The relative toxicity of three alkylamines and two alkanolamines to *Cryptostegia grandiflora* leaf discs

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SUMMARY

Leaf discs of rubber-vine (*Cryptostegia grandiflora* R. Br.) were floated on 0.1% aqueous solutions of dimethylamine, diethylamine, triethylamine, diethanolamine, and triethanolamine. The rates and extent of injury caused by the amines were determined as the change in impedance ratio (low frequency impedance/high frequency impedance) of the treated discs with time. Phytotoxicity decreased in the order dimethylamine>diethylamine = triethylamine>diethanolamine > triethanolamine. It is suggested that the observed differences arise from differences in the rate of penetration of the amines as well as from differences in toxicity per se.

INTRODUCTION

Cryptostegia grandiflora R. Br., known locally as rubber-vine, is a serious weed in Queensland north of 24°S latitude. The estimated area of infestation exceeds 121 410 ha, mainly along some 10 143 km of rivers and streams (Caltabiano, 1973).

The plant is toxic (Everist, 1974) but the main economic loss and nuisance value of the plant are due to mustering difficulties and restriction of access to water (Caltabiano, 1973).

Rubber-vine is susceptible to low concentrations of the phenoxyalkanoic acid herbicides (i.e. 2,4-dichlorophenoxyacetic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid) as esters in diesel distillate using basal bark application. However, higher concentrations of these same herbicides applied as over-all sprays of the amine salts in water are relatively ineffective (Queensland Lands Department reports, unpublished).

Compared with over-all spray applications, basal bark application is labour-intensive, time-consuming, and costly, and in the case of rubber-vine, often impractical. There is thus a need for more effective over-all spray formulations, either of new or old herbicides.

As the first step in a program to develop such formulations, a study has been made of the relative phytotoxicity of five common amines. Although these amines, particularly dimethylamine and triethanolamine, are commonly used in herbicide formulations, a search of the literature failed to reveal any earlier studies on the phytotoxicity of these compounds. However, a number of studies has been made of more complex amines, and substituted or unsubstituted amine groups are fairly common in pesticides.

MATERIALS AND METHODS

The five amines studied were dimethylamine (DMA), diethylamine (DEA), triethylamine (TEA), diethanolamine (DEOA), and triethanolamine (TEOA). All chemicals were B.D.H. laboratory reagent grade. From preliminary trials (see Figure 1) it was found that 0.1% solutions of these amines were suitable for the experimental procedure adopted; more concentrated solutions caused too rapid injury, and less concentrated solutions were too slow. Distilled water was included as a control.

Freshly cut leaf discs, 5 mm diameter, were floated, lower epidermis down, on 0.1% solutions of the amines in distilled water, in covered petri dishes. All experiments were performed in the laboratory at room temperature (approximately 20°C) and without supplementary lighting.

Samples of 10 discs were withdrawn from each solution at intervals of 1, 3, 6, 24, and 48 hours, and the impedance ratio of each disc determined using a direct reading variation of the wide-range a.c. bridge described by de Plater and Greenham (1959).

A balanced incomplete block design (Cochran and Cox, 1957) was adopted for the experiment as only three of the six treatments could be handled at one time. The mean of each set of 10 readings at each time was used as data, and the results for each time period were analysed by analysis of variance.

RESULTS

The results are depicted graphically in Figure 2 (lower values of the impedance ratio indicate greater injury (de Plater and Greenham, 1959)). DMA is clearly faster-acting and more injurious than the other amines; DEA and TEA are approximately equally phytotoxic, DEOA next, and TEOA least phytotoxic. This graphical picture is supported by the results of the statistical analysis (see Table 1).

Table 1. Split-plot balanced incomplete block analysis of variance regarding chemicals as main effects and time as subtreatments

Source of variation	Degree of freedom	Mean square
Blocks	9	30.70
Chemicals	5	234.73 **
Error (a)	15	4.59
Time	4	43.15 **
Chemicals x Time	20	4.30 **
Error (b)	95	0.64

** Statistically significant, P<0.01

Visual observation of the treated leaf discs supported the above results, although such observations were purely subjective and not quantitative. Discs affected by the treatments darkened appreciably, from the outer cut surfaces inwards with time. Injured

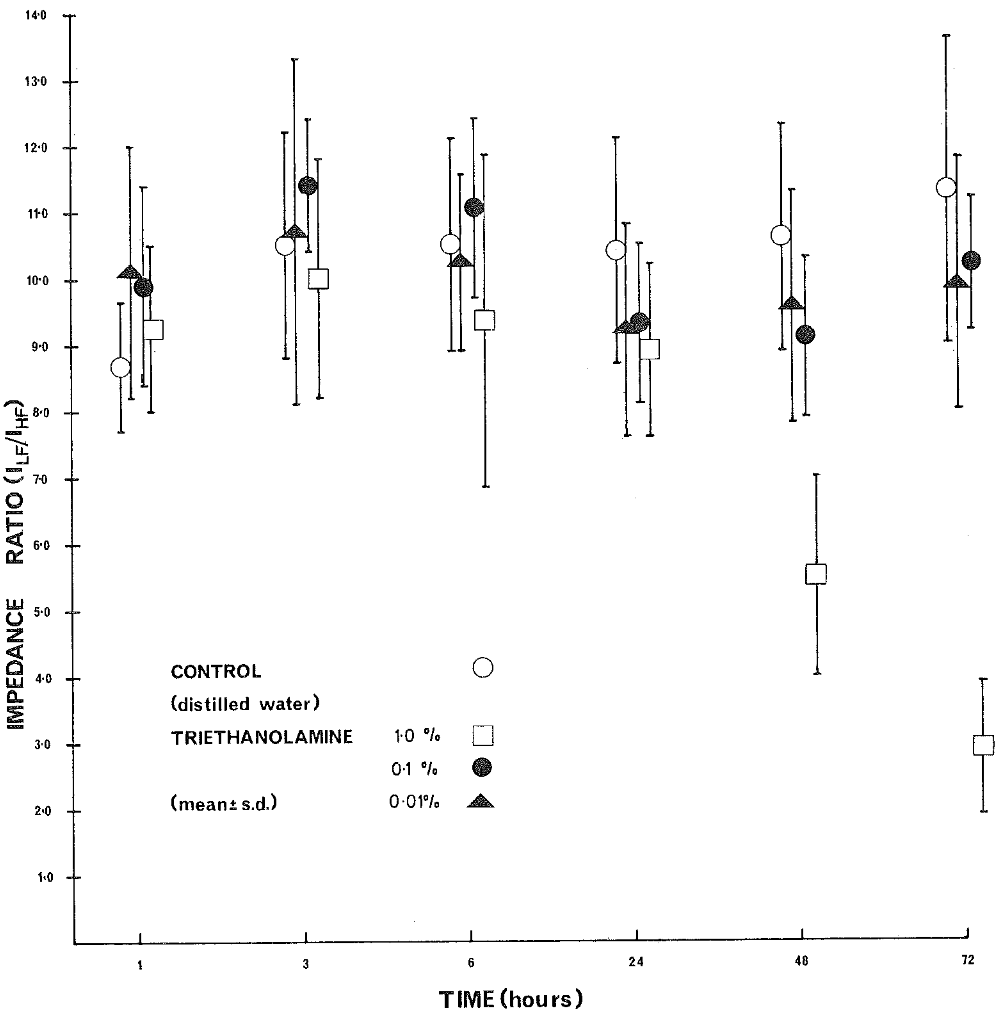

Figure 1. Impedance ratios of rubber vine leaf discs after treatment with triethanolamine at three concentrations. Each point represents the mean ± standard deviation of readings from 10 discs.

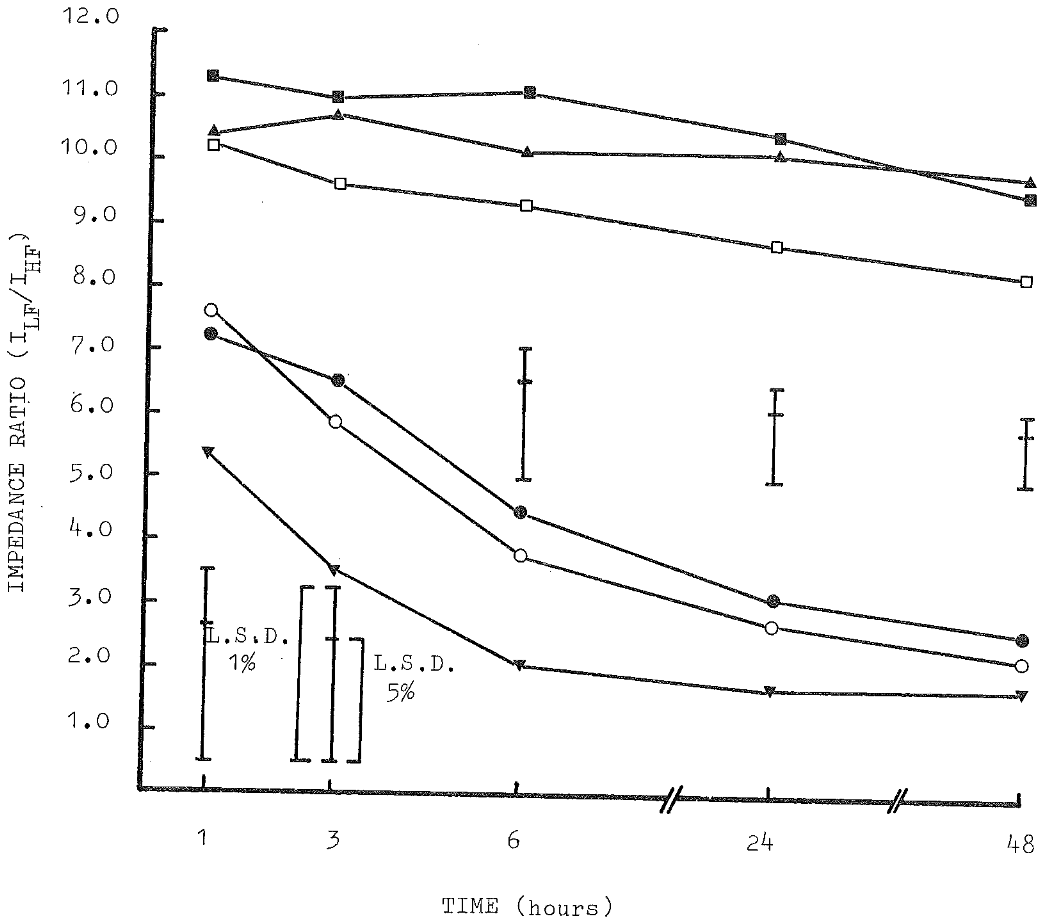

Figure 2. Impedance ratios of C. grandiflora leaf discs after treatment with distilled water (▲), triethanolamine (■), diethanolamine (□), dimethylamine (▼), diethylamine (●), and triethylamine (○). Each point the mean of 10 measurements.

discs assumed a water-logged appearance, as would be expected if the cell membranes were disrupted through injury, but no discs sank after any treatment (c.f. da Silva, Fadayomi, and Warren, 1976; Gawronski, Callihan, and Pavek, 1977). The control and TEOA-treated discs were still visually unaffected after 48 hours.

DISCUSSION

The absolute values of the impedance ratios reported here should be treated with caution. Although the impedance ratio should, theoretically, be unity at death, such a value is reached only under ideal conditions (Greenham, 1966). Bachelard and Ayling (1971), using Greenham's prototype machine, report resistance ratios of approximately unity for herbicide-treated *Pinus radiata* needle segments, but Greenham (1957) notes that the resistance ratio of a particular plant can vary from experiment to experiment, and comparisons of values should be restricted to comparisons of treatments and untreated controls within a single experiment.

The steady state value of 2 after 6 hours for the DMA-treated discs indicates that this is the minimum value of the impedance ratio for rubber-vine leaf discs under the particular conditions of this experiment, and that maximum injury (death?) occurred approximately 6 hours after treatment with DMA. The DEA- and TEA-treated discs approached this condition after 48 hours, while the DEOA-treated discs were only mildly affected, and the TEOA-treated discs were apparently unaffected, after this time.

The results are surprising, not because the amines proved phytotoxic, but because the extent of injury is so obviously related to chemical structure (see Table 2). There are two possible explanations for this - differences in toxicity per se. and/or differences arising from the rates of penetration of these amines.

Table 2. Chemical formulae, molecular weights, phytotoxicity and (mammalian) toxicity of the five amines used in the reported experiment

Chemical	Chemical formulae	Molecular* weight	Order of phytotoxicity after 1 hr [a]	Toxicity* (acute local)[b]
Triethanolamine	$(HOCH_2CH_2)_3N$	149	1	0
Diethanolamine	$(HCOH_2CH_2)_2NH$	105	2	1
Triethylamine	$(C_2H_5)_3N$	101	3	3
Diethylamine	$(C_2H_5)_2NH$	73	4	2-3
Dimethylamine	$(CH_3)_2NH$	45	5	2

* From Sax (1968)

a 1 = least phytotoxic 5 = most phytotoxic

b 0 = no toxicity 3 = severe toxicity

The order of phytotoxicity is strongly and inversely correlated with molecular weight, except for TEA, which is more toxic (after 3 hours or longer, but not initially) than DEA, though the corresponding molecular weights are 101 and 73, respectively. This suggests that the higher toxicity of the lower molecular weight amines may result from more rapid penetration of these substances into the plant cells (Stadelmann, 1969; Collander, 1959). However no association could be found between injury and nitrogen content of the rubber-vine leaf discs, owing to the variation in natural nitrogen content of the leaf discs with age during the course of the experiment.

Given, however, that differences in permeability of the leaf discs to the different amines do exist, the results indicate that there are also differences in toxicity per se between the amines. The consistently high readings for TEOA-treated discs, which suffered the least injury, suggest uptake of non-injurious solute from the medium. Phytotoxicity decreases in the order methylamine > ethylamines>ethanolamines, showing clearly that there is a relationship between phytotoxicity and chemical structure, but the chemical basis of this relationship can only be speculated upon.

It is noteworthy that the order of phytotoxicity of these amines is much the same as, and could have been predicted from, the order of mammalian toxicity of these compounds (Table 2) (Sax, 1968). However, since it is possible, or even probable, that the same chemical and/or physical factors determine the order of mammalian and phytotoxicity, such coincidence does not help to explain the results.

The important consideration arising from this experiment is whether the phytotoxicity of these amines affects the results obtained with amine salts of acid herbicides. This problem will be investigated in further experiments.

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