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THE SELECTIVITY AND ACTIVITY OF *s*-TRIAZINE HERBICIDES IN BANANA PLANTS

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THE SELECTIVITY AND ACTIVITY OF s-TRIAZINE HERBICIDES IN BANANA PLANTS

Abstract

Field screening trials in Hawaii showed ametryne (2-methylmercapto-4-ethylamino-6-isopropylamino-s-triazine) to be a potentially important herbicide for bananas. Ametryne exhibited more effective weed control than atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). Furthermore, atrazine caused injury to bananas in 2 of 3 locations used. Investigations were conducted to explain the differential selectivity of the 2 closely related s-triazine herbicides to bananas.

Sand culture experiments showed that both herbicides at concentrations of 0.4, 4, and 16 ppm in 2 liters of nutrient solution injured the plants. Plants grown in pots containing soils from the experimental fields showed increasing phytotoxicity to the herbicides in the following order: Poamoho soil, Waimanalo soil, and Kauai soil. Ametryne was less injurious than atrazine but differences were not as pronounced as that under field conditions where ametryne did not exhibit phytotoxicity.

Phytotoxicity was directly related to leachability of the herbicides and negatively related to adsorption capacity of each soil for the herbicides. Organic matter content seemed to partly explain the response observed. Selectivity appeared to be largely attributed to differential position of roots of banana plants in relation to herbicide placement rather than to inherent selectivity. Apparently, atrazine being less adsorbed by soil and more readily leached, moved to a greater depth upon receipt of a given amount of water resulting in crop injury.

Laboratory studies confirmed results of greenhouse experiments that absorption, translocation and degradation of the herbicides by the plants

did not account for the selectivity observed. Both of the herbicides were partly metabolized by the plants to their common hydroxy derivative and 2 other unidentified metabolites after 3 and 7 days of exposure to nutrient solutions containing C^{14}-labelled ametryne and atrazine. When applied to the leaves with a surfactant the herbicides were absorbed and translocated in an acropetal direction to a very limited extent.

Higher leaf content of free ammonia, free nitrate, and non-protein nitrogen, and lower chlorophyll content, were associated with plants receiving phytotoxic concentrations of ametryne and atrazine after 1 and 4 weeks from treatment. It appears that phytotoxic concentrations of these herbicides resulted in disturbance of the incorporation of nitrogen into more complex nitrogen containing plant constituents as proteins. In these experiments atrazine at 2 and 10 lb/A and ametryne at 10 lb/A applied to soil were phytotoxic to banana plants grown in pots. Ametryne at 2 lb/A did not injure the plants.

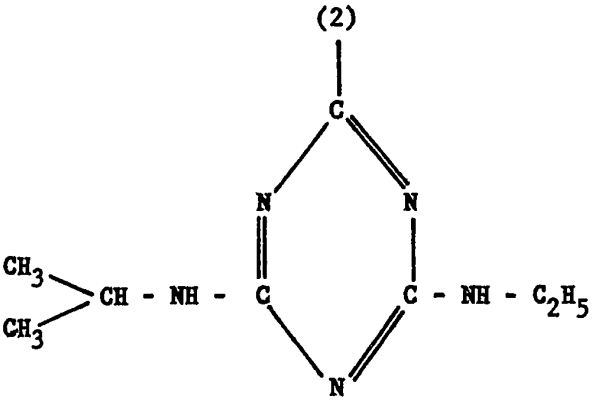
THE SELECTIVITY AND ACTIVITY OF s-TRIAZINE HERBICIDES IN BANANA PLANTS

INTRODUCTION

Since the introduction of 2,4-D for weed control in 1945, the use of herbicides has increased tremendously. New herbicides have continued to appear and several of these are now being used on millions of crop acres. In temperate regions herbicides surpassed insecticides in importance during the past year. The widespread use of herbicides has not been adopted in tropical agriculture where weeds play a more important role in limiting crop production because of the diversity of weeds and the longer periods of favorable climate for weed growth.

Herbicide screening trials were initiated with bananas in Hawaii in 1962. Trials were conducted at 3 locations where ametryne and atrazine were among the herbicides tested most extensively (64). Ametryne did not exhibit injury to the crop and it showed superiority over atrazine in both initial effect and duration of weed control. In atrazine treated plots, severe symptoms of marginal leaf chlorosis were observed at Poamoho, moderate at Kauai and none at Waimanalo. These observations indicated that selectivity was influenced by environmental factors with respect to atrazine. The observed differences in selectivity between the 2 herbicides are interesting since both compounds differ only in substituents attached to a carbon atom of the triazine ring (Figure 1).

The studies reported in Section A were initiated with the following objectives: a) to determine the influence of soil on differential selectivity of bananas to ametryne and atrazine, b) to determine the



(2)

SCH_3 - Ametryne

Cl - Atrazine

Figure 1. Structural formula of ametryne and atrazine.

inherent phytotoxicity of the herbicides to bananas using sand culture, c) to determine adsorption and leaching of the 2 herbicides on 3 soil types and to relate these findings with selectivity observed in the field and in greenhouse pot experiments, and d) to determine if selectivity between the 2 herbicides is influenced by differential absorption, translocation, and metabolism of the herbicides by banana plants.

The investigation reported in Section B was conducted to determine the influence of ametryne and atrazine on the N constituents of plants adequately supplied with soil N. Recent evidences indicate that the *s*-triazines have an effect on the N metabolism of plants (19, 24).

Ametryne was chosen on the basis of its proven potential as an economically important herbicide for bananas and atrazine to serve as a basis for comparison because of their differences in response under field conditions. These 2 compounds represent an important group of herbicides, the *s*-triazines.

LITERATURE REVIEW

Properties of the *s*-Triazine Herbicides

Triazine compounds were first tested as herbicides in Switzerland in 1952 (26, 27). Screening trials showed 2-chloro-4,6-bis-(diethylamino)-*s*-triazine (chlorazine) to be non toxic to corn but injurious to many species of germinating plants.

The parent compound of *s*-triazine is a six-membered ring consisting of alternating carbon and nitrogen atoms joined by alternating single and double bonds. The atoms are numbered consecutively around the ring beginning with a hetero or nitrogen atom (69).

Gysin (27) classified triazine derivatives according to whether they have a) 2 chlorine atoms and one substituent; b) 1 chlorine atom and 2 other substituents; and c) no chlorine atoms.

Most of the triazines possessing herbicidal activity have alkyl-substituted amino groups in the 4 and 6 positions (51). The selectivity can be modified somewhat by varying the alkyl substituents on these amino groups. However, major changes in selectivity are brought about by varying the substituents in the 2 position. Chlorine, methoxy, and methylmercapto substitutions in the 2 position have shown the most herbicidal activity (26, 51).

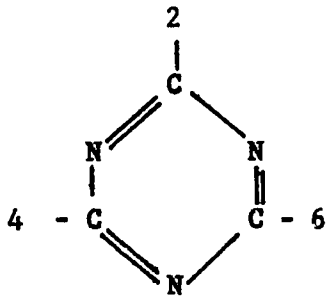
The 2-chloro, 2-methoxy, and 2-methylmercapto triazines may each be divided into 2 sub-groups with reference to the type of substitutions at the 4 and 6 positions. The symmetrically substituted group contains the same alkylamino group at both positions while the asymmetrically substituted group contains different alkylamino groups at each of the

4 and 6 positions. The more important triazine herbicides are presented in Figure 2.

By far the most important triazine herbicide belongs to the chloro-bis-alkylamino-s-triazines with amine having alkyl groups containing 2 or 3 carbon atoms. Corn is tolerant to all members of the 2-chloro triazines but shows practically the same degree of sensitivity as other plants to the 2-methoxy derivatives (27). Simazine can be used as a selective weed killer on a large number of crops such as maize, grapes, trees, sugarcane, citrus, and tree rubber. In higher amounts, it can be used as a non selective herbicide in industrial sites, railroads, etc. Among the asymmetric chloro-triazine herbicides, the most widely used is atrazine which has found use in maize. It is active as a preemergence and an early post-emergence herbicide. The chlorotriazines have a water solubility of 5 to 70 parts per million (ppm). Within this the symmetrically substituted group (chlorazine, simazine, propazine) has a solubility range of 5 - 10 ppm whereas the asymmetrical group (trietazine, ipazine, atrazine) has a solubility range of 20 - 70 ppm (26).

The 2-methylmercapto triazines show a different spectrum of herbicidal activity (27). Crops such as barley, wheat, cotton, and rye are fairly tolerant to prometryne. Ametryne has been shown to be promising for sugarcane (37, 39) and bananas (43, 64). The methylmercapto triazines have a water solubility of 48, 185 and 450 ppm for prometryne, ametryne and simetryne, respectively (26).

The methylmercapto triazines have been reported to have a much shorter residual property in soils than the chlorotriazines. The



	2	4	6
Chlorotriazines			
Simazine	Cl	$NH-C_2H_5$	$NH-C_2H_5$
Atrazine	Cl	$NH-C_2H_5$	$NH-i-C_3H_7$
Propazine	Cl	$NH-i-C_3H_7$	$NH-i-C_3H_7$
Methoxytriazines			
Simetone	OCH	$NH-C_2H_5$	$NH-C_2H_5$
Atratone	OCH	$NH-C_2H_5$	$NH-i-C_3H_7$
Prometone	OCH	$NH-i-C_3H_7$	$NH-i-C_3H_7$
Methylmercapto triazines			
Simetryne	SCH	$NH-C_2H_5$	$NH-C_2H_5$
Ametryne	SCH	$NH-C_2H_5$	$NH-i-C_3H_7$
Prometryne	SCH	$NH-i-C_3H_7$	$NH-i-C_3H_7$

Figure 2. Structure of the s-triazine herbicides.

shorter residual properties of the latter cannot be explained on the basis of their water solubility since the more soluble alkoxy-derivatives have much longer residual properties than the methyl-mercapto derivatives. Gysin (27) attributes this to the ease with which the methylmercapto group is oxidized to sulfoxide or sulfone.

Water solubility of the 2-methoxy s-triazines is much higher than that of the other 2 groups. Solubilities of prometone, atratone and simetone are 750, 1800 and 3200 ppm, respectively. In addition to their being more water soluble, compounds belonging to this group have a considerable lipoid solubility; hence they may be especially valuable for post emergence leaf applications (26).

Herbicidal Effect and Mode of Action of the s-Triazines

The primary toxicity mechanisms of the triazines to plants is believed to involve impairment of photosynthesis (6, 26, 53). Experiments to determine the mechanism by which triazines inhibit photosynthesis have centered around the Hill or light reaction (13, 26, 36). The Hill reaction which involves the splitting of water in the plant chloroplast using energy from light is represented as follows:

$$A + H_2O \xrightarrow[\text{Light}]{\text{Chloroplast}} AH_2 + O_2$$

where A is an H acceptor (14). Chloroplasts illuminated in the absence of CO_2 cleave water in the presence of an H acceptor, such as organic dye or Fe^3, to form O_2. However, inhibition of the Hill reaction is very likely not the only mechanism of toxicity in that the inhibition of the Hill reaction does not always correlate with the herbicidal activity (26). Moreland et al (53) using the Hill reaction showed that

photochemical activity of barley chloroplasts was reduced by 50% at a simazine concentration of 4.6×10^{-6}M; also the barley plants could be kept alive in the presence of lethal concentration of simazine by supplying the plant with an exogenous source of glucose. In similar studies, Allen and Palmer (3, 4) obtained protection of barley from simazine injury with several metabolites including L-glutamine and a-ketoglutarate. Eastin et al (20) found that both glucose and sucrose supplied to the treated plant through a severed leaf tip, gave protection to a susceptible inbred line of corn against the toxic effects of atrazine and simazine. Simazine inhibited starch accumulation in Coleous plants (26). However, chloroplasts of Coleous leaves were able to form starch in the presence of both sugars and simazine indicating that simazine inhibited the formation of sugars.

Simazine inhibited CO_2 fixation in kidney bean leaves and the amount of inhibition increased with exposure to time and simazine concentration, but $C^{14}O_2$ fixation could never be inhibited completely by these compounds suggesting that an alternate $C^{14}O_2$-fixation reaction might remain unaffected (6). Similar inhibition of photosynthetic $C^{14}O_2$ fixation was exhibited by atrazine (76). However, processes involved in CO_2 fixation in the dark were unaffected by atrazine. In the same study atrazine treatment resulted in complete blockage of sucrose synthesis.

Ashton and Uribe (7) found that atrazine caused a decrease in serine, alanine, and glyceric acid synthesis and an increase in aspartic acid and glutamic acid biosynthesis when C^{14}-labelled sucrose was the substrate in red kidney bean leaves. Darkness caused a

decrease in serine and glyceric acid biosynthesis and a corresponding increase in aspartic acid biosynthesis from sucrose. Since darkness did not cause a decrease in alanine or an increase in glutamic acid, these workers concluded that other metabolic processes entirely independent of photosynthetic blocks were responsible for these differences. Jordan et al (42) found that herbicidal concentrations of atrazine and simazine inhibited growth of non photosynthesizing tobacco callus, but non herbicidal triazines (hydroxy analogues of atrazine and simazine) had no effect. They concluded that toxicity was due to interference with activity other than photosynthesis.

Metabolism of the s-Triazines by Plants

Studies have been undertaken to determine the factors responsible for the tolerance of certain plants to the triazines (11, 12, 15, 16, 50, 51, 52, 53). According to Montgomery and Freed (51) several factors could be involved in resistance of plants to triazines: a) the apparently resistant plant absorbs very little herbicide, b) the biochemical systems inhibited in the sensitive plants are not affected in the resistant plant, and c) the resistant plant is able to detoxify the herbicide.

Studies have shown that the low absorption of the herbicide by apparently resistant plants is a factor in the selectivity of certain triazines on a limited number of plants (26, 51). This is illustrated by the selective use of simazine on deep rooted crop plants such as citrus, apple, and peach. Simazine is strongly adsorbed by soils so that very little herbicide is found below 3 or 4 inches of soil (50). This property and the fact that simazine is adsorbed mainly by roots

causes minimum exposure of deep rooted plants to the herbicide. Another possibility for decreased exposure of a plant to the herbicide is that the resistant plant, for some physiological reasons, takes up less chemical. However with the triazines, this behavior has not been noted. In studies where the amount of herbicide taken up by resistant and susceptible plants has been compared, little difference was found between these two types of plants (15, 26, 51). In most cases the resistance of plants to the triazine herbicides cannot be explained by the amount of chemical absorbed.

Differences on the effects of triazine herbicides on biochemical processes between resistance and susceptible species have not been confirmed or disproved since considerable discrepancies exist concerning the effects on various enzyme systems (19, 24). Funderburk and Davis (24) did not find any relationship between the effects on several respiratory enzymes and susceptibility of 8 plant species to atrazine injury. Even the effects of chlorotriazines on the photosynthetic mechanism is similar in both resistant and susceptible plants (49, 53).

Metabolism of the herbicides by resistant plants seems to be the predominant factor in the resistance of some plants to the triazine herbicides. However, this fact is no more important than the rate at which the herbicide is metabolized. If the rate of absorption is much greater than the rate of metabolism, a lethal concentration of herbicide may accumulate. With triazines, different plants possess varying capacities for detoxification. A number of reports have shown that a good correlation exists between the extent of metabolism and

degree of resistance to the triazine herbicides (11, 12, 15, 23, 26). In experiments with C^{14}-labelled atrazine, Montgomery and Freed (51) reported that atrazine is converted to a new compound 2-hydroxy-atrazine by expressed corn juice. Castelfranco et al (11) found corn to contain a non-enzymatic system which converts simazine to hydroxy simazine. The constituent was subsequently isolated and identified as the cyclic hydroxamate, 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one. In vitro conversion of simazine to the hydroxy analogue was demonstrated by using a crystalline sample of the cyclic hydroxamate or its glucoside. The hydroxy analogue was also found in extracts of corn plants exposed to simazine, demonstrating in vivo conversion (28). This reaction is probably common for all of the triazines since propazine undergoes the same conversion when incubated with corn juice. The rapid conversion of the chlorotriazines to the hydroxy analogues appears to be the primary factor in the high tolerance of corn to this herbicide. Using intact plants, Davis et al (15) found that corn, cotton, and cucumber took up the same amount of simazine; however approximately 95, 75, and 50 per cent was converted to the hydroxy analogue, respectively. Corn is quite tolerant to simazine, cotton moderately sensitive, and cucumber very sensitive. However, some doubt has been raised as to the relationship between degradation and tolerance in several species (31). Sorghum, which was quite resistant, was found to be lacking in benzoxazinone and no conversion of the chlorotriazine to the hydroxy derivative occurred, although other susceptible species, containing benzoxazinone were capable of affecting such a conversion. Recently, Shimabukuro et al (67) reported that pea

plants dealkylated atrazine to 2-chloro-4-amino-6-isopropylamino-s-triazine. Hydroxy-atrazine, the major metabolite reported to occur in other plants was not detected. Results indicate that an alternate pathway other than the degradation of 2-chlorotriazine to the hydroxy analogue exists in higher plants.

Further oxidation of the ring (59) and the side chain of atrazine (23) has been demonstrated. Montgomery and Freed (51) found that corn was ca. 7 times as active as oats in metabolizing C^{14}-atrazine to $C^{14}O_2$.

The metabolism of triazine other than the chloro-substituted members has been studied very little. Corn is moderately sensitive to the methoxy and methylmercapto triazines (51). Incubation of corn juice with C^{14}-prometryne did not reveal any significant amounts of hydroxypropazine. Apparently, the system responsible for conversion of the chlorotriazine to the hydroxytriazine is not active on the methylmercapto derivative. Furthermore, corn plants exposed to labelled prometryne did not give off significant amounts of $C^{14}O_2$, although some of the absorbed herbicide was metabolized. Carrot, moderately resistant to prometryne, was found to degrade the herbicide in a manner analogous to that in which corn degrades atrazine (51). Whitenberg (74) suggested that hydroxypropazine found in soil grown plants was converted to this form before absorption by the plants and that cotton did not metabolize the herbicide to any detectable extent. He suggested that the resistance of cotton to prometryne results from the binding or complexing of the chemical at sites which differ from those necessary for the expression of herbicidal activity. Prometryne was found to be accumulated in the lysigenous glands and these glands may be involved in the tolerance of cotton to certain s-triazines (29, 74).

Some Aspects of Behavior of Triazine Herbicides in Soil

The behavior of herbicides in soil is important as they affect the degree of weed control, crop residue build up, contamination of ground water and streams, etc. Triazine herbicides have been removed from the soil by volatilization, photodecomposition, microbial breakdown, chemical breakdown and leaching (22).

Adsorption of Triazines in Soil

Adsorption is a key factor in the behavior of herbicides in soil. Adsorption reduces the effectiveness of an herbicide. Sheets (65) showed that the phytotoxicity of simazine is less in soil than in solution cultures. Oats grown in solution needed 0.028 ppmw of simazine compared to 0.43 ppmw in clay for a 50 per cent reduction in growth. The values for soybeans were 0.069 in solution compared to 1.64 in clay soil. Harris and Warren (32) showed that pH influenced the adsorption of triazines especially on montmorillonitic clays. The degree of adsorption had been found to be high on montmorillonite, low on kaolinite and medium to low on illite (47).

Burnside and Behrens (10) reported that increasing the temperature from 59 to 86 F increased the phytotoxicity of simazine. Talbert (70) showed that the adsorption of simazine was more affected by soil temperature than atrazine. He also found that desorption was faster with increasing temperature.

Soil organic matter is a very important source of adsorption of herbicides. Upchurch and Mason (71) found that the concentration of herbicide necessary to decrease fresh weight by 50 per cent (GR 50) was closely related to per cent soil organic matter. The concentration for GR 50 at 20 per cent organic matter was 5 times greater than that required at 4 per cent organic matter. Talbert (70) found a high

correlation with 3 chloro-triazines between adsorption and the per cent organic matter although the correlation was not as good with prometone and prometryne. Sheets et al (66) found that GR_{50} values were more highly related to soil organic matter than any other soil property measured. Organic matter content was better to predict GR_{50} values than were K values, textural properties, CEC, or pH. Hilton and Yuen (37) reported that the easily oxidized organic fraction of Hawaiian sugarcane soils and the true carbon arising from cane leaf burning highly contributed to adsorption. They found also that the clay fraction contributed to adsorption but not to the extent of the 2 previously mentioned factors. Adsorption was found to be greater on muck than on mineral soil according to Harris and Warren (32). Harris and Warren (32) found that dalapon was easily desorbed from soil, and simazine was much slower indicating differences in adsorption and desorption. Harris and Warren (32) and Tablert (70) found that the adsorption of triazine herbicides on mineral soils was reversible with water. In muck and peat soils, however, these authors found desorption to be slow or incomplete. Harris (33) found that representative chlorotriazines were less adsorbed than that of methylmercapto derivatives. He concluded that the $-SCH_3$ group in prometryne influences the electron density of the molecule to cause strong binding forces between soil and the herbicide. The effect of chloro substitution, conversely, appears to lessen the attractive forces between herbicide and soil particles.

Leaching of Triazine Herbicides in Soil

The leaching of an herbicide depends upon the degree of adsorption. The depth of leaching will also depend on rainfall, soil texture and herbicide concentration (47).

Sheets (65) studied leaching in columns of a sandy loam and clay loam as affected by rate of simazine and amount of water applied. In sandy loam he found no leaching below 3 inches of soil when 2 inches of water were applied after treatment with 1 lb/A and additional leach- was limited when 10 inches of water were applied. At 20 lb/A leaching occurred in the whole column. Downward movement was much less in the clay loam soil.

Montgomery and Freed (49) found that most of the C^{14}-simazine remained in the 0 - 1 inch layer after applying 12 inches of water through a sandy loam soil over a 3 day period. Under the same conditions the greatest concentration of C^{14}-atrazine occurred at the 7 - 8 inch depth.

Rogers (63) found atrazine to be readily leached in sandy soils, simazine and atratone intermediate, and ipazine the least. There was, however, little leaching of any of these triazines below the 1 inch zone in muck soil.

Influence of Herbicides on the Nitrogen Constituents of Plants

Comprehensive reviews dealing with the effects of herbicides on the nitrogen metabolism of plants have been presented by Gramlich (24) and Eastin (19) during the past 2 years. These investigators studied the influence of atrazine on the nitrogen constituents of some resistant and susceptible species.

Gramlich (24) citing Russian workers, stated that 2,4-D treatment of 4 day old lupine seedlings resulted in protein hydrolysis and deamination of amino acids with the release of free ammonia. In maize the protein content was slightly lowered and the ammonia and amino acid contents were increased considerably. In peas, 2,4-D treatment sharply reduced the protein content and increased the content of amino acids and ammonia. In bean, herbicidal concentrations of 2,4-D stopped nitrogen uptake, sharply reduced synthesis and enhanced the decomposition of proteins. This was not observed in oats, a resistant species, which lead Gramlich (24) to suggest that the effect on nitrogen metabolism may account for at least part of the differences in susceptibility which exist between grasses and broadleaf plants to injury by 2,4-D.

McWhorter (48) reported that amitrole treatment resulted in increased concentrations of manganese, free ammonia and total nitrogen (TN), and that protein nitrogen (PN) content of chlorotic tissue was usually reduced whereas free amino acids were increased. Other workers (35, 41) have found that amitrole interferes with histidine metabolism.

Investigators with both corn and orchard crops have shown that applications of simazine and atrazine caused an increase in nitrogen uptake by these plants (18, 60, 61). Bartley (9) observed that corn was taller and greener when simazine was applied at 16 lb/A. Other workers found an increase of 230 to 370 per cent of forage corn with simazine rates of 1 to 5 lb/A. De Vries (18) found an increase in nitrogen uptake by corn in simazine treated soil. Ries and Gast (60)

found that the addition of simazine increased N content of corn plants regardless of the level of N used while hydroxysimazine had no effect.

Gramlich (24) suggested that the chlorotic appearances of seedling plants emerging from soil treated with 3-(p-chlorophenyl)-1,1-dimethylurea (monuron) indicated interference with N metabolism. Audus (8) stated that monuron treatment resulted in decreased per cent ammonium and nitrate N and increased PN, but the latter effect was apparently the result of reduced growth.

Gramlich (25) using up to 8 lb/A atrazine treatment found that corn and Johnson grass were reduced in size compared to checks, but percentages of N were higher. Atrazine treated corn plants contained higher percentage of both protein and non protein N than the checks, Nitrate percentages were also increased but free ammonia content was not significantly affected.

SECTION A

MATERIALS AND METHODS

PHYTOTOXICITY OF AMETRYNE AND ATRAZINE TO BANANAS

Greenhouse Soil Culture Experiments

Soils obtained from the three field test locations were used for the greenhouse soil culture experiments to determine the influence of soil type on the differential response of the plants to ametryne and atrazine. The Waimanalo Experimental Farm has a dark magnesium clay soil which belongs to Waimanalo Silty Clay type, the Poamoho Experimental Farm has a soil which belongs to the Low Humic Latosol group which consists of a mineralogical composition of kaolinitic clay and iron oxides. The Halii soil series at the Kauai Branch Station is a strongly leached soil which has been depleted of silica. Other details for the three soils are contained in Table 1.

Banana (*Musa acuminata* variety "Dwarf Cavendish") suckers were planted in the equivalent of 1.5 kg oven dried soil in gallon cans which were lined with polyethylene bags. The plants were watered daily to field capacity. Uniform plants about 12 inches tall were allowed to get established prior to treatment with the equivalent of 0, 4, 16 and 40 ppm (active ingredient) of the herbicides on an oven dried soil basis. The herbicide was mixed in 100 ml water and applied around the plants washing all traces of the herbicide into the soil with additional water. The soil was watered following herbicide application and henceforth watering was done to field capacity daily. Each treatment was replicated 3 times with one plant per pot constituting a replication. The plants were observed at regular intervals.

Table 1. Description of the test locations.

Experiment Station	Island	Elevation (Feet)	Soil Type	Organic Matter (Per Cent)	Median Annual Rainfall (Inch)	Soil pH[1/]
Kauai Branch Station	Kauai	530	Halii Gravelly Silty Clay	7-8	92	5.0
Waimanalo Experimental Farm	Oahu	70	Waimanalo Silty Clay	4-5	45	6.5
Poamoho Experimental Farm	Oahu	870	Wahiawa Silty Clay	2	45	5.8

[1/] 1 part soil-1 part water ratio, w/v.

Greenhouse Sand Culture Experiments

To further explain the differential selectivity of bananas to atrazine among the three test locations and to determine why ametryne was not injurious to plants under field conditions at the rates used, experiments were initiated using sand as the rooting medium. The use of herbicides in controlled sand culture experiments would eliminate the influence of soil variables. The results obtained should indicate the herbicidal effect *per se* including the effect of concentration of each herbicide.

Banana suckers were grown in 2 gallon glazed jars filled with washed builders sand. The plants were supplied weekly with 500 ml of 1/2 strength Hoagland's Solution A (40) by applying the solution and collecting run-off water until the solution was depleted. Between applications of nutrient solution, the plants were watered daily with tap water until treatment time. Prior to treatment each pot was flushed with 4 liters of distilled water to remove the soluble salts.

Concentrations of ametryne and atrazine (commercial preparation - 80% wettable powder) were prepared in 1/2 strength Hoagland solution to give an active equivalent of 0, 0.4, 4, and 16 ppm, respectively. At the initiation of the experiment, 2 liters of solution containing the herbicide concentrations were applied to each pot. Drain-off solution was collected in individual beakers, applied the following day, and the cycle continued. Thereafter, the pots were watered to approximately field capacity daily with distilled water. The excess water was collected and returned to the pots during the next watering period.

One liter half strength Hoagland's solution without the herbicide was supplied to each pot at two-week intervals.

SOIL ADSORPTION AND LEACHING CHARACTERISTICS OF AMETRYNE AND ATRAZINE

Some soils high in organic matter require increased rates for a given herbicidal activity. This effect is possibly due to high adsorptive capacity of herbicides to these soil constituents. As soil applied herbicide is generally considered to enter plant roots as solutes with the soil water, the degree of adsorption modifies the availability of the herbicides to plant or the activity of the herbicide. Soil adsorption of herbicides, therefore, is basic to understanding the behavior of herbicides in the soil (70). In addition, the quantity of organic matter (OM) and clay regulate leaching (65).

This study was initiated to compare some adsorption and leaching properties of ametryne and atrazine in the three soils used for the field studies and to relate, if possible, findings with observed responses of the field grown banana plants to these herbicides.

Soil Adsorption

Ametryne- and atrazine-C^{14} (1.79 ppm, 2 microcuries per 100 ml activity) were prepared by pipetting from a stock solution containing 2 microcuries per ml in dimethyl formamide and diluting with 0.01 M calcium chloride containing 0.1 ml Triton-X-100 per 100 ml. The original activity of C^{14}-atrazine was 1.28 ppm (2 microcuries per 100 ml) but the activity per given weight was made to correspond with that of ametryne by the addition of a 99 per cent non labelled atrazine[1/]. Based on preliminary trials, the surfactant did not affect adsorption.

[1/]Samples obtained gratis from the Geigy Agricultural Chemical Co.

One gram soil samples, oven dried equivalent, were placed into 40 ml centrifuge tubes and 5 ml of the adsorption solution were pipetted into each tube after distilled water was introduced into each weighed soil sample with a micro syringe to make the soil moisture content equal to 10 per cent. The soil was previously ground in a mortar and pestle to pass a No. 40 mesh screen. The tubes were sealed with rubber stoppers covered with aluminum foil and attached to a Burrell Wrist Action Shaker set at maximum speed and shaken for 12 hours. One ml aliquots were pipetted into glass scintillation vials with 20 ml scintillation solution. The solution contained 4 gm 2,5-diphenyloxazole (PPO) and 0.1 gm 1,4-bis-2(5 phenyloxazolyl)-benzene (POPOP) dissolved in a liter of 70 per cent toluene and 30 per cent absolute ethanol.

A Packard Tri Carb Scintillation Counter (Model 3101) was set at its maximum operating efficiency for the solution and each sample was counted for ten minutes. Before counting each vial was shaken and immediately lowered into the counting chamber. Standards, counted in the same manner, consisted of 1 ml aliquots of the s-triazine-C^{14} solution without the soil. Background counts were obtained from the supernatant containing everything as the labelled solutions except the C^{14} herbicides. The difference between the amount of herbicide found in the standard solution and the supernatant of the sample was assumed to have been adsorbed. Adsorption in this case is the process in which the dissolved herbicide becomes physically or chemically bonded to colloidal surfaces, thereby causing a net decrease in the concentration of the herbicide in the solution phase (70).

The procedure gave a counting efficiency close to 60 per cent for both herbicides. Variations between several countings of the same sample were slight and counts were high.

Soil Leaching

Ametryne (13.9 mg) and atrazine (12.8 mg) were dissolved in 25 ml methanol to give an activity of 4 microcuries per ml. Neutral atrazine (98.8%) and ametryne (98.5%) were added to make a working solution containing 944.5 micrograms per ml and 1 microcurie of radioactivity in 50 per cent methanol.

Top soils obtained from fields located on Kauai, Waimanalo and Poamoho, as previously described, were air dried and ground in mortar and pestle to pass a No. 40 mesh screen. Moisture content was determined and 100 gram oven dried equivalent samples were weighed and placed in glass columns (250 ml alkali dispensing burettes) on top of a layer of one-half inch silica sand. The bottom of the tube was lined with a loose ball of glass wool and the outlet was provided with rubber and glass tubings with adjustable clamps to control the flow of water. After the soil was introduced into the columns, it was tapped gently to remove air channels. The bottom portion was immersed in distilled water until the soil was completely saturated after which excess water was allowed to drain. The soil column was about 6 inches for each soil type.

After 2 hours, 1 ml of the s-triazine-C^{14} leaching solutions containing 944.5 micrograms herbicide were pipetted onto the top of the soil column and allowed to stand for 6 hours. After 6 hours equilibration one-half inch of sand was introduced onto the top of the soil and leaching was initiated. A continuous supply of water was applied

at the top of the column and the rate of flow at the bottom was regulated for each tube. Increments of 20 ml corresponding to 1 inch of water were collected and placed in vials. Increments of every 2 inch equivalent of water were collected after the 12th inch of water was leached and the process was terminated after 24 inches of water were collected. One-half ml aliquots were pipetted from each increment collected and placed in both planchets and scintillating vials. Activity in planchets was counted with a G-M Gas Flow Automatic Counter and the scintillator was described previously.

Samples from the leaching solution and leachate from the soil column without C^{14} were counted for standard and background counts, respectively. Herbicide recovered per water increment was expressed as percentage based on total applied by using values from the standard.

The experiment was replicated 3 times, each column constituting a replication and duplicate radioactive counts were made per sample.

ABSORPTION, TRANSLOCATION AND METABOLISM OF AMETRYNE AND ATRAZINE IN BANANA PLANTS

The apparent resistance of a few crop plants to the triazines has been attributed to the low absorption of the herbicide. Often this is due to the inability of the herbicide to reach the roots rather than a reduced uptake. The triazines are easily absorbed by most plants so that the resistance to the triazines, in most cases, points towards the ability of the plants to degrade the compound to prevent accumulation of toxic concentrations either after absorption as in corn (26) or around the root system as in sugarcane (39).

Because of the toxicity of atrazine to field grown banana plants and the apparent resistance of the plants to ametryne, this study was

initiated to compare absorption, translocation and degradation of the herbicides in bananas. Furthermore, studies of this nature have not been reported for bananas and this study appears to be of academic and practical importance in view of the promising results obtained with some triazines in weed control of banana orchards.

Autoradiography

Suckers were grown in sand culture for approximately 2 months and then transferred to one-half strength Hoagland's solution in 250 ml plastic containers. Plants under this condition grew normally for at least a month without aeration if the nutrient solution was changed every 2 days. After the plants were established, the roots were washed in distilled water, blotted with tissue paper and transferred to a new nutrient solution containing 100 ml and 2 microcuries of either C^{14}-ametryne or atrazine. The plants were placed in growth chambers with night and day temperatures set to approximately 75 and 85 F, respectively. Light was supplied with incandescent and fluorescent lamps with 12 hour light and dark cycles. To reduce evaporation, the nutrient containers were covered with aluminum foil with openings for the plant stems. Aeration was not provided to reduce contamination. At selected intervals, plants were sampled, pressed between blotters after rinsing the root system with distilled water and dried in an oven at 70 - 75 F for 12 - 24 hours. The plants were exposed to X-ray film for 1 week and then developed.

Aliquots were obtained from radioactive solutions on which the plants were growing and counted for activity. The amount of solution absorbed between sampling dates was also recorded. This served as a

preliminary study to determine the trend of change in radioactivity in nutrient solution in the vicinity of roots. The result of another similar experiment is discussed in the next topic.

Metabolism Studies

Preliminary trials were made to determine suitable methods for extraction, cleanup and chromatography of extracts for identification of the herbicides and their degradation products. Water, carbon tetrachloride, ether, pentane, ethanol, methanol and chloroform were used. The comparison was not done to any great detail but promising solvents were repeatedly compared. The procedure was to chop and mix whole plants previously grown in C^{14}-ametryne solution, and then extract 5 gram samples with 50 ml solvent twice. The extracts were filtered, evaporated, made to volume with 10 ml solvent and 1 ml subsequently pipetted into aluminum planchets for radioactive counts with a gas flow automatic G-M counter.

Adsorption of the herbicides was studied on exchange resins together with basic Woelm alumina grade V and Darco activated charcoal. Methanol extracts of banana plants previously grown in C^{14}-ametryne and atrazine were sorbed unto, and eluted from, 2 x 15 cm columns of the resin bedded in 50 per cent methanol. The columns were washed with 100 ml water and eluted with 4N NH_4OH or 3N HCl in 50 per cent methanol until activity was reduced to background. Exchangers used were: Amerlite 1R-120H; Dowex 50W-X4 (cation resins); Amberlite IR-45, OH form (anion resin). Charcoal was tested in the same manner as the ion exchangers. Basic alumina was used dry and packed into the column by gentle tapping. The sorbed herbicide was eluted with 50 ml

methanol, 100 ml of 80 per cent methanol, and 80 ml distilled water, respectively, following the method of Roberts (62).

In chromatography, several solvents were tried to find a suitable method for banana extracts. Paper chromatography (descending, 1 dimensional) using Whatman No. 1 paper and the following solvents were used: n-butanol-acetic acid-water (5:1:4 v/v/v), n-propanol-20% NH_4OH-water (73:20:7 v/v/v) and isoamyl alcohol saturated with 3N HCl. Thin layer chromatography (TLC) using silica gel pre-coated sheets (Distillation Products Industries, Inc.) were tried with the following solvents: benzene-acetic acid-water (10:10:1 v/v/v); ethyl acetate-chloroform-acetic acid (3:4:3 v/v/v) and chloroform-acetone (9:1 v/v). Based on the results from these preliminary experiments, methanol and chloroform were chosen as extracting solvents, and the chloroform-acetone mixture solvent on TLC was adopted for chromatography.

Plants previously growing in silica sand were transferred to plastic containers containing 150 ml half-strength Hoagland's solution and C^{14} activity of .02 microcuries per ml with an herbicide concentration of 1.79 ppm. The lid of the container served to support the plant after a hole was made in the center to accommodate the stem and a radial cut was made so that the plant could be inserted.

The plants were set in the greenhouse in a portion that admitted 50 per cent of the sunlight. Solution containers were shaded so that only the leaves and stem of plants were exposed to sunlight. Some plants were placed in partial shade in which the plants were not exposed to direct sunlight and the light intensity approximately 10 per cent of intensity outside the greenhouse. Two ml aliquots were removed at pre-

determined time intervals up to 7 days from the nutrient solutions containing the herbicides. Solution was not added to the original during the duration of the experiment. One-half ml aliquots were pipetted into planchets and duplicate samples were counted in a G-M automatic gas flow counter. Samples were also counted in the scintillator. Background counts were made from the same sample as the treatments without the C^{14} herbicides. Nutrient solutions with the herbicides and without the plants were sampled in a similar manner to determine if degradation, or change in concentration occurred differently in the presence of plant roots. Activity was converted to percentage based on total herbicide in the nutrient solution at the start of the experiment. Aliquots were evaporated in a hood and chromatographed as the plant extracts. Plants were sampled after 3 and 7 days and separated into roots and leaves for extraction and chromatography. Each section was chopped with a Waring Blender with 80 ml chloroform for 1 hour. The homogenate was filtered through a coarse porosity sintered glass filter and the residue returned to the blender. The procedure was repeated once with 80 ml chloroform with 30 minutes blending followed by blending the residue 2 times in 80 ml of methanol for 1 hour and 30 minutes, respectively, and finally blending the residue for 1 hour with 80 ml of chloroform-methanol (1:1 v/v) mixture. The residue after filtration was transferred to a 500 ml flask using 80 ml of the chloroform-methanol mixture. The flask was stoppered and shaken in a Wrist Action shaker for 6 hours and filtered. This was evaporated separately from the combined filtrated from extraction of the same sample. The two were combined after each was evaporated to less than 50 ml, during which time chlorophyll and other pigments

have precipitated, and made to 100 ml volume. One ml aliquots were taken for counting to determine the approximate C^{14} compounds recovered.

The extract was further evaporated to 2 ml. The addition of 10 ml methanol to this portion precipitated some brown pigments, presumably containing sugar. The solution was filtered into a centrifuge tube, centrifuged for 10 minutes at 10,000 rpm and transferred into small vials. Pellets from the residue were washed with small amounts of water, centrifuged and decanted into the same vial containing the supernatant of the sample. The solution was evaporated to near dryness and 10 microliters of this was spotted on TLC sheets kept in a desiccator. The chromatogram was run to approximately 13 cm solvent travel, air dried, and autoradiographed. Another set was chromatographed, cut to strips and counted in a Packard Radiogram Scanner, Model 7200.

RESULTS AND DISCUSSION

PHYTOTOXICITY OF AMETRYNE AND ATRAZINE TO BANANAS

Greenhouse Soil Culture Experiments

The results of the pot experiments which compared rates of ametryne and atrazine with 3 soil types are presented in Table 2 (Tables 2-a, 2-b). Plant injury was scored by rating from 1 (no injury) to 5 (plant dead). Symptoms of injury were noticeable on the plants 2 weeks after treatment and phytotoxicity gradually increased with time.

Injury was influenced by soil type, kind of herbicide, concentration of herbicide, and time of sampling (Table 2). Both herbicides injured the plants and the extent of injury was related to concentration. In general, at any sampling date, slightly more injury was observed with atrazine compared to ametryne. Compared to the results obtained in the sand culture experiments, ametryne was less phytotoxic than atrazine when applied to the soil indicating that phytotoxicity was reduced by soil factors. Decreasing order of injury generally follows this order: Poamoho soil, Waimanalo soil, and Kauai soil.

Values for phytotoxicity to plants obtained 10 weeks after treatment were statistically analyzed for comparison. These values were chosen as representative of the response of the plants to treatment. The analysis of variance is shown in Table 2-a. The results show that the influence of both soil and herbicide concentration were highly significant.

Although injury due to atrazine was significant over that of ametryne, injury was not totally absent with ametryne treatment even at the lowest concentration used in contrast to plants grown in the field. However, the values obtained indicate that pot conditions modified the

Table 2. Subjective ratings of leaf injury of banana plants treated with ametryne and atrazine in greenhouse soil cultures.[1/] [2/]

Treatments		Weeks After Treatment					
		1	2	4	6	10	14
				Kauai Soil			
Check		1.0	1.0	1.3	1.0	1.0	1.3
Ametryne	4 ppm	1.0	1.0	1.0	1.7	2.0	2.3
	16 ppm	1.0	1.3	2.3	2.3	3.0	3.0
	40 ppm	1.0	1.7	2.7	2.7	4.0	4.3
Atrazine	4 ppm	1.0	1.0	1.3	2.0	2.0	2.7
	16 ppm	1.3	1.3	2.3	2.7	2.7	4.0
	40 ppm	1.0	2.0	3.0	3.7	4.3	5.0
				Waimanalo Soil			
Check		1.0	1.0	1.3	1.0	1.0	1.3
Ametryne	4 ppm	1.0	1.3	2.0	2.0	2.0	2.7
	16 ppm	1.0	2.0	1.7	2.7	3.0	3.7
	40 ppm	2.0	2.7	2.7	2.7	3.7	4.0
Atrazine	4 ppm	1.0	1.7	1.7	1.7	2.3	2.7
	16 ppm	1.3	2.0	2.0	2.3	3.7	4.3
	40 ppm	1.3	3.3	2.7	3.7	4.7	5.0
				Poamoho Soil			
Check		1.0	1.0	1.3	1.0	1.3	1.7
Ametryne	4 ppm	1.0	1.0	2.3	2.3	4.0	4.0
	16 ppm	1.0	1.7	2.3	3.3	4.3	4.3
	40 ppm	1.3	2.0	3.3	3.7	4.7	4.7
Atrazine	4 ppm	1.0	1.0	2.3	2.3	3.7	4.3
	16 ppm	1.3	1.3	2.7	3.0	4.3	4.7
	40 ppm	1.0	2.0	4.0	4.3	5.0	5.0

[1/] Each value an average of 3 replications.

[2/] Rating Scale:

1 - No apparent injury, 2 - Slight tipburn and/or marginal chlorosis on older leaves, 3 - Moderate tipburn and/or marginal chlorosis on older leaves, 4 - Severe tipburn and/or chlorosis on young and old leaves, 5 - plants dead.

Table 2-a. Analysis of variance for Table 2, 10 weeks after treatment.

Source of Variation	df	MS[1/]
Replication	2	1.69*
Treatment Combinations	17	
Soil	(2)	6.91**
Herbicide	(1)	1.50*
Concentration	(2)[2/]	16.75**
Soil x Herbicide	(2)	0.50
Soil x Concentration	(4)	0.32
Herbicide x Concentration	(2)	0.23
Soil x Herbicide x Conc.	(4)	0.14
Error	34	0.332
Total	53	

[1/] *Significant at the 5% level (F-test) and **1% level.

[2/] Check not included in the analysis of variance.

Table 2-b. Mean rating values for main effects described in Table 2.

Treatments	Weeks after treatment					
	1	2	4	6	10	14
Soils						
Kauai	1.0	1.3	2.0	2.3	2.7	3.2
Waimanalo	1.2	2.0	2.0	2.3	2.9	3.4
Poamoho	1.1	1.4	2.6	2.8	3.9	4.1
Herbicide Concentrations						
Check	1.0	1.0	1.3	1.0	1.1	1.4
4 ppm	1.0	1.2	1.8	2.0	2.7	2.7
16 ppm	1.0	1.6	2.2	2.7	3.5	4.0
40 ppm	1.3	1.8	3.1	3.5	4.4	4.7
Herbicides						
Ametryne	1.1	1.6	2.2	2.5	3.4	3.7
Atrazine	1.1	1.7	2.4	2.9	3.6	4.2

soil effect to an extent quite different from that obtained in the field. An explanation for this will be discussed under the section devoted to "Soil Adsorption and Leaching Studies."

Greenhouse Sand Culture Experiments

Symptoms of injury were readily noticeable on the plants 2 weeks after treatment. Slight marginal burn and/or chlorosis were observed on some plants receiving 16 ppm ametryne and atrazine treatments after 1 week. All levels of both herbicides showed injury to the plants and the degree of damage was related to the concentrations of the herbicide and the length of exposure. The results are presented in Table 3 (Table 3-a). Statistical analysis using Duncan's Range Test for comparison was made on values obtained 8 weeks after treatment.

Ametryne showed a slightly less toxic effect than atrazine based on the scores in Table 3. However, no statistical difference existed between the two herbicides at any selected concentration when compared using Duncan's Range Test.

The similarity in phytotoxicity of both herbicides to plants grown in sand indicated that bananas cannot metabolize ametryne to a degree that would render the herbicide non toxic as was suspected when injury was not obtained among treated plants in the field. Although injury ratings of plant treated with atrazine were higher compared to ametryne at each concentration, the differences were not statistically significant.

The results obtained with the sand culture experiments suggest that soil characteristics may be the dominant factors in the differential response of field grown plants to ametryne. Some soil factors may prevent the plants from accumulating toxic levels of ametryne and the plant

Table 3. Subjective ratings of leaf injury of banana plants treated with ametryne and atrazine in greenhouse sand cultures.[1/] [2/]

Treatments		Weeks After Treatment			
		1	2	4	8[3/]
Check		1.0	1.0	1.0	1.3[d]
Ametryne	0.4 ppm	1.0	1.3	1.3	1.7[c]
	4.0 ppm	1.0	2.3	3.0	3.3[b]
	16.0 ppm	1.1	3.0	3.7	4.7[a]
Atrazine	0.4 ppm	1.0	1.3	2.0	2.0[c]
	4.0 ppm	1.0	2.3	3.7	3.7[b]
	16.0 ppm	1.1	2.3	4.3	5.0[a]

[1/] Each value an average of 3 replications.

[2/] Rating Scale:
1 - No apparent injury, 2 - Slight tipburn and/or marginal chlorosis on older leaves, 3 - Moderate tipburn and/or marginal chlorosis on older leaves, 4 - Severe tipburn and/or chlorosis on young and old leaves, 5 - Plants dead.

[3/] Means not followed by a common letter are statistically different at Duncan's Multiple Range Test 5% level.

Table 3-a. Analysis of variance for Table 3, 8 weeks after treatment.

Source of Variation	df	MS[1/]
Treatment	6	6.41**
Replication	2	0.10
Error	12	0.44
Total	20	

[1/] **Significant at the 1% level (F-test).

is not resistant to the herbicide. Because of the results of these experiments, soil adsorption of the herbicide was suspected to play a role in the differential response observed in the field; consequently, this topic was studied in depth.

SOIL ADSORPTION AND LEACHING CHARACTERISTICS OF AMETRYNE AND ATRAZINE

Soil Adsorption

Adsorption of ametryne in any given soil was approximately 3 times the values obtained for atrazine (Table 4) (Table 4-a). Comparison of adsorption capacities between the three soils for both herbicides shows that Kauai soil was the most adsorptive followed by Waimanalo and Poamoho, respectively.

Adsorption of ametryne was greater than atrazine in any given soil. This substantiates the findings of Hilton and Yuen (37) that ametryne was adsorbed more than atrazine in Hawaiian soils. Likewise adsorption of atrazine and ametryne are greatest in Kauai soil followed by Waimanalo and Poamoho, respectively. The main effects, however, were not independent and additive since there was a highly significant herbicide x soil interaction.

Soil Leaching

The total percentages of ametryne and atrazine leached through the 6 inch columns of the three different soils are presented in Tables 5 and 6. Percentage herbicide recovered per water increment is presented in Table 6.

As shown in the results the amount of herbicide leached by each soil was inversely related to the adsorptive capacity of the soil in question. In any given soil the retention of ametryne was greater than

Table 4. Micrograms of ametryne and atrazine adsorbed per gram of soil (amount adsorbed from 1.79 ppm initial concentration of solution)

Soil	Herbicide	ug Adsorbed
Kauai	Ametryne	4.90[1/]
	Atrazine	1.77
Waimanalo	Ametryne	3.50
	Atrazine	1.11
Poamoho	Ametryne	2.82
	Atrazine	0.94

[1/]Each value an average of 4 replications.

Table 4-a. Analysis of variance for Table 4.

Source of Variation	df	MS[1/]
Treatment	5	9.4**
Soil	(2)	4.47**
Herbicide	(1)	36.49**
Soil x Herbicide	(2)	0.78**
Error	18	0.0014
Total	23	

[1/]**Significant at the 1% level (F-test).

Table 5. Per cent ametryne and atrazine recovered by leaching 100 grams of soil with up to 24 inches of water. Approximately 1 mg of herbicide was applied to column surface.[1/]

Soil	Herbicide	% Recovered	
		12" leachate	24" leachate
Kauai	Ametryne	0.31	5.36
	Atrazine	26.31	50.32
Waimanalo	Ametryne	29.36	54.47
	Atrazine	60.63	79.19
Poamoho	Ametryne	35.87	68.34
	Atrazine	78.03	93.60

[1/]Each value an average of 3 replications.

Table 6. Per cent herbicide per inch increment of leachate recovered. Approximately 1 mg of herbicide was applied to 100 grams of soil.[1]

Inch Water Increment	Kauai Soil		Waimanalo Soil		Poamoho Soil	
	Ametryne	Atrazine	Ametryne	Atrazine	Ametryne	Atrazine
1	-[2]	-	-	-	-	-
2	-	.01	-	.03	.10	.06
3	-	.03	.01	.05	.07	.06
4	-	.09	.11	1.46	.13	.08
5	-	.84	.97	10.04	1.35	6.49
6	-	2.19	1.65	10.50	2.35	15.27
7	-	3.93	3.88	7.95	4.67	15.90
8	.01	2.95	5.49	7.31	5.66	11.68
9	.03	3.80	4.79	7.01	6.31	9.07
10	.05	4.48	5.54	6.02	5.54	6.95
11	.10	4.06	4.30	5.58	4.71	6.42
12	.11	3.93	3.62	4.68	4.99	6.06
13 - 14	.19	6.11	5.37	6.92	7.82	5.92
15 - 16	.30	4.52	5.39	4.11	6.92	4.28
17 - 18	.27	3.31	4.16	3.04	5.37	2.07
19 - 20	.45	3.22	3.48	2.04	4.62	1.48
21 - 22	1.65	3.23	3.67	1.37	4.13	1.18
23 - 24	2.25	3.62	3.03	0.99	3.61	0.64
Total 12"	.35	26.31	29.33	60.63	35.87	78.03
Total 24"	5.36	50.32	54.19	79.01	68.34	93.60

[1] Each value an average of 3 replications.

[2] Less than 0.01%.

atrazine, Retention of herbicide was greatest in Kauai soil followed by Waimanalo and Poamoho, respectively. Comparison of the results of leaching and adsorption of herbicide indicates that the amount of herbicide leached was not strictly inversely proportional to adsorption. This indicates that the differential capacity of the soil to release "adsorbed" herbicide was not entirely dependent upon the amount of water applied. Effects of herbicide and soil are both statistically significant and each soil is significantly different from the other. Values for herbicide x soil interactions are also significant.

Apparently the differential selectivity of field grown bananas to ametryne and atrazine can be attributed at least in part to the differences in adsorption and leaching of these two herbicides and to the differences in these characteristics as influenced by soil type.

Inherent selectivity of bananas to the two herbicides has not been obtained in greenhouse sand and soil culture experiments. The greenhouse phytotoxicity studies using soil, the injury observed was related to the adsorptive capacity of the soil, hence greater injury was observed in Poamoho soil compared to Waimanalo and Kauai. Likewise, atrazine was more injurious to bananas than ametryne; however, bananas did not escape injury from ametryne in contrast to field observations where 8 lb active/A ametryne was completely safe to the plants. Likewise, atrazine was safe to use at Waimanalo whereas injury was observed with even the lowest concentration of herbicide in pots using soil from that location. Several factors might have influenced the observations but it is apparent that leaching and adsorption played an important role. As described by Upchurch (72), selectivity may be obtained by placement of herbicide in

such a way that roots of the crop are not exposed to the herbicide. In the field the amount of water used at any given time might not have been sufficient to leach phytotoxic concentrations of the herbicide where injury was not obtained. This in turn was related to the adsorptive capacity of the soil for the particular herbicide in question. Banana roots are distributed in greatest amount at least a few inches below the soil surface (68). Data on soil leaching indicate that it takes a considerable amount of water to leach 50 per cent of ametryne in any soil type used below 6" soil. Atrazine, on the other hand was more easily leached.

Injury to pot grown banana plants was at least partly due to the fact that the roots occupied the whole portion of the soil; also new roots were occasionally exposed on the soil surface. Surface roots would undoubtedly facilitate uptake by direct contact exchange with the soil-adsorbed herbicide. Also in pot grown plants, the minimum amount of moisture would bring the desorbed herbicide within the vicinity of the roots.

Better control of most weeds was obtained (in both immediate effect and duration of control) with ametryne in the three soils under field conditions. The greater adsorptive capacity for ametryne appears to play a role in that the herbicide is retained in the surface layer where weed roots are most abundant. For bananas and other deep rooted crop plants this phenomena is desirable in that selectivity can be obtained by failure of the herbicide to reach the roots of the crop plants.

The results obtained are in agreement with observations made by several investigators. For instance, Gysin and Knusli (27) pointed out that 3 processes are important with respect to action of soil borne

herbicides: a) adsorption as it determines availability, b) leaching as it determines placement and eventual loss, and c) breakdown as it relates to persistence in the soil. Hilton (38) mentioned the importance of soil adsorption in preventing leaching to root zones of sugarcane plants when herbicides injurious to the crop are used. According to Upchurch (71), where tolerance of a crop is inherently poor, selectivity can be obtained by placing the herbicide (through physical incorporation or leaching) in a precise position for maximum weed injury and minimum crop injury.

ABSORPTION, TRANSLOCATION AND METABOLISM OF AMETRYNE AND ATRAZINE IN BANANAS.

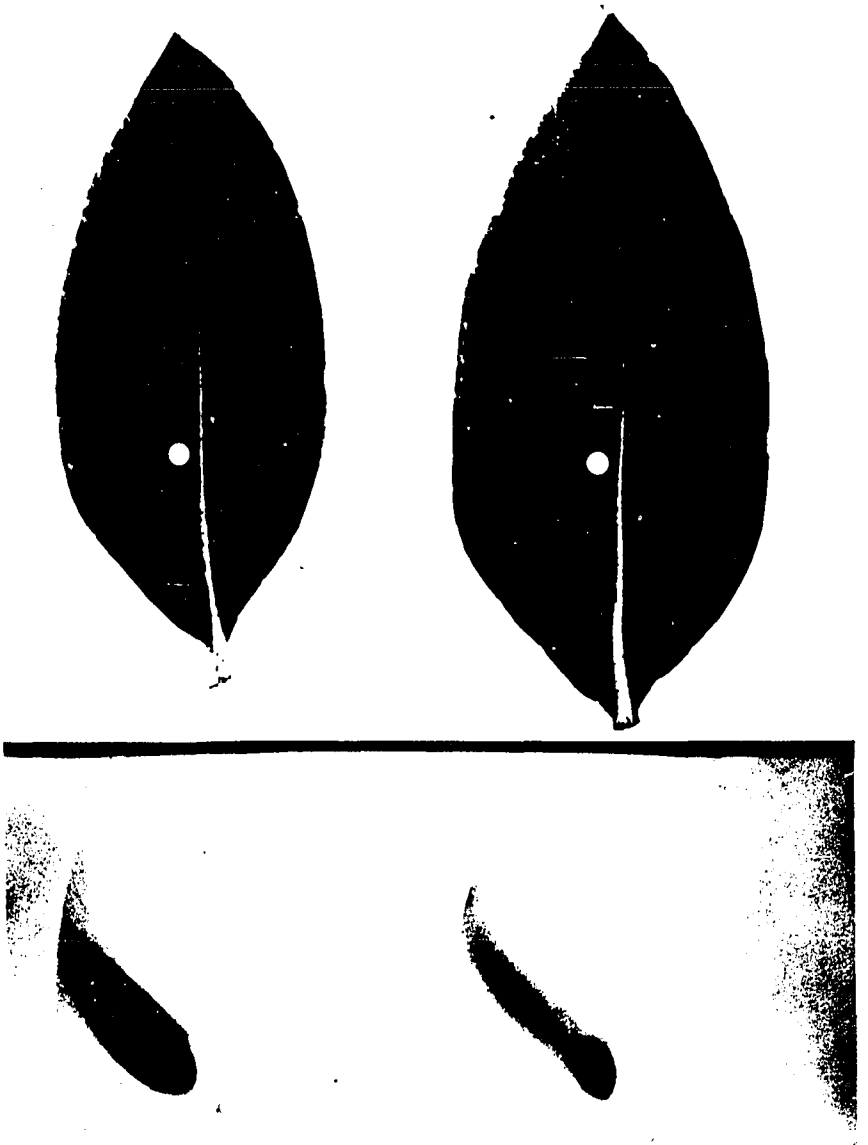
Autoradiography

Autoradiography showed that bananas absorbed C^{14} ametryne and atrazine readily from nutrient solution. Radioactivity was more pronounced along the leaf midribs and veins. The amount of radioactive compound absorbed and translocated was proportional to length of exposure. No difference, by visual observations, was found between radioautographs of ametryne and atrazine treated plants. Radioactivity was distributed throughout the plant after 1 day exposure to either herbicide.

Comparisons showed that oven drying did not alter the distribution pattern of the radioactive compound when compared with freeze dried plants prior to autoradiography.

Herbicide applied to the leaf surface was absorbed to a limited extent after 1 week exposure and movement was always acropetal. Herbicide applied to one side of the midrib did not get into the midrib but there was a limited movement towards the leaf margin (Figure 3). Herbicide applied to the midrib moved towards the tip of the leaf, also to a very limited extent. The same pattern was observed for both herbicides.

Figure 3. Autoradiograph (bottom) of banana leaves (top) treated with foliar application of C^{14}-ametryne (left) and atrazine (right). Herbicide was applied near the midrib (white dots) and sampled after 1 week.



Metabolism Studies

Methanol was found to be the most efficient extracting solvent based on the preliminary survey. Results were based on radioactive counts of aliquots of extracts which were counted with a gas flow G-M counter after evaporation in planchets. Combined extraction with chloroform increased recovery of C^{14}. Efficiency of any of the other solvents, except ethanol, was not consistently obtained and no attempt was made to pursue this comparison further. Detailed comparisons of several extracting solvents (alone or in combinations) were planned for another experiment. Although water might seem suitable as co-extractant for water soluble degradation products, such as the hydroxy compounds, this was not subsequently used because of the co-extracted plant pigments.

All the ion exchange resins used including activated charcoal retained more than 90 per cent of the radioactivity after washing with water, and this was readily eluted with 4N NH_4OH or 3N HCl in 50 per cent methanol. The eluate, however, was not free of pigments including chlorophyll and the degree of cleanup did not improve over that of subjecting the extract to filtration, evaporation to small volumes to precipitate chlorophyll, and subsequent centrifugation. Using exchangers for cleanup was not adopted primarily because of the extreme pH of the eluant needed that might alter the C^{14} compounds. Likewise, heat was not used for evaporation for the same precaution.

Paper chromatography was not very successful because of trailing of the radioactive spots as shown by autoradiography of chromatograms. In this manner, only the hydroxyl and the parent compounds were readily distinguishable. The difficulty was apparently due to interference of

co-extracted plant substances. Abbott and Thompson (2) stated that TLC procedures, in general, are much more tolerant of co-extracted materials than are paper or gas chromatographic systems. In fact, very often, TLC is used for clean-up purposes before application of other analytical methods of determination. TLC was more successful in the preliminary experiments and the use of chloroform-acetone mixture gave the clearest and most consistent separation. Benzene-acetic acid gave adequate separation of the hydroxy and parent triazines giving Rf values of about .43 for the hydroxy derivative and .98 for ametryne. The trailing of some spots may have masked the presence of other metabolites. Ethyl-acetate-chloroform-acetic acid gave very clear separation for the hydroxy triazine but a clear circular spot was not obtained with the parent triazine which travelled with the solvent front. Some spots appeared at about Rf .80 but the spot was masked by trailing areas in the auto-radiograph of the chromatogram. Chloroform-acetone mixture was utilized for all succeeding experiments.

Extraction, separation and identification of triazines and their metabolites have been reported by several investigators (1, 11, 12, 15, 16, 17, 23, 28, 29, 44, 45, 46, 50, 51, 55, 56, 58, 62, 67, 74, 75).

Loss of the herbicides in nutrient solution with and without plants is shown in Table 7. It is evident that the plants rapidly took up the herbicides and in 45 minutes of exposure time concentration of the herbicides dropped to between 60 and 70 per cent. Table 8 shows that the plants took up the herbicides much faster than could be accounted for by the amount present per volume of treatment solution absorbed by the plants. The rapid uptake reached equilibrium in less than 45 minutes and

Table 7. Per cent herbicide in the nutrient solution (C^{14} activity per ml) at each sampling date based on 100 per cent initial concentration (1.79 ppm) of solution before treatment.[1]

Length or Exposure	Plant in Shade	Plant in Sun	Nutrient Solution Without Plant
		Ametryne	
45 min	68	68	100
3 hr	66	71	95
12 hr	54	71	95
24 hr	68	82	-
72 hr	68	93	101
168 hr	77	130	100
		Atrazine	
45 min	73	84	100
3 hr	72	75	97
12 hr	63	75	97
24 hr	83	83	-
72	88	98	99
168 hr	100	116	97

[1] Each value an average of 3 replicates.

Table 8. Total herbicide absorbed by banana plants at each sampling date. Values based on total amount of herbicide (100 per cent) in 150 ml of treatment solution.[1]

Length of Exposure	Ametryne				Atrazine			
	Plant in Shade		Plant in Sun		Plant in Shade		Plant in Sun	
	Per Cent Herbicide Absorbed	ml H_2O Used	Per Cent Herbicide Absorbed	ml H_2O Used	Per Cent Herbicide Absorbed	ml H_2O Used	Per Cent Herbicide Absorbed	ml H_2O Used
45 min	38	3	35	3	35	3	35	3
3 hr	40	5	33	5	27	5	32	6
12 hr	55	8	33	7	42	7	48	11
24 hr	44	11	30	23	27	11	20	17
72 hr	49	23	37	45	30	22	32	46
168 hr	55	54	68	105	38	47	75	95

[1] Each value an average of 2 replicates.

further loss of herbicide from the nutrient solution was slow. The herbicide removed from the nutrient solution may have been immediately translocated by the plant or merely adsorbed (or absorbed) by the roots and gradually translocated. Both seem to be true to a certain extent since autoradiograph of plants after 1 hour of treatment with labelled ametryne and atrazine showed radioactivity to be concentrated around the roots with traces found midway in the pseudostems. No radioactivity showed in the leaves. The roots of plants have been compared to ion exchangers that attract molecules, particularly strongly charged molecules. The increase in concentration of herbicide in the nutrient solution at times, particularly between 12 and 24 hours from treatment, indicates that the plants released the herbicides at certain times and the rate of herbicide uptake by the plant was not dependent upon the amount of solution uptake. At the termination of the experiment, however, plants that transpired readily took up the greatest amount of herbicide. Plants grown without shade belonged to this category. Per cent uptake was based on the total amount of herbicide supplied to the growing solution before treatment. Nutrient solution without plants did not fluctuate in concentration to any extent. Evaporation was minimized by using cotton to seal any openings in the containers.

To determine if degradation occurred in nutrient solution around the plant roots, aliquots were sampled and evaporated prior to chromatography. Chromatograms of nutrient solutions up to 7 days showed only Rf values corresponding to that of standards from chromatograms of the parent triazines. Chromatograms of standards and nutrient solutions without plants are presented in Figures 4, 5, and 6. Solutions where

Figure 4. Chart showing radioactivity in chromatograms.
C^{14}-ametryne (top) and atrazine (bottom) standard solutions.

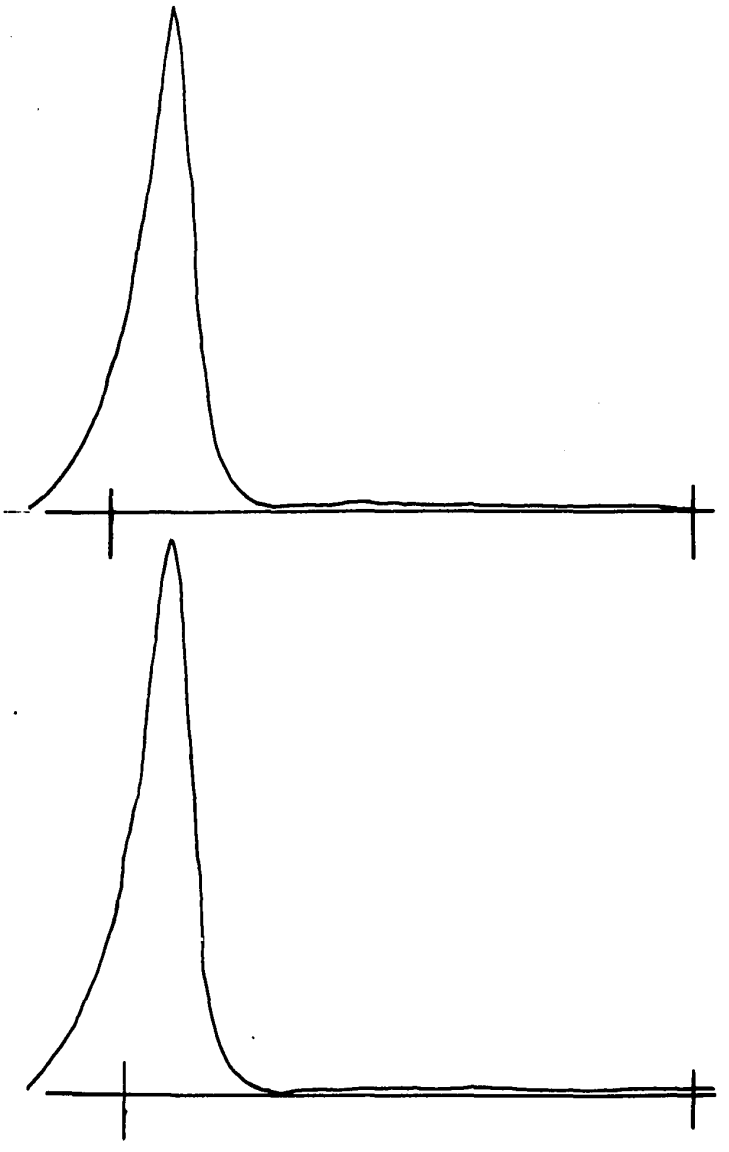


Figure 5. Chart showing radioactivity in chromatogram. C^{14}-hydroxyatrazine standard solution.

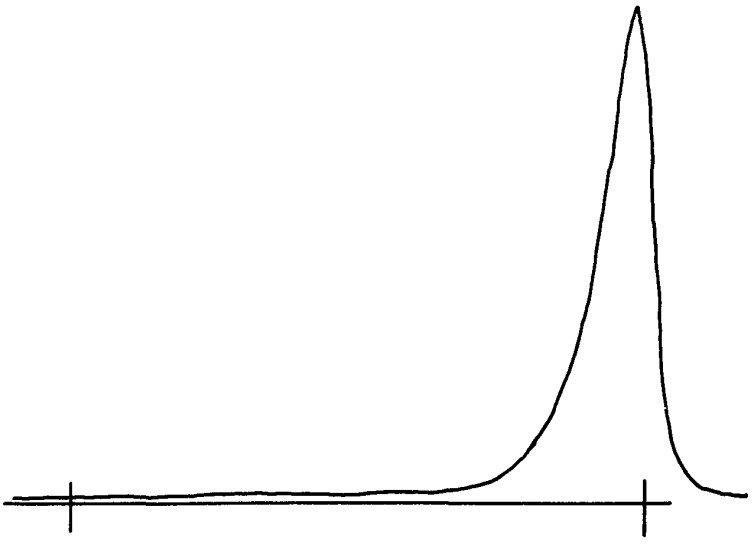
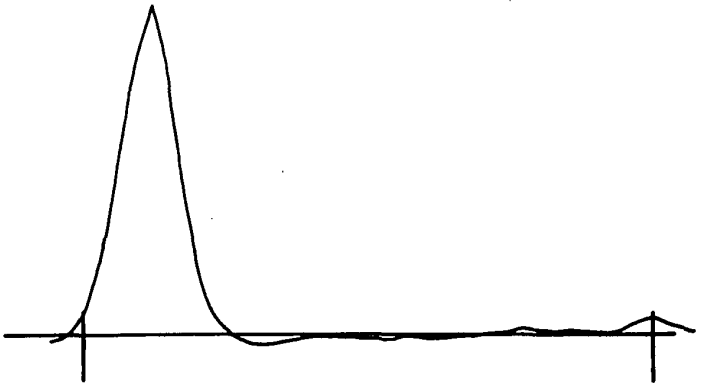


Figure 6. Chart showing radioactivity in chromatograms. Nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom) without plants after 7 days.



plants were growing showed very slight evidence, if any, of degradation after 3 days of exposure (Figure 7) but 3 metabolites besides the parent triazines appeared as peaks in the chromatogram chart after 7 days from start of treatment (Figure 8).

The charts shown in Figures 4 to 12 were traced from those made with a Packard Radiogram Scanner. Settings for the standards (Figures 4 and 5) were as follows: Scale - 1000, Slit - 2.5 mm, Time Constant - 100, Chart Speed - 0.5 cm/min. Settings for all other samples (Figures 6 to 12) were the same as that of the standards except for scale which was reduced to 300.

In looking at the charts, the 2 vertical half-inch lines indicate the origin or spots where the samples was spotted (right) and end of the solvent front (left). Rf values were obtained by dividing the distance between origin and location of radioactivity by the distance between origin and solvent front (distance between the 2 vertical lines on the chart).

Rf values for ametryne standard ranged from .90 to .92, atrazine from .91 to .93 and hydroxyatrazine from 0 to .02. Extracts of banana plants and nutrient solutions receiving ametryne treatments gave the following chromatogram Rf values (ranges of 4 peaks obtained): .89 to .93, .47 to 50, .32 to .38, and 0 to .02. Those that received atrazine treatments gave the following Rf values (ranges for 4 spots detected): .89 to 93, .47 to .50, .32 to .38, and 0 to .02. The charts presented should not be used to estimate amounts of C^{14} compounds in the samples corresponding to the sizes of the peaks in the chromatogram charts. Rather, the charts can be used only to compare relative amounts or proportion of

Figure 7. Chart showing radioactivity in chromatograms. Nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom). Banana plants were grown in the solution for 3 days.

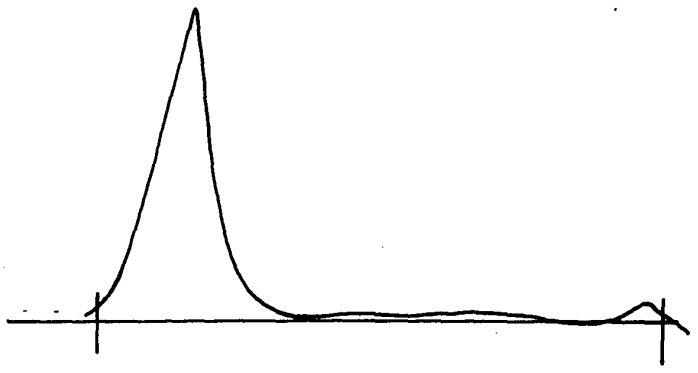
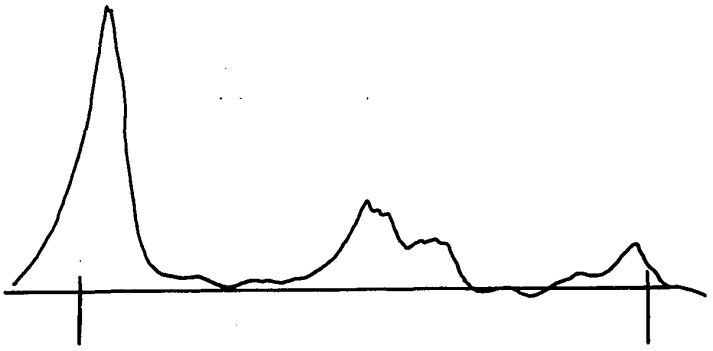


Figure 8. Chart showing radioactivity in chromatograms. Nutrient solution containing C^{14}-ametryne. Banana plants grown in the nutrient solution for 7 days in open shade (top) and sun (bottom).



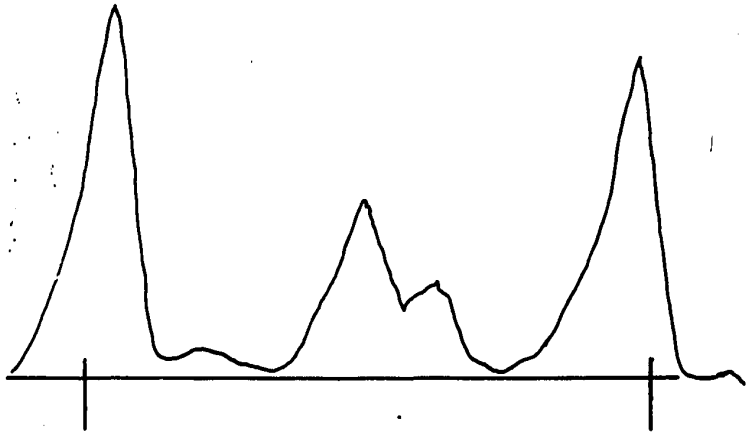


Figure 9. Chart showing radioactivity in chromatograms. Leaf extracts of banana plants grown for 3 days in nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom).

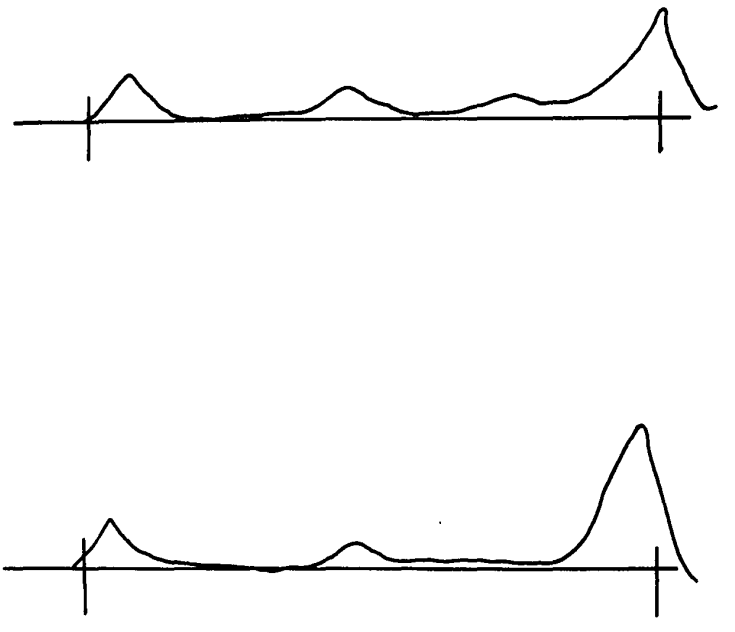
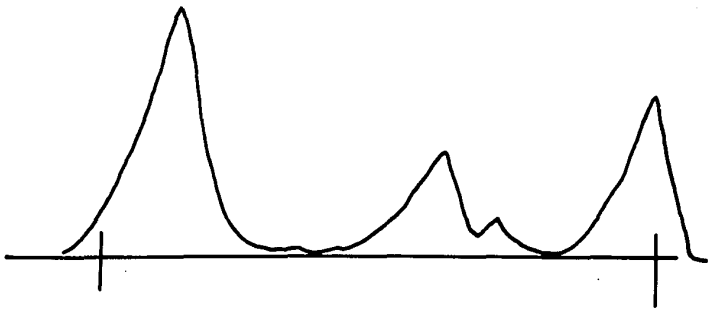


Figure 10. Chart showing radioactivity in chromatograms. Root extracts of banana plants grown for 3 days in nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom).



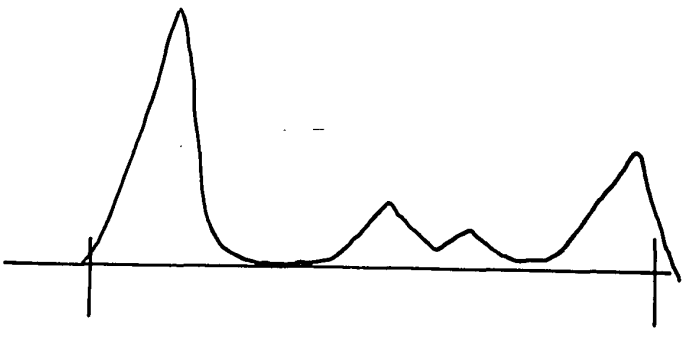


Figure 11. Chart showing radioactivity in chromatograms. Leaf extracts of banana plants grown for 7 days in nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom).

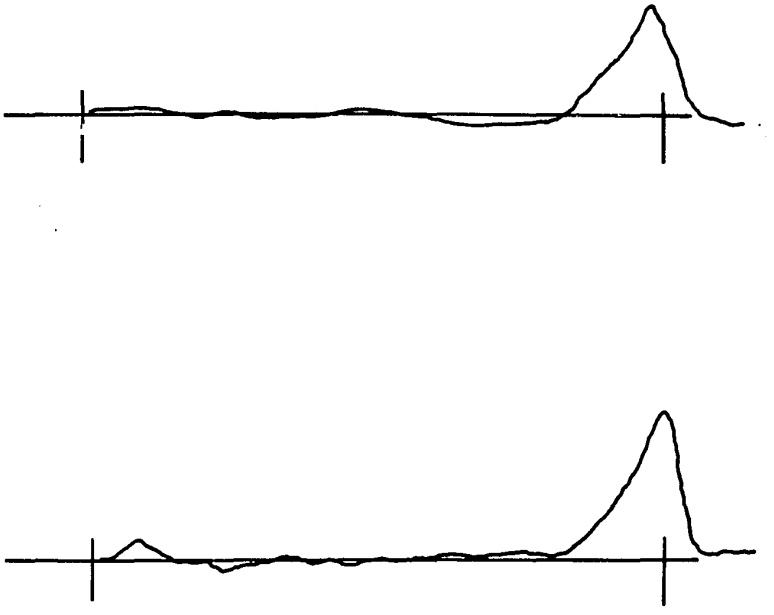
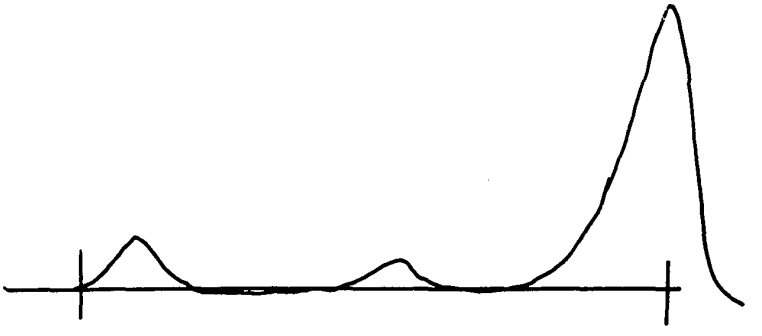
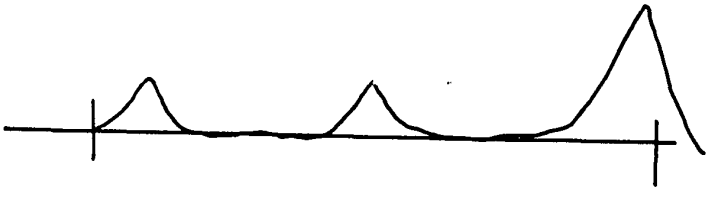


Figure 12. Chart showing radioactivity in chromatograms. Root extracts of banana plants grown for 7 days in nutrient solution containing C^{14}-ametryne (top) and atrazine (bottom).





each metabolite in relation to others found in the same chromatogram. Because of low values of some metabolites, in some samples their corresponding extracts were evaporated to near dryness and used for chromatography using as much of the sample as possible. Obviously, it was rather difficult to spot a consistently proportionate amount.

A comparison of the degradation of the nutrient solution around the plant roots was made between plants grown in sun and shade at 7 days after treatment. The charts for ametryne (Figure 8) showed the same metabolites except that there was a greater proportion of the compound corresponding to the hydroxyl derivative in chromatograms from the nutrient solution with plants grown in the sun. The trend was similar for the atrazine treatment.

Extracts of plants showed a much higher degree of degradation of the applied herbicides than occurred in the ambient solution surrounding the roots. Leaf extracts of 3-day treated plants showed that the predominant metabolite was the hydroxy derivative. The parent compound was also present together with one other metabolite (Figure 9). This was true for either ametryne or atrazine. After 7 days only the hydroxyl compound was present in chromatograms of leaf extracts from ametryne and atrazine treatments (Figure 11).

Root extracts of plants exposed to the herbicides for 3 days showed the predominance of the parent compound while the hydroxy compound and the 2 other metabolites were present in approximately equal proportions but less than the parent compounds in both ametryne and atrazine treatments (Figure 10). The proportions shifted to the hydroxy derivative the greatest and less of the parent compounds and one other metabolite

after 7 days of treatment (Figure 12). One metabolite, present in 3 day treated plants was not detected after 7 days of treatment.

Root extracts after 7 days showed a greater proportion of the hydroxy derivative in relation to the parent compound and another metabolite in the case of ametryne treatment when compared with root extracts of atrazine treated plants.

The results obtained are typical and readily reproduced under the conditions used. The only difficulty encountered was in showing metabolites other than the hydroxy or parent compounds in leaf extracts. No doubt detection was not easily obtained because of the trace amounts of these metabolites. Loading the chromatogram with extracts resulted in accumulation of pigments that affected separation. The small quantities of breakdown products from a small quantity of a parent compound applied to plants make often difficult or impossible to isolate and identify compounds from a living system that has the ability to disperse organic molecules (55).

In both measurements and extraction of herbicides, 3 replicates were made. The results presented herein were based on the first 2 replicates of each treatment. Several trials were carried out before an experiment was initiated to ascertain that the results were reproducible and predictable. The experiments reported herein were planned and initially based on earlier findings.

Although interesting to point out differences favoring the faster metabolism of ametryne than atrazine, a conclusion that this is so must be taken with reservation. For instance Figure 12 shows a higher proportion of the hydroxy derivative of ametryne than atrazine in root

extracts of 7-day treated plants. However, no differences in degradation rate was observed in 3-day treated plants or in solutions with or without plants growing.

Hilton (39) found sugarcane to metabolize ametryne and atrazine to 3 compounds within a period of hours. He suggested that these other 2 metabolites, besides the hydroxy derivative, were further degradation products of hydroxyatrazine, based on sequence and rate of formation of these metabolites.

The metabolites found in bananas, other than the hydroxy derivative, were not identified due to the absence of reference standards.

The presence of metabolites other than the hydroxyl compound is interesting in view of experiments in plants (67) and microorganisms (cited in 24) that metabolites resulting from dealkylation of the triazines have been detected.

The results of these experiments raise interesting question as regards residue and clearance problems. Most of the procedures reported for the analysis of triazines (1, 44, 45, 46, 59, 75) are specific for the parent compounds. A sample containing breakdown products may show no residue for the compound being analyzed using some of the methods described.

SECTION B

THE INFLUENCE OF AMETRYNE AND ATRAZINE ON THE NITROGEN CONSTITUENTS OF DWARF CAVENDISH BANANA PLANTS

MATERIALS AND METHODS

An initial symptom of atrazine injury to banana plants is chlorosis indicating a lack of normal formation of chlorophyll or rapid breakdown of existing chlorophyll. In field trials plants treated with ametryne seemed greener than untreated plants.

Gramlich (24) suggested that the chlorotic appearance of seedling plants emerging from soil treated with monuron, a phenylurea herbicide, indicates interference with nitrogen metabolism. The s-triazines closely resemble the phenylureas in adsorption and translocation as well as ability to cause inhibition of photosynthesis (13, 24). In view of these evidences, it seems that the s-triazines might affect the N metabolism of plants.

The present experiment was conducted in an attempt to determine the effect of ametryne and atrazine on the N constituents of banana plants adequately supplied with soil N. It is hoped that the results may contribute to the fundamental knowledge of the mode(s) of action of the s-triazine herbicides.

Field Experiments

Leaf samples of banana plants treated with ametryne and atrazine at 8 lb active/A were analyzed for N constituents. The leaf samples were obtained August 21, 1965, from an experiment which was in progress at the Poamoho Experimental Station. The details of the experiment have been reported by Romanowski et al (64). Since symptoms of injury in-

variably start from older leaves, both mature and immature leaves were obtained from the same plants 9 weeks after soil application. Immature leaf samples consisted of the youngest fully opened leaf and mature leaf samples consisted of the 7th youngest leaf. Samples from 4 replicates were labelled, placed in plastic bags and subsequently chopped with a Buffalo meat chopper located at the Experiment Station of the Hawaiian Sugar Planters' Association. Twenty grams were obtained from each freshly chopped sample and oven dried for moisture content determinations. Total N was determined using the moisture samples after drying. Another 10 gram fresh sample was obtained from the same composite sample for determination of alcohol soluble N.

Greenhouse Pot Experiments

To substantiate the results obtained from field grown plants, a series of plants were grown in gallon size glazed pots using Manoa clay loam-compost (15:1 v/v) mixture. Pots which contained 10 inch plants were treated with 0, 2, and 10 lb/A active equivalent of ametryne and atrazine. The desired amount of herbicide was dissolved in 50 per cent ethanol and each pot received 10 ml of the solution. Check treatments also received 10 ml of the solvent. The herbicide solution was applied with a pipette around the plant and sufficient water was added to saturate the soil. The drain holes were plugged with rubber stoppers to prevent leaching of the herbicides and nutrients. Thereafter, using a weighing method, watering was done daily to a field capacity. Distilled water was used to avoid accumulation of soluble salts. A week before the treatments were started the plants were supplied with the equivalent of 100 lb/A N from ammonium sulfate. The experiment was replicated 4 times

in a completely randomized arrangement of treatments on a greenhouse bench. The position of each pot was rearranged daily at random during watering.

Leaf samples were obtained 1 day, 1 week and 4 weeks after date of treatment. The second and third fully opened leaves, count starting with the first fully opened leaf, were obtained at each sampling date. Samples were weighed and immediately dried in the oven at 70 C for 24 hours. Dried samples were weighed and ground in a Wiley Mill to pass a No. 30 mesh screen.

Alcohol soluble (PN) and non-alcohol soluble (NPN) nitrogen contents were obtained from one sample. Total N was the summation of these values. Another sample from the same composite sample was used for determination of free ammonia and free nitrate N. N remaining after removal of these soluble N fractions were also analyzed and designated as residue N. The sum of these constituted the total recoverable N analysis of a sample. Detailed methods for analysis are described in the Appendix Section.

Leaf punches were obtained from the second fully opened leaves 4 weeks after herbicide treatment and analyzed for chlorophyll content.

RESULTS AND DISCUSSION

Field Experiments

The results of the total N content of field grown banana plants are shown in Table 9 (Table 9-a). The data indicate that there were no appreciable differences in total N contents as influenced by herbicide treatments. Immature leaves showed slightly higher total N content than mature leaves but the differences between treatments were negligible. Atrazine treated plants had higher non protein N (NPN) than either control or ametryne treated plants and this corresponded to the visual phytotoxic symptoms of the herbicides. Mature leaves from atrazine treated plants were significantly different than the other treatments. The higher NPN values existing among atrazine treated plants is perhaps due to a reduction in protein formation resulting from the injurious effects of the herbicide.

Greenhouse Pot Experiments

Leaf samples from the control treatment showed a higher percentage of ammonia at 1 day after treatment than the triazine treated plants (Table 10) (Table 10-a). Only the ammonia values differed considerably at the 1 day samplings.

One week after treatment drastic differences in free ammonia and nitrate contents were observed (Table 10). Check samples and the low rate of ametryne treated plants had a decidedly lower free ammonia content than the other treatments. Both treatments did not show any signs of herbicidal toxicity. Free nitrate exhibited a similar trend in that both treatments had lower percentage nitrate contents than the injured plants. No appreciable differences could be detected in percent total PN among plants 1 week after herbicide treatment.

Table 9. Total and non-protein N content of banana leaves 9 weeks after soil treatment with 8 lb/A ametryne and atrazine.[1]

Treatment	Age of Leaves	Total N	NPN[2]
Check	Immature	2.39	.20[b]
	Mature	2.07	.15[b]
Ametryne 8 lb/A	Immature	2.26	.18[b]
	Mature	2.10	.17[b]
Atrazine 8 lb/A	Immature	2.43	.23[ab]
	Mature	2.23	.27[a]

[1] Values average of 4 replications.

[2] Means not followed by a common letter are statistically different at Duncan's Multiple Range Test 5% Level.

Table 9-a. Analysis of variance for NPN, Table 9.

Source of Variation	df	MS[1/]
Treatment	5	.00857*
Replication	3	.00232
Error	15	.0023
Total	23	

[1/] *Significant at the 5% level (F-test).

Table 10. Free ammonia, free nitrate and total N content of banana leaves treated with soil applications of 2 and 10 lb/A of ametryne and atrazine.[1]

Treatment	1 Day after Treatment				1 Week after Treatment				4 Weeks after Treatment[2]			
	NH_4	NO_3	N Residue	Total N	NH_4	NO_3	N Residue	Total N	NH_4	NO_3	N Residue	Total N
Check	.072	.033	2.188	2.293	.051	.088	2.553	2.691	.066[c]	.067[c]	2.665[a]	2.798[b]
Ametryne 2 lb/A	.026	.037	2.532	2.595	.064	.106	2.685	2.854	.067[c]	.100[c]	2.826[a]	2.993[a]
Ametryne 10 lb/A	.021	.025	2.270	2.316	.113	.101	2.618	2.832	.168[b]	.383[a]	2.384[bcd]	2.935[ab]
Atrazine 2 lb/A	.046	.053	2.370	2.469	.102	.122	2.606	2.830	.170[b]	.266[b]	2.356[cd]	2.790[b]
Atrazine 10 lb/A	.029	.034	2.366	2.429	.139	.154	2.461	2.754	.229[a]	.343[a]	2.252[d]	2.823[b]

[1]Values average of 4 replications.

[2]Means not followed by a common letter are statistically different at Duncan's Multiple Range Test 5% Level.

Table 10-a. Analysis of variance for Table 10, 4 weeks after treatment.

Source of Variation	df	MS[1]			
		NH_4	NO_3	N Residue	Total N
Treatment	4	.0203**	.0109*	.230*	.047*
Error	15	.0007	.0020	.059	.012
Total	19				

[1]*Significant at the 5% level (F-test) and ** 1% level.

Four weeks after treatment the plants were analyzed for chlorophyll content to give an indication of the condition of the plants (Table 11) (Table 11-a). In all cases check plants and those receiving 2 lb/A ametryne were more vigorous in growth than those receiving other treatments. Stunting effect of the lower rate of atrazine and higher rate of ametryne were noticeable, while plants receiving the higher rate of atrazine exhibited drying of leaves and no additional growth. The values for per cent chlorophyll correlated well with the observed visual symptoms.

Values for N analysis from plants 4 weeks after treatments are presented in Table 10. Check and 2 lb/A ametryne treated plants had approximately the same values for free ammonia and free nitrate as the 1 week samples. Both ammonia and nitrate contents of the other treatments increased over that of samples obtained from the same plants 1 week after treatment. At the 4 week sampling, check and 2 lb/A ametryne treatments had a significantly lower free ammonia and free nitrate content than any other treatment.

An inverse relationship was obtained in values of N (residue N) obtained from the same tissue after removal of free ammonia and free nitrate. The differences were statistically significant being highest in the low ametryne and check treatments. The results indicated that ametryne and atrazine treatments which were phytotoxic to the plants resulted in higher concentrations of free N ions which indicates a block in the incorporation of these components into plant proteins. Perhaps, it is a consequence of reduced ability of the plants to utilize these nitrogen fractions as a result of injury by the herbicides in question, hence a secondary effect. It is interesting to note, however, that

Table 11. Per cent chlorophyll content of banana leaves, 4 weeks after treatment with ametryne and atrazine.

Treatment	Per Cent Chlorophyll[1/]
Check	.041[a2/]
Ametryne 2 lb/A	.041[a]
Ametryne 10 lb/A	.018[b]
Atrazine 2 lb/A	.021[b]
Atrazine 10 lb/A	.014[b]

1/Values average of 4 replications.

2/Means not followed by a common letter are statistically different at Duncan's Multiple Range Test 5% Level.

Table 11-a. Analysis of variance for Table 11.

Source of Variation	df	MS[1/]
Treatment	15	.000667**
Error	4	.0000185
Total	19	

[1/]**Significant at the 1% level (F-test).

changes approximate phytotoxic injuries of plants as observed visually. Slight differences were observed in the TN contents of the treated samples. There appeared to be a higher per cent TN among samples from ametryne treated plants.

Analyses for PN and NPN are presented in Table 12 (Table 12-a). Per cent constituents of the samples 1 day after treatment appear to be without apparent differences. Differences begun to appear 1 week after treatment and the trend continued with the samples taken 4 weeks after treatment. When considering TN values 4 weeks after treatment (Table 12), there were no appreciate differences in per cent TN between treatments; however, the 2 lb/A ametryne treatment gave a significantly higher per cent N than values obtained from check and atrazine treated plants (Table 10).

The NPN values showed a different trend in that each value was significantly different from the other when tested with Duncan's Multiple Range Test. Check plants contained the lowest amount of NPN followed by 2 lb/A ametryne, 2 lb/A atrazine, 10 lb/A ametryne and 10 lb/A atrazine, respectively. These values support previous speculations based on the analysis of free ammonia and free nitrate mainly, that herbicidal injuries may be due to a reduced capacity of the plant to incorporate N into protein constituents. This is further supported by values for PN where check and 2 lb/A ametryne treated plants contained a higher percentage of PN compared to the rest of the treatments.

On a percentage basis the TN contents were similar. Although TN seemed to be unaffected, protein utilization of non-injured plants was expected to be more efficient. Because of reduced growth, as mentioned by Audus (8), affected plants may actually show a higher per cent of N but not a higher rate of protein synthesis or total protein synthesized.

Table 12. Per cent protein and non-protein N content of banana leaves treated with soil applications of 2 and 10 lb/A of ametryne and atrazine.[1]

Treatment	1 Day after Treatment			1 Week after Treatment			4 Weeks after Treatment		
	NPN	PN	TN	NPN	PN	TN	NPN	PN	TN
Check	.309	1.938	2.247	.326	2.727	3.053	.334e[2]	2.538a	2.872
Ametryne 2 lb/A	.230	2.209	2.439	.445	2.916	3.361	.391d	2.702a	3.093
Ametryne 10 lb/A	.219	2.139	2.358	.492	2.419	2.911	.798a	2.186b	2.984
Atrazine 2 lb/A	.274	2.174	2.448	.546	2.566	3.112	.605c	2.209b	2.814
Atrazine 10 lb/A	.252	2.173	2.425	.596	2.513	3.109	.787b	2.051b	2.838

[1] Values average of 4 replications.

[2] Means not followed by a common letter are statistically different at Duncan's Multiple Range Test 5% Level.

Table 12-a. Analysis of variance for Table 12, 4 weeks after treatment.

Source of Variation	df	MS[1]	
		NPN	PN
Treatment	4	.187**	.2942*
Error	15	.002	.0108
Total	19		

[1] *Significant at the 5% level (F-test) and **1% level.

In amitrole treated plants McWhorter (48) reported an increase of free ammonia and total N, also the protein content of chlorotic corn tissue was usually reduced whereas free amino acids were increased.

SUMMARY

In herbicide screening trials for bananas in Hawaii ametryne was found superior to atrazine in terms of initial effect and duration of weed control. Atrazine injured the crop in 2 of 3 locations studied while no injury was observed with ametryne treatments.

Greenhouse and laboratory studies were initiated to determine causes of this selectivity. A separate investigation was conducted to relate herbicidal action of the s-triazines with changes in N constituents of treated plants.

Although injury to bananas by atrazine was greater than by ametryne in greenhouse soil experiments at any given concentration of 4, 16 and 40 ppmw (oven dried soil basis), injury by ametryne was not totally absent even at the lowest concentration. The values obtained indicate that pot conditions modified the soil effect quite differently from that obtained in the field where injury to ametryne was not observed.

The similarity in phytotoxicity of ametryne and atrazine to plants grown in sand culture with nutrient solution containing 0, 0.4, 4, and 16 ppm of the herbicides shows that banana plants cannot metabolize ametryne to a degree that would render the herbicides nontoxic as was previously suspected. The results obtained in the sand culture experiments seem to indicate strongly that soil characteristics may be the dominant factors in the differential selectivity of plants to ametryne and atrazine in the field.

Laboratory studies showed that selectivity is attributed at least in part to differences in adsorption and leaching as influenced by soil factors. According to Upchurch (72) when tolerance of the crop is in-

herently poor, selectivity can be obtained by proper placement of the herbicide where roots of weeds and crops are differentially located. Under field conditions, the majority of the roots of a banana plant are distributed several inches below the surface. Leaching studies showed that ametryne was not readily moved to sub-surface layers of the soil by applied moisture. Injury to pot grown plants by ametryne was at least partially due to the fact that roots occupied the whole portion of the soil including the top inch layer where herbicide was applied.

Laboratory studies using C^{14}-labelled ametryne and atrazine treated plants confirmed results of greenhouse experiments that differences in absorption, translocation and metabolism of the herbicides did not explain selectivity of banana plants. The activities of the two herbicides were similar. Absorption of the herbicides by roots was observed in 45 minutes and gradual translocation followed so that labelled C^{14} was distributed throughout the plant after 24 hours. Uptake up to 7 days did not increase appreciably and treatment (1.79 ppm in nutrient solution) resulted in reduced transpiration. Uptake of the herbicides was not directly related to the amount of water transpired.

Plants degraded a portion of the herbicide to the hydroxy derivative and two other unidentified metabolites in 3 days. Degradation increased up to 7 days at which time only the hydroxy analog was readily detectable in leaf extracts. A greater ratio of the metabolites to the parent compound was detected in plant extracts than in nutrient solution around banana roots. Degradation did not occur in nutrient solution without plants in 7 days.

Ametryne and atrazine at 8 lb/A did not influence the per cent protein nitrogen (PN) and total N (TN) content of banana leaves from the experimental orchard at Poamoho. Atrazine treatment increased NPN content of leaves.

Leaf samples were obtained from pot grown plants treated with ametryne (0, 2 and 10 lb/A) and atrazine (2 and 10 lb/A) at 1 day, 1 week, and 2 weeks for analysis of N constituents. No appreciable differences were obtained at the 1 day sampling. Results were similar at the 1-week and 4-week samplings and results of the latter were statistically compared. At this period chlorophyll content of leaves of check and 2 lb/A ametryne treatments were higher than the others. These treatments did not exhibit injury to the banana plants. At the 4-week sampling, check and 2 lb/A treatments had lower free ammonia and free nitrate contents than any of the other treatments. An inverse relationship was observed in values of N obtained from the same tissue after removal of free N. The results indicate that phytotoxic concentrations of ametryne and atrazine resulted in higher free N suggesting a disturbance in the incorporation of these compounds to more complex N containing plant constituents as proteins. This speculation is further supported by analysis of NPN which gave higher values with plants receiving phytotoxic concentrations of the herbicides.

APPENDIX

(Tables 1-8)

Appendix Table 1. Micrograms of ametryne and atrazine adsorbed per gram of soil (Detailed observations for Table 4).

Soil	Herbicide	Replication[1/]				Average
		1	2	3	4	
Kauai	Ametryne	4.93	4.90	4.87	4.87	4.90
	Atrazine	1.78	1.79	1.75	1.76	1.77
Waimanalo	Ametryne	3.58	3.55	3.46	3.42	3.50
	Atrazine	1.11	1.10	1.10	1.11	1.11
Poamoho	Ametryne	2.82	2.80	2.82	2.82	2.82
	Atrazine	0.94	0.91	0.99	0.91	0.94

[1/] Each value an average of 2 determinations.

Appendix Table 2a. Per cent ametryne and atrazine recovered by leaching 6" column of 100 grams of soil with 12" water (Detailed observations for Table 5).

Soil	Herbicide	Replication			Average
		1	2	3	
Kauai	Ametryne	.35	.27	.31	.31
	Atrazine	25.78	26.39	26.76	26.31
Waimanalo	Ametryne	30.70	27.99	29.40	29.36
	Atrazine	59.76	61.64	60.50	60.63
Poamoho	Ametryne	34.62	36.04	36.96	35.87
	Atrazine	78.69	76.35	79.06	78.03

Appendix Table 2b. Per cent ametryne and atrazine recovered by leaching 6" column of 100 grams of soil with 24" water (Detailed observations for Table 5).

Soil	Herbicide	Replication			Average
		1	2	3	
Kauai	Ametryne	6.12	4.68	5.28	5.36
	Atrazine	48.95	50.72	51.28	50.32
Waimanalo	Ametryne	55.88	53.00	54.54	54.47
	Atrazine	77.49	80.77	79.01	79.19
Poamoho	Ametryne	66.12	68.29	70.61	68.34
	Atrazine	93.60	91.58	95.63	93.60

Appendix Table 3. Per cent free ammonia and free nitrate N content of banana leaves 1 day after soil treatment with ametryne and atrazine.[1/] (Detailed observations for Table 10.)

Treatment	Rep.	NH_4	NO_3	Residual N	TN
Check	1	.063	.028	2.208	2.299
	2	.075	.041	2.360	2.486
	3	.077	.033	2.263	2.363
	4	.072	.031	1.919	2.022
	Ave.	.072	.033	2.188	2.293
Ametryne 2 lb/A	1	.021	.033	2.238	2.292
	2	.032	.030	2.846	2.908
	3	.030	.047	2.314	2.391
	4	.021	.038	2.729	2.788
	Ave.	.026	.037	2.532	2.595
Ametryne 10 lb/A	1	.021	.028	2.241	2.290
	2	.020	.023	2.314	2.457
	3	.024	.023	2.070	2.020
	4	.019	.025	2.454	2.495
	Ave.	.021	.025	2.270	2.316
Atrazine 2 lb/A	1	.053	.069	2.537	2.669
	2	.049	.046	2.256	2.216
	3	.042	.054	2.426	2.512
	4	.038	.045	2.261	2.434
	Ave.	.046	.053	2.370	2.469
Atrazine 10 lb/A	1	.028	.030	2.366	2.134
	2	.035	.033	2.579	2.647
	3	.026	.036	2.459	2.811
	4	.028	.036	2.060	2.124
	Ave.	.029	.034	2.366	2.429

[1/] Per cent oven dried basis. Each value an average of 2 determinations.

Appendix Table 4. Per cent free ammonia and free nitrate N content of banana leaves 1 week after soil treatment with ametryne and atrazine.[1/] (Detailed observations for Table 10.)

Treatment	Rep.	NH_4	NO_3	Residual N	TN
Check	1	.062	.108	2.740	2.910
	2	.048	.081	2.599	2.728
	3	.054	.086	2.639	2.779
	4	.040	.075	2.232	2.347
	Ave.	.051	.088	2.553	2.691
Ametryne 2 lb/A	1	.063	.111	2.714	2.888
	2	.075	.112	2.645	2.832
	3	.045	.108	2.720	2.873
	4	.071	.092	2.659	2.822
	Ave.	.064	.106	2.685	2.854
Ametryne 10 lb/A	1	.106	.091	2.714	2.911
	2	.097	.105	2.554	2.756
	3	.132	.098	2.614	2.844
	4	.117	.110	2.588	2.815
	Ave.	.113	.101	2.618	2.832
Atrazine 2 lb/A	1	.080	.122	2.549	2.751
	2	.114	.140	2.775	3.029
	3	.120	.115	2.559	2.797
	4	.093	.110	2.540	2.743
	Ave.	.102	.122	2.606	2.830
Atrazine 10 lb/A	1	.126	.136	2.475	2.737
	2	.144	.158	2.526	2.828
	3	.162	.172	2.536	2.870
	4	.125	.151	2.306	2.582
	Ave.	.139	.154	2.461	2.754

[1/] Per cent oven dried basis. Each value an average of 2 determinations.

Appendix Table 5. Per cent free ammonia and free nitrate N content of banana leaves 4 weeks after soil treatment with ametryne and atrazine.[1/] (Detailed observations for Table 10.)

Treatment	Rep.	NH_4	NO_3	Residual N	TN
Check	1	.084	.089	2.734	2.907
	2	.053	.056	2.236	2.345
	3	.075	.047	2.936	3.058
	4	.053	.077	2.753	2.883
	Ave.	.066	.067	2.665	2.798
Ametryne 2 lb/A	1	.067	.107	2.817	2.991
	2	.066	.101	3.205	3.372
	3	.060	.091	2.734	2.885
	4	.075	.100	2.548	2.723
	Ave.	.067	.100	2.826	2.993
Ametryne 10 lb/A	1	.119	.293	2.683	3.095
	2	.203	.459	2.365	3.027
	3	.144	.399	2.254	2.797
	4	.205	.378	2.232	2.835
	Ave.	.168	.383	2.384	2.935
Atrazine 2 lb/A	1	.195	.263	2.389	2.847
	2	.187	.239	2.538	2.964
	3	.123	.292	2.487	2.902
	4	.173	.268	2.008	2.449
	Ave.	.170	.266	2.356	2.790
Atrazine 10 lb/A	1	.233	.344	2.048	2.625
	2	.214	.328	2.430	2.972
	3	.244	.354	2.317	2.915
	4	.223	.345	2.212	2.780
	Ave.	.229	.343	2.252	2.823

[1/] Per cent oven dried basis. Each value an average of 2 determinations.

Appendix Table 6. Per cent protein and non-protein N content of banana leaves 1 day after soil treatment with ametryne and atrazine.[1] (Detailed observations for Table 12.)

Check	1	.316	2.061	2.377
	2	.368	2.061	2.429
	3	.333	2.068	2.401
	4	.219	1.560	1.779
	Ave.	.309	1.938	2.247
Ametryne 2 lb/A	1	.222	1.934	2.156
	2	.229	2.409	2.638
	3	.265	2.488	2.753
	4	.207	2.004	2.211
	Ave.	.230	2.209	2.439
Ametryne 10 lb/A	1	.295	1.971	2.276
	2	.232	2.314	2.546
	3	.225	1.945	2.168
	4	.217	2.324	2.541
	Ave.	.219	2.139	2.358
Atrazine 2 lb/A	1	.309	2.644	2.953
	2	.264	1.807	2.071
	3	.302	2.144	2.446
	4	.220	2.100	2.320
	Ave.	.274	2.174	2.448
Atrazine 10 lb/A	1	.239	2.000	2.239
	2	.289	2.398	2.687
	3	.245	2.526	2.501
	4	.235	1.767	2.272
	Ave.	.252	2.173	2.425

[1] Per cent oven dried basis. Each value an average of 2 determinations.

Appendix Table 7. Per cent protein and non-protein N content of banana leaves 1 week after soil treatment with ametryne and atrazine.[1/] (Detailed observations for Table 12.)

Treatment	Rep.	NPN	PN	TN
Check	1	.309	2.913	3.222
	2	.279	2.780	3.059
	3	.367	2.749	3.116
	4	.348	2.467	2.815
	Ave.	.326	2.727	3.053
Ametryne 2 lb/A	1	.436	2.721	3.157
	2	.447	2.822	3.269
	3	.440	3.212	4.252
	4	.456	2.909	2.765
	Ave.	.445	2.916	3.361
Ametryne 10 lb/A	1	.455	2.230	2.685
	2	.442	2.754	3.196
	3	.557	2.351	2.908
	4	.513	2.340	2.853
	Ave.	.492	2.419	2.911
Atrazine 2 lb/A	1	.585	2.308	2.893
	2	.541	2.585	3.126
	3	.507	2.877	3.384
	4	.551	2.495	3.046
	Ave.	.546	2.566	3.112
Atrazine 10 lb/A	1	.495	2.429	2.924
	2	.638	2.624	3.262
	3	.653	2.376	3.029
	4	.598	2.622	3.220
	Ave.	.596	2.513	3.109

[1/] Per cent oven dried basis. Each value an average of 2 determinations.

Appendix Table 8. Per cent protein and non-protein N content of banana leaves 4 weeks after soil treatment with ametryne and atrazine.[1] (Detailed observations for Table 12.)

Treatment	Rep.	NPN	PN	TN
Check	1	.304	2.561	2.865
	2	.372	2.670	3.042
	3	.355	2.422	2.777
	4	.305	2.500	2.805
	Ave.	.334	2.538	2.872
Ametryne 2 lb/A	1	.403	2.625	3.028
	2	.439	2.781	3.220
	3	.368	2.643	3.011
	4	.352	2.760	3.022
	Ave.	.391	2.702	3.093
Ametryne 10 lb/A	1	.748	2.300	3.048
	2	.792	2.157	2.949
	3	.831	2.168	2.999
	4	.819	2.121	2.940
	Ave.	.798	2.186	2.984
Atrazine 2 lb/A	1	.591	2.265	2.856
	2	.586	2.192	2.778
	3	.558	2.236	2.794
	4	.686	2.144	2.830
	Ave.	.605	2.209	2.814
Atrazine 10 lb/A	1	.786	1.882	2.668
	2	.731	2.258	2.989
	3	.877	2.108	2.985
	4	.752	1.959	2.711
	Ave.	.786	2.051	2.838

[1] Per cent oven dried basis. Each value an average of 2 determinations.

Analytical Methods for N Determinations

Field Experiments

Macro Kjeldahl Method for Total Nitrogen

The procedure used was a modification of the method used by Metson (1955). Approximately 500 mg oven dried samples were placed into a 500 ml Kjeldahl flask and moistened. Twenty ml of sulfuric acid-salicylic acid mixture (60:2 w/w) were added while rotating the flask. This was allowed to stand for about an hour with intermittent shaking and allowed to stand for several more hours or overnight. About 2 grams of sodium thiosulfate were added through a long stemmed funnel to prevent salt from adhering to the neck of the flask. The flask was heated gently for about 5 minutes until dense fumes appeared after which 4 grams of potassium sulfate mixture (2 parts K_2SO_4: 1 part $CuSO_4 \cdot 5H_2O$: 2 parts $FeSO_4$) were added through the special funnel. Two pieces of selenized (Hengar's) granules were added and the flask was heated until the solution was clear. Heating was continued for an hour to complete digestion. The flask was cooled and 150 ml water were added followed by 50 ml of 50% NaOH to make strongly alkaline. The flask was immediately connected to a distillation apparatus. The distillate was collected in a 100 ml volumetric flask containing 10 ml of 0.025 sulfuric acid until about 75 ml were collected. An aliquot was taken which approached the color of a standard containing 0.2 mg/100 ml when developed with Nessler's reagent. The aliquot was transferred to a 100 ml volumetric flask, made to about 50 ml and 5 ml of Nessler's reagent added while shaking. This was made to volume and read in a Klett Sommerson Colorimeter using a No. 54 (green filter) together with a known standard. The per cent N was computed based on this color comparison with a standard.

Preparation of Nessler's Reagent

Two hundred and forty-seven grams of potassium iodide were mixed in a liter of water. Saturated mercuric chloride were added slowly and cautiously to make a permanent bright red color (about 1.5 liters). The red precipitate was dissolved by adding exactly 3 grams of powdered potassium iodide. Next, 600 grams of potassium hydroxide dissolved in 1 liter of water were added. The volume was made to 4 liters with distilled water and mixed thoroughly. The mixture was allowed to stand several days before use.

Determination of Alcohol Soluble N

Ten gram fresh samples were obtained from the same composite samples which were used for total N analysis and placed in 125 ml flasks containing 50 ml of acidified 80% ethanol. These were heated in a steam bath for 10 minutes and allowed to stand for 1 week before blending with a Waring Blender. The mixture was filtered quantitatively using additional methanol and the filtrate was collected in a 100 ml volumetric flask. Twenty-five ml aliquots were used for the determination of alcohol extractable (non-protein) N and the residue after filtration was used to determine protein N following the method described for total N analysis. Aliquots from alcohol extraction were pipetted into 500 ml Kjeldahl flask containing 4 grams potassium sulfate and the mixture was evaporated in a steam bath. The residue was analyzed for nitrogen as described previously and % N computed on an oven dried basis.

Pot Experiments

Semi Micro Kjeldahl for Total N

The procedure was a modification of the method used by Gramlich (1965). The plant sample was placed in 300 ml Kjeldahl flask and moistened. Fifteen ml sulfuric acid-salicylic acid mixture were added while rotating the flask. The flask was rotated or shaken at intervals during a period of several hours and then left overnight. About 2 grams of sodium thiosulfate were added with a long necked funnel. The mixture was heated for about 5 minutes till dense white fumes appeared. About 4 grams of digestion mixture (20 parts K_2SO_4: 1 part $CuSO_4 \cdot 5H_2O$: 1 part $FeSO_4$) ground to pass a No. 30 mesh screen, were added through the long stemmed funnel together with 1 piece of Hengar's granule. The flask was heated until clear and digestion was continued for an additional hour. The mixture was cooled, diluted with 125 ml distilled water and 45 ml of 50% NaOH. The flask was immediately connected to a distillation apparatus and distillate collected in 100 ml volumetric flask containing 10 ml of 0.025 N sulfuric acid. Temperature of the distilling apparatus was controlled individually by a variable transformer. The distillate was made to volume after collecting about 80 ml and aliquot obtained for color development with Nessler's reagent as described in Macro Kjeldahl Method.

Determination of Alcohol Soluble N

Alcohol soluble N was extracted from approximately 200 mg of dried plant sample with 25 ml of acidified 80% ethanol by shaking the mixture in a flask, heating in a steam bath for 10 minutes and filtering quantitatively into a 50 ml volumetric flask. Additional methanol was used to wash the residue until 50 ml of the solution were collected. The entire

filtrate was analyzed by evaporating to about 5 ml in a 300 ml Kjeldahl flask containing about 2 grams potassium sulfate and analyzing for total N as previously described. The residue was analyzed by digesting it in a Kjeldahl flask together with the filter paper and proceeding with the analysis as previously described.

Determination of Free Ammonia and Free Nitrate Nitrogen

In another set of samples taken from the same composite sample used for the PN and NPN analyses, free ammonia and free nitrate were extracted with 4% boric acid solution by placing about 200 g dried sample in a 125 ml flask containing 25 ml boric acid extractant and shaking for 2 hours with a Burrell Wrist Action Shaker. This mixture was filtered into a Kjeldahl flask, washed with an additional 5 ml of distilled water and the filtrate analyzed for free ammonia and free nitrate.

1) Free ammonia: Five ml of 10 N NaOH were added into the Kjeldahl flask containing the filtrate and attached to a distilling apparatus. The distillate was collected into a 50 ml test tube receiver containing 5 ml of 0.025 N sulfuric acid. Two and one-half ml Nessler's reagent were added. The mixture was made to volume, and the color read together with a standard for comparison. Procedure is as previously described.

2) Free nitrate: After removal of ammonia N from the sample, the Kjeldahl flask was removed from the heater, cooled somewhat, and 200 mg of Devarda alloy (50 parts copper: 45 parts aluminum: 5 parts zinc) were rapidly added. The flask was rapidly attached to the distillation apparatus provided with a new receiver and the ammonia nitrogen liberated

was determined as previously described. The heat during distillation was carefully controlled by a variable transformer so that distillation took about 2 hours.

The plant residue after removal of free ammonia and free nitrate was determined for N as previously described.

Determination of Chlorophyll

About 1 gram fresh leaf punches were weighed, ground in a mortar and pestle with small amounts of calcium carbonate and quantitatively filtered. The filtrate was diluted to 50 ml volume and read in a Klett Sommerson Colorimeter using a No. 66 (red) filter. The values were compared to a standard curve and the amount of chlorophyll was determined.

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