The data showed a rapid decrease of atrazine: 10.90 to 6.89 ppm between 12 hours and 1 day, 6.89 to 2.17 ppm between 1 and 2 days, and 2.17 to 0.77 ppm between 2 and 4 days after the spraying (Table 14 and Fig. 8). The decrease in atrazine leveled off after 4 days. However a

Table 14. Concentration of Atrazine Found in Cucumber Leaf Tissue Harvested at Various Time Intervals.

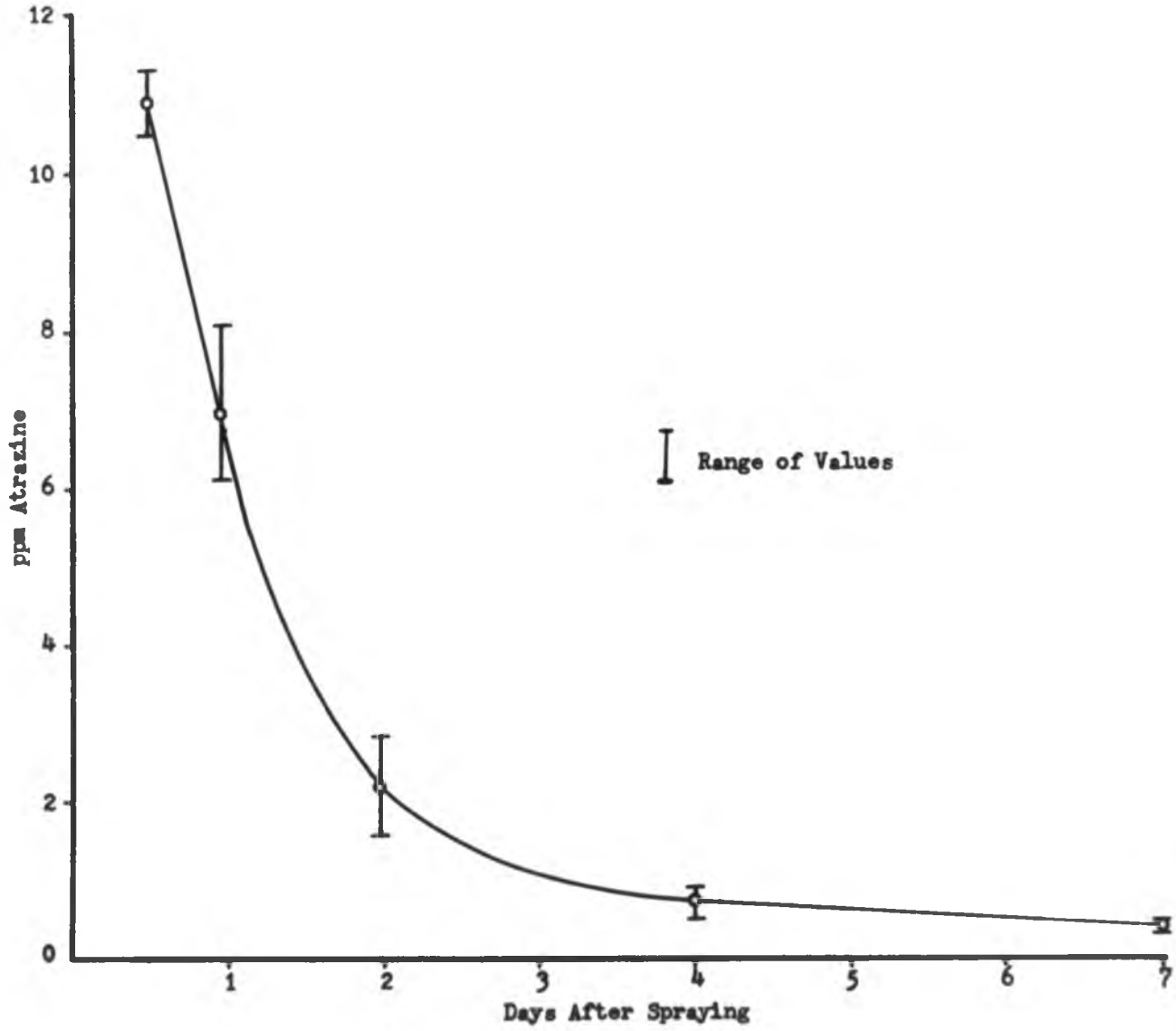
Treatment (Days Between Spraying and Harvesting)	ppm (Fresh Weight Basis) 1	2	3	Average[a]
0.5	11.28	10.52	10.90	10.90
1	8.10	6.12	6.44	6.89
2	2.84	1.64	2.02	2.17
4	0.95	0.63	0.74	0.77
7	0.50	0.41	0.46	0.46

[a] Means connected by the line are not statistically different at 1% level (Duncan's Multiple Range Test).

nonsignificant decrease from 0.77 to 0.46 ppm was measured between 4 and 7 days after the spraying.

Since physical symptoms would start to occur between 2 and 3 days

Figure 8. Loss of Atrazine From Cucumber Plants Sprayed With 0.1 lb/A Atrazine.


12
10
8
6
4
2
0
ppm Atrazine
Range of Values
1
2
3
4
5
6
7
Days After Spraying

after a drift of atrazine occurred on cucumber plants, sampling of damaged tissue would most likely be initiated at 3 days or later after the drift. Although higher results would be obtained from samples harvested earlier than 5 days after a drift, more consistent results would be obtained by harvesting samples at 5 to 7 days. Samples should not be harvested much later than 8 days since many of the damaged leaves would start to fall at that time.

Correlation of Spray-Concentration to Tissue Ananlysis:

Physical symptoms were recorded and samples harvested 7 days after cucumber plots were sprayed with 0, 0.005, 0.05, 0.1 and 0.2 lb/A atrazine. The samples were then analysed chemically using the H.S.P.A. Method (centrifuge step left out) with and without alumina columns (Table 15 and Fig. 9).

All treatment means were statistically different from all other treatments except between the 0 and 0.005 lb/A treatment. Although no statistical difference was found between the 0 and 0.005 lb/A treatments, small but definite amounts of strazine were detected in all five of the replications of the 0.005 lb/A treatment. Atrazine detected from cucumber tissues of this experiment was much higher than the equivalent treatments in the initial screening phytotoxicity experiment. This may have been attributed to rain shortly after the atrazine was sprayed on the plants in the initial screening experiment.

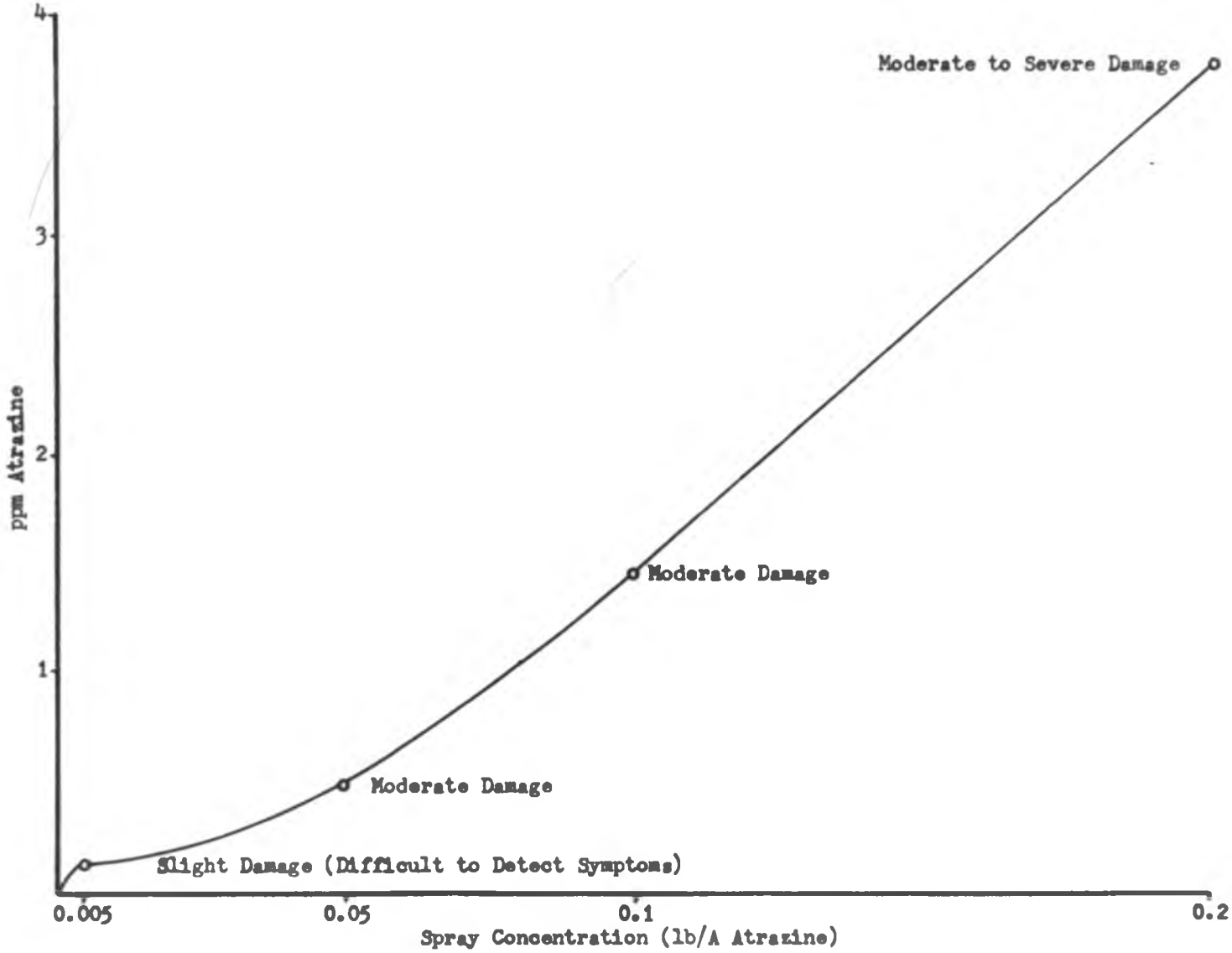
The physical symptoms were the same as the physical symptoms in the initial screening experiment except that the symptoms were slightly more severe and the initial symptoms occurred one day later than the initial screening experiment. The delay of the initial symptoms may be

Table 15. Correlation of Spray-Concentration to Tissue Analysis. Cucumber plants were used as the test crop.

Atrazine Spray-Concentration (lb active/A)	ppm (Fresh Weight Basis)					
	1	2	3	4	5	Average[a]
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.005	0.12	0.09	0.17	0.18	0.12	0.14
0.05	0.42	0.32	0.60	0.68	0.43	0.49
0.1	1.01	1.39	1.74	1.45	1.67	1.45
0.2	3.57	4.50	3.63	3.98	3.23	3.78

[a] Means connected by a line are not statistically different at the 1% level (Duncan's Multiple Range Test).

Figure 9. Correlation of Spray-Concentration to Tissue Analysis. Refer to Table 15.


4
3
2
1
ppm Atrazine
Moderate to Severe Damage
Moderate Damage
Moderate Damage
Slight Damage (Difficult to Detect Symptoms)
0.005
0.05
0.1
0.2
Spray Concentration (lb/A Atrazine)

contradictory at first because the samples in this experiment contained more atrazine at the end of 7 days than the initial screening experiment. Two possible reasons are postulated. The actual droplets of water and the high humidity caused by the rain in the initial screening experiment may have increased absorption of atrazine into the leaves earlier than normal. Increased foliar absorption of water and other chemicals with increasing humidity have been shown by many workers (8, 41, 43). Also, the initial screening experiment was performed during the summer when the average temperatures were higher (see Appendix for environmental conditions during the experiments). Increase in temperature has been shown by Wax et al. (42) to increase foliar uptake of atrazine applied to the foliage.

Lower Limit of Detection:

Up to this point 0.005 lb/A atrazine was the lowest concentration sprayed on cucumber plots. At this concentration atrazine was detected at low but definite levels in all cases.

In this experiment 0.0005 lb/A atrazine was sprayed on five plots and samples analyzed chemically using the H.S.P.A. Method without the centrifuge step. Although in one replication a minute amount of atrazine (0.04 ppm) was detected, no atrazine was found in the other four replications. For all practical purposes, atrazine was not detected in cucumber tissue after being sprayed with 0.0005 lb/A atrazine. No physical symptoms were noted on any of these plots.

Physical Symptoms On Cucumber Plants at 1.0 lb/A Atrazine:

A concentration of 1.0 lb/A atrazine was sprayed on cucumber plots to observe any change of physical symptoms which might not have appeared

at the lower concentrations. One of the expected changes was an earlier appearance of the initial symptoms. Both 0.1 lb/A and check plots were also used as a means of comparison.

Data from this experiment indicated no difference in the general physical symptoms between the 1.0 and 0.1 lb/A treatment. The physical symptoms appeared at the same time (third day after the spraying) in both treatments. The normal stages of damage (yellow chlorosis changing to a bleached white color, and then to necrosis) observed earlier at the lower concentrations were also observed at the 1.0 lb/A concentration. However, the damage was more extensive at 1.0 lb/A concentration resulting in the death of all plants three weeks after the spraying.

GENERAL DISCUSSION

Although the physical symptoms observed from atrazine sprays on cucumber and snapbean plants were not unlike many types of other physical and chemical damages, these symptoms can still be used as secondary evidence in an actual drift case. Intervenal and/or marginal chlorosis appeared 2 or 3 days after atrazine was sprayed on cucumber and snapbean plants. These chlorotic symptoms turned briefly to a bleached-white color on the leaves, which in turn became necrotic within 5 days after the spraying. These stages of damage were observed when cucumber and snapbean plants were sprayed with 0.05, 0.1, 0.2 and 1.0 lb/A atrazine. At the 0.05, 0.1 and 0.2 lb/A spray-concentrations, physical symptoms gradually disappeared as damaged leaves fell and new leaves took their place. At 1.0 lb/A all plants died.

A second method of diagnosing a suspected drift case which probably is more accurate and objective is tissue analysis. An ultraviolet spectrophotometric method used successfully by Hilton and Uyehara (24) to analyze sugar cane tissue for atrazine residue was found suitable for the atrazine analysis of cucumber tissue. This method which is referred to in this thesis as the H.S.P.A. Method is very similar to the ultraviolet spectrophotometric method mentioned by Knüsli (25) in the "Advances in Pest Control Research (Volume IV)". Atrazine could be detected consistently as hydroxyatrazine on the DK-2 spectrophotometer in cucumber plants which were sprayed with the low concentration of 0.005 lb/A atrazine. At this spray-concentration physical symptoms were very difficult to detect, and the cucumber plants were considered undamaged.

The H.S.P.A. Method was not found to be completely satisfactory with

snapbeans due to the high interfering background. But with the addition of alumina columns, the modified H.S.P.A. Method was found to be suitable for snapbean tissue. Laboratory studies showed that very little or no atrazine was loss due to the alumina columns. Alumina columns may be useful as an additional cleanup step with most vegetable crops.

Although the per cent recoveries were low with check cucumber samples spiked with atrazine, the results were very consistent. The per cent recoveries increased from 56.5% at 0.1 ppm to 78.8% at 0.5 ppm but did not increase above 0.5 ppm. To account for this, two types of losses are postulated. The first type of loss affected the per cent recoveries equally at all concentrations and involved the incomplete separation of the extracting solvent (pentane) from the cucumber tissue after the blending and mixing. No attempt was made to squeeze all the extracting solvent out due to the increase of pigments. By increasing the number of times that the cucumber tissue is "washed", such loss of atrazine can probably be decreased. But this type of loss did not explain the gradual increase in the per cent recoveries observed from 0.1 to 0.5 ppm. If a definite amount of atrazine were loss irrespective of concentration due to a specific phenomena, the per cent recovery would be less affected as the concentrations increased. It may be that available sites, which can bind atrazine physically or chemically, are present on cucumber tissue. If this were the case and the number of sites were low enough that even the 0.1 ppm concentration would easily saturate all the sites, it could explain the gradual increase in the per cent recoveries observed from 0.1 to 0.5 ppm. For example, if 2 ug of atrazine were tied up in this fashion, there would be a 20% decrease in the per cent recovery at the 0.1 concentration

and only approximately a 3% decrease in the per cent recovery at the 0.6 ppm concentration.

A check tissue sample that is to be used as a reference sample with the DK-2 spectrophotometer should be the same as the sample in question without the chemical that is being analyzed. This assumption is not usually fulfilled in atrazine drift cases. Natural variation between plants due to genetic variability and variable environmental conditions is even more important in drift cases because check tissue samples must be obtained away from the drift area. Also, the necrotic condition of the damaged crop is not easy to duplicate and is not usually attempted. Check cucumber tissue varied from 0 to -10 mm on the DK-2 spectrophotometer when water was used as the reference sample. Although this shows considerable variation, it is comforting to see that none of the readings were positive. To be safe, water should be used as the reference sample and only positive atrazine readings accepted. The above consideration becomes important only when very small amounts of atrazine are involved.

Sampling technique is important. In a majority of the field experiments, 200 gram samples were taken from plots 5 by 20 feet. Only the most damaged leaf tissue were sampled for several reasons. It was postulated that such samples would contain more atrazine and be more consistent. Also, the danger of bias sampling technique when a random sample is called for is largely removed. The sampling of only the most damaged leaves proved to be very satisfactory.

The sampling time experiment showed a rapid decrease of atrazine from the time of spraying to 4 days after the spraying and very little decrease of atrazine from 4 to 7 days. For consistency, samples should be taken 4

or more days after the suspected drift, although earlier sampling would give higher results, but should not be taken much more than 7 days after the suspected drift since the damaged leaves started to fall 7 days after the spraying. Most of the data in this thesis are based on samples harvested 7 days after spraying.

As much as is practical, field samples should be chopped in thin slices, weighed, placed in polyethylene bags, sealed, labeled, and frozen in storage and transportation.

Although the presence of atrazine in cucumber tissue can be detected easily with tissue analysis, it may not be easy to distinguish between degrees of damage. But together with visual observations of the physical symptoms and damage, tissue analysis can be used with confidence to diagnose not only the presence of atrazine but also the extent of damage. The H.S.P.A. Method with alumina columns as an additional cleanup step can probably be used to analyze other vegetable crops for atrazine.

CONCLUSION

1. Atrazine physical symptoms on cucumber and snapbean plants were similar to general visual symptoms due to other physical and chemical damages. But the physical symptoms were distinct enough to be used as secondary evidence and to estimate damage after tissue analysis substantiated the presence of atrazine.
2. Although recoveries were low they were consistent.
3. Since check cucumber samples were variable, water should probably be used as the reference sample and only positive readings accepted. This is important when dealing with very minute amounts of atrazine.
4. The H.S.P.A. Method was suitable to detect the presence of atrazine in cucumber tissue at spray-concentrations so low that essentially no visual symptoms could be seen.
5. With the addition of alumina columns the H.S.P.A. Method was also found suitable for snapbeans. The addition of alumina columns to the H.S.P.A. Method did not cause a reduction of atrazine and can probably be used with most vegetables crops to reduce background.
6. Sampling of only the most damaged leaves was found to be very satisfactory.
7. Although samples taken earlier would give higher results, cucumber samples would probably be most consistent and accurate when harvested from 4 to 7 days after a suspected drift.
8. The H.S.P.A. Method with the addition of alumina columns can be used with confidence to detect atrazine drift onto cucumber and snapbean plants but not necessarily to accurately differentiate between degrees of damage. Visual observation of the physical symptoms may be of use

to estimate the extent of damage.

9. See Appendix, pages 54 to 57, for the recommended sampling procedure and the analytical procedure.

APPENDIX

SAMPLING PROCEDURE USED IN THE EXPERIMENTS

1. Two hundred and twenty-five grams of the most damaged leaves were picked from each plot (5 by 20 feet) and placed in polyethylene bags. The bags were sealed with rubber bands and labeled.
2. The field samples were chopped into approximately 5 by 2 cm strips and weighed. Two hundred grams from each sample were placed into polyethylene bags, sealed, labeled and frozen.

RECOMMENDED SAMPLING PROCEDURE

1. Sample as soon as possible after the necrotic symptoms appear.
2. Take at least 5 samples from different parts of the suspected area.
3. For each sample, harvest from an area approximately 100 square feet.
4. Follow step 1 of the sampling procedure above.
5. Follow step 2 of the sampling procedure above.
6. Use extra 25 grams for moisture sample to express final figures on a dry weight basis (if desired).

ANALYTICAL PROCEDURE

H.S.P.A. Method

1. Extraction With Pentane

Let frozen samples, which have already been cut into 5 cm lengths, stand at 72°F for 12 hours. Place one unfrozen 200 gram sample into a blender and add 400 ml of n-pentane (technical grade). Press plant material firmly to the bottom to enable proper blending. Blend at the lowest speed for 30 seconds (may need to be altered with type and condition of tissues). Mix for 5½ minutes and filter over a 500 ml glass sintered funnel (coarse grade) into a suction flask. Wash with 100 ml n-pentane. Stop filtration when green pigments start to filter through.

2. Centrifuge Filtrate

3. Evaporate to Dryness

Pour filtrate into a 500 ml volumetric flask and bring to volume with n-pentane. Take a 250 ml volumetric aliquot and evaporate to approximately 15 ml in a 250 ml beaker. Pour into 50 ml round-bottom flask. Wash beaker with a few ml of n-pentane and pour into round-bottom flask. Evaporate to dryness.

4. Hydrolysation

Place 10 ml of 1 N sulfuric acid into round-bottom flask and place in water bath for 3 hours at a water temperature of from 80 to 100°C. Remove and cool to room temperature.

5. Washing With Organic Solvents

Pour the cooled solution over a 50 ml glass sintered funnel (coarse grade) into a 125 ml separatory flask. Add 25 ml of 20% ethyl ether in chloroform and mix vigorously.

Allow layers to separate and eliminate the chloroform (bottom layer). Chloroform interferes with the reading of hydroxyatrazine on the DK-2 spectrophotometer. Add 25 ml of ethyl ether, mix, and allow layers to separate. Collect bottom layer, which would contain the possible hydroxyatrazine.

6. Reading on the DK-2 Spectrophotometer

Read on the DK-2 spectrophotometer for hydroxyatrazine at 240 mu wave length using the base-line technique. Use a check tissue blank as the reference sample when working with large amounts of atrazine and water as the reference sample when working with small amounts of atrazine.

H.S.P.A. Method With Alumina Columns

(To be tried when high interfering background is present)

1. Extraction With Pentane (same)
2. Centrifuge Filtrate (same)
3. Evaporate to Dryness

Pour filtrate into a 500 ml volumetric flask and bring to volume with n-pentane. Take a 250 ml aliquot and evaporate to approximately 20 ml in a 250 ml beaker. Pour into a 100 ml beaker and evaporate to dryness.

4. Column Cleanup With Alumina

Prepare Woelm basic alumina, activity grade V, the night before using by mixing thoroughly 85 grams of Woelm basic alumina, activity grade I, with 15 ml of water in a tightly closed bottle.

Add 25 grams of the Woelm basic alumina, activity grade V, to a

18 x 200 mm column and rap gently to eliminate channeling and achieve uniform packing of the alumina.

Dissolve residue from step 3 by adding 10 ml of carbon tetrachloride to the 50 ml beaker with the residue. Pour the solution into the column. Wash the beaker with 10 ml of carbon tetrachloride and add to the column. Repeat with another 5 ml of carbon tetrachloride. Add 80 ml of carbon tetrachloride to column just after the previous 5 ml of carbon tetrachloride penetrates the top of the alumina.

Just before the 80 ml of carbon tetrachloride has penetrated the top of the alumina, place a clean 250 ml beaker under the column as a receiver. Add 125 ml of 5% ethyl ether in carbon tetrachloride to the column and collect completely in the 250 ml beaker receiver. Evaporate to approximately 15 ml and add to 50 ml round-bottom flask. Evaporate this to dryness.

5. Hydrolysation (same)
6. Washing With Organic Solvents (same)
7. Reading on the DK-2 Spectrophotometer (same)

Table 16. Subjective ratings[a] for leaf burn and chlorotic symptoms on cucumber plants from the 4 week spray application of the initial screening for atrazine phytotoxicity.

	Concn (lb/A)	1st Rep.	2nd Rep.	3rd Rep.	Ave.
1st Day After Spraying	0.0	1	1	1	1.0
	0.005	1	1	1	1.0
	0.05	1	1	1	1.0
	0.1	1	1	1	1.0
2nd Day After Spraying	0.0	1	1	1	1.0
	0.005	1	1	1	1.0
	0.05	2	2	2	2.0
	0.1	2	2	2	2.0
3rd Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	3	3	2.7
	0.1	3	3	3	3.0
4th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	3	3	2.7
	0.1	3	3	3	3.0
5th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	3	3	2.7
	0.1	3	3	3	3.0
6th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	3	3	2.7
	0.1	3	3	3	3.0
7th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	3	3	2.7
	0.1	3	3	3	3.0

[a]Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 17. Subjective ratings[a] for leaf burn and chlorotic symptoms on snapbean plants from the 4 week spray application of the initial screening for atrazine phytotoxicity.

	Concn (lb/A)	1st Rep.	2nd Rep.	3rd Rep.	Ave.
1st Day After Spraying	0.0	1	1	1	1.0
	0.005	1	1	1	1.0
	0.05	1	1	1	1.0
	0.1	1	1	1	1.0
2nd Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	2	2	2.0
	0.1	2	2	2	2.0
3rd Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	2	3	2.3
	0.1	3	3	3	3.0
4th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	2	3	2.3
	0.1	3	3	3	3.0
5th Day After Spraying	0.0	1	1	1	1.0
	0.005	1	2	1	1.3
	0.05	2	2	3	2.3
	0.1	3	3	3	3.0
6th Day After Spraying	0.0	1	1	1	1.0
	0.005	2	2	2	2.0
	0.05	3	3	3	3.0
	0.1	3	3	3	3.0
7th Day After Spraying	0.0	1	1	1	1.0
	0.005	2	2	2	2.0
	0.05	3	3	3	3.0
	0.1	3	3	3	3.0

[a] Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 18. Subjective ratings[a] for leaf burn and chlorotic symptoms on cucumber plants from the 6 week spray application of the initial screening for atrazine phytotoxicity.

	Concn (lb/A)	1st Rep.	2nd Rep.	3rd Rep.	Ave.
1st Day	0.0	1	1	1	1.0
After	0.005	1	1	1	1.0
Spraying	0.05	1	1	1	1.0
	0.1	1	1	1	1.0
2nd Day	0.0	1	1	1	1.0
After	0.005	1	1	1	1.0
Spraying	0.05	2	2	1	1.7
	0.1	2	2	2	2.0
3rd Day	0.0	1	1	1	1.0
After	0.005	2	2	2	2.0
Spraying	0.05	2	2	3	2.3
	0.1	2	3	3	2.7
4th Day	0.0	1	1	1	1.0
After	0.005	2	2	2	2.0
Spraying	0.05	3	3	3	3.0
	0.1	3	4	3	3.3
5th, 6th	0.0	1	1	1	1.0
and 7th	0.005	2	2	2	2.0
Day After	0.05	3	3	3	3.0
Spraying	0.1	3	4	3	3.3

[a] Rating Scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 19. Subjective ratings[a] for leaf burn and chlorotic symptoms on snapbean plants from the 6 week spray application of the initial screening for atrazine phytotoxicity.

	Concn (lb/A)	1st Rep.	2nd Rep.	3rd Rep.	Ave.
1st Day After Spraying	0.0	1	1	1	1.0
	0.005	1	1	1	1.0
	0.05	1	1	1	1.0
	0.1	1	1	1	1.0
2nd Day After Spraying	0.0	1	1	1	1.0
	0.005	2	2	2	2.0
	0.05	2	2	3	2.3
	0.1	2	2	3	2.3
3rd Day After Spraying	0.0	1	1	1	1.0
	0.005	2	2	3	2.3
	0.05	2	3	3	2.7
	0.1	2	3	3	2.7
4th, 5th, 6th and 7th Day After Spraying	0.0	1	1	1	1.0
	0.005	2	3	3	2.7
	0.05	3	3	3	3.0
	0.1	3	4	3	3.3

[a]Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

Table 20. Subjective ratings[a] for leaf burn and chlorotic symptoms on tomato plants from the 6 week spray application of the initial screening for atrazine phytotoxicity.

	Concn (lb/A)	1st Rep.	2nd Rep.	3rd Rep.	Ave.
1st Day	0.0	1	1	1	1.0
After	0.005	1	1	1	1.0
Spraying	0.05	1	1	1	1.0
	0.1	1	1	1	1.0
2nd Day	0.0	1	1	1	1.0
After	0.005	1	1	1	1.0
Spraying	0.05	1	2	1	1.3
	0.1	2	2	1	1.7
3rd Day	0.0	1	1	1	1.0
After	0.005	1	1	1	1.0
Spraying	0.05	1	2	1	1.3
	0.1	2	2	1	1.7
4th, 5th,	0.0	1	1	1	1.0
6th and	0.005	1.5	1.5	1.5	1.5
7th Day	0.05	2	2	2	2.0
After	0.1	2	2	2	2.0
Spraying					

[a] Rating scale: 1- no burn, 2- slight burn and/or chlorosis, 3- moderate burn, 4- severe burn, 5- dead.

RAINFALL AND TEMPERATURE DATA

Each rainfall datum represented the amount of rain that fell from 8 AM that morning to 8 AM of the next morning. Temperature data were taken at 8 AM. The maximum temperature would usually be around 7 degrees higher. Rainfall and temperature data for each field experiment were obtained only for the time period between and including the day of spraying and the day of sampling.

Initial Screening for Atrazine Phytotoxicity Experiment

4 Week Spraying Experiment:

Date	Rainfall (inches)	Temperature (°F)
7/8/65 (Sprayed)	1.50	76
7/9/65	0.21	78
7/10/65	0.40	76
7/11/65	0.37	77
7/12/65	Trace	79
7/13/65	Trace	80
7/14/65 (Sampled)	0.06	78

6 Week Spraying Experiment:

Date	Rainfall (inches)	Temperature (°F)
7/22/65 (Sprayed)	0.06	74
7/23/65	0.03	75
7/24/65	0.01	77
7/25/65	0.01	77
7/26/65	0.02	79

6 Week Spraying Experiment: (cont.)

Date	Rainfall (inches)	Temperature (°F)
7/27/65	0.16	78
7/28/65	0.21	78
7/29/65 (Sampled)	Trace	78

Delayed Freezing of Field Samples Experiment

Date	Rainfall (inches)	Temperature (°F)
10/23/65 (Sprayed)	Trace	74
10/24/65	0.01	75
10/25/65	0.02	73
10/26/65	0.31	76
10/27/65	0.25	74
10/28/65	0.02	75
10/29/65	0.01	72
10/30/65 (Sampled)	0.03	75

Sampling Time Experiment

Date	Rainfall (inches)	Temperature (°F)
10/23/65 (Sprayed)	Trace	74
10/24/65 (Sampled)	0.01	75
10/25/65 (Sampled)	0.02	73
10/26/65	0.31	76
10/27/65 (Sampled)	0.25	74
10/28/65	0.02	75
10/29/65	0.01	72
10/30/65 (Sampled)	0.03	75

Correlation of Spray-Concentration to Tissue Analysis Experiment

Date	Rainfall (inches)	Temperature (°F)
10/21/65 (Sprayed)	Trace	74
10/22/65	0.0	72
10/23/65	Trace	74
10/24/65	0.01	75
10/25/65	0.02	73
10/26/65	0.31	76
10/27/65	0.25	74
10/28/65 (Sampled)	0.02	75

Lower Limit of Detection and Physical Symptoms on Cucumber Plants at 1.0 lb/A Atrazine Experiment

Date	Rainfall (inches)	Temperature (°F)
4/6/66 (Sprayed)	0.0	73
4/7/66	0.0	72
4/8/66	0.12	74
4/9/66	0.05	73
4/10/66	0.10	74
4/11/66	0.09	72
4/12/66	0.08	74
4/13/66	Trace	72

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