STUDIES IN THE FOLIAR UPTAKE AND TRANSLOCATION OF PESTICIDES

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CONTENTS

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DECLARATION

Preliminary reports of much of the data forming Chapters Two, Three and Four have already been, or are in the process of being published (Babiker; Cook and Duncan, 1976; Cook; Babiker and Duncan, 1977; Cook and Duncan, 1978 (in press); Cook and Duncan (accepted for publication)).

SUMMARY

This thesis is principally an investigation of the factors affecting the foliar uptake and translocation of pesticides with particular emphasis on the influence of additives on the herbicide aminotriazole. The work was sub - divided as follows :-

1. A discussion of the various classes of additives which may influence foliar penetration and/or translocation with reference to their mode of action.

2. An investigation of the influence of adjuvants and humidity on the uptake of aminotriazole. The findings can be summarised as follows :-

Penetration of bean leaves (Phaseolus vulgaris var. Canadian Wonder) was greatly enhanced under high humidity conditions (10% penetration in 17h at the low humidity level (LHL) compared with 100% at the high humidity level (HHL)). The addition of polyoxyethylene 20 sorbitan monolaurate (polysorbate 20) to the spray fluid increased penetration on all occasions at the LHL. The increase obtained was found to be dependent on the concentration of polysorbate 20. Indeed, the w/w ratio of aminotriazole/polysorbate 20 seemed to be of primary importance. A 1/2 ratio appeared to bring about optimum penetration. The polysorbate 20 itself was not found to be taken up by the leaf to any great extent (5.4% penetration in 5h compared with 77.4% of the aminotriazole). The inclusion of polysorbate 20 at the HHL resulted in an increase in aminotriazole penetration at low polysorbate 20 concentrations (0.2-12.8g/litre) and a non-significant decrease over the aqueous control at a concentration of 40g/litre. Although aminotriazole penetration in the presence of polysorbate 20 (6.4g/litre) was increased at the HHL compared with the LHL, polysorbate 20 penetration was reduced.

The addition of glycerol to the spray solution increased aminotriazole penetration on all occasions at the LHL while at the HHL none of the concentrations tested enhanced penetration.

A polysorbate 20 plus glycerol combination (6.4g + 0.6ml/litre) gave the same order of penetration (98.4 and 94.0%) at the HHL and LHL respectively. In both cases, penetration exceeded that obtained with the corresponding polysorbate 20 and glycerol controls.

3. An investigation of the uptake by bean leaves (P. vulgaris var. Canadian Wonder) of aminotriazole from humectant-surfactant combinations and the influence of humidity on their effects. The findings can be summarised as follows :-

Aminotriazole penetration was not greatly influenced by the addition to the spray solution of dimethyformamide (DMF), dimethyl sulphoxide (DMSO), ethylene glycol and polypropylene glycol 400 (PPG 400). However, the addition of polysorbate 20 (0.2 - 1.0g/litre) to spray solutions of the above additives and glycerol (5.0ml/litre, except for DMF, 50.0ml/litre) substantially increased uptake to 80-100% in all cases at $50^{+}_{-}10\%$ relative humidity (r.h.). Similar trends were found when a range of polysorbate surfactants (0.2g/litre) were applied to solutions containing either DMSO or glycerol (5.0ml/litre). Humidity was found to have a critical effect upon such humectant-surfactant combinations. With DMSO-polysorbate 20 the following uptake figures were recorded : $< 30\%$r.h. - 3.1% ; $45^{+}_{-}10\%$ r.h. - 86.8% ; 55-65% r.h. - 48.2% and 100%r.h. - 0.3%. Similar trends were recorded with other combinations. Further studies revealed that the adverse effect of humidity on DMSO-polysorbate 20 mixtures could be at least partially overcome by regulating the DMSO concentration.

4. An investigation of the mode of action of thiocyanate and iodide in aminotriazole formulations. The findings are summarised as follows :-

Ammonium thiocyanate (NH_4SCN) was shown to inhibit aminotriazole oxidation in two free radical generating systems, namely (a) riboflavin photosensitised oxidation, (b) oxidation by hydroxyl radicals. Evidence from in vitro studies is presented to show that NH_4SCN could enhance aminotriazole performance by being preferentially oxidised within the leaf, thereby preventing aminotriazole free radical formation and subsequent conjugation with amino acids and other plant

constituents. This opens up the possibility of a whole new range of additives which could enhance translocation by inhibiting free radical reactions. A comparison of possible inhibitors revealed that iodide and perhaps bromide and cyanide could be of use in this respect. Asulam was also found to be oxidised by the two free radical generating systems mentioned above. Again, the degree of oxidation was decreased by thiocyanate, iodide and also by ferrocyanide. This would suggest that additives such as NH_4SCN may be of wider use than is at present recognised. In addition to inhibiting free radical reactions, NH_4SCN, KSCN, NaSCN, NaI and KI were shown to have a considerable effect on aminotriazole uptake by bean leaves (P. vulgaris var. Canadian Wonder). MgI_2, CaI_2 and $Ca(SCN)_2$ had little effect on uptake.

5. A field investigation into the effects of various herbicide formulations on bracken control. The findings are summarised as follows :-

Aminotriazole in the presence of DMSO - polysorbate 20 and polysorbate 20 - glycerol additive combinations was much less effective in reducing frond densities than the commercial product (Weedazol TL, aminotriazole + NH_4SCN). This appeared to be due to the influence of NH_4SCN on translocation rather than uptake. The incorporation of NH_4SCN into an aminotriazole formulation containing polysorbate 20 + glycerol was more effective than the commercial formulation although the difference was not significant. A study of the overall penetration/translocation process of aminotriazole in bracken revealed that this could take two weeks and possibly much longer from penetration of the fronds to accumulation in the rhizome buds.

The incorporation of NH_4SCN into an Asulox formulation decreased scorching, thus indicating the possible use of NH_4SCN in such formulations. However, the results of its effect on bracken suppression by Asulox are not yet available. NaI caused considerable scorching of bracken. Again however, its effects on bracken suppression by aminotriazole are not yet available.

6. An investigation of the uptake of amitrotriazole by bracken growing in different environments. The results may be summarised as follows :-

(i) Penetration of the upper surfaces of bracken pinnules with thick cuticles was significantly greater than penetration of pinnules with thin cuticles. This appeared to be due to the degree of spreading of the spray solutions.

(ii) Penetration of the lower surfaces appeared to be correlated with stomatal density and/or the degree of hairyness.

7. The possibility of using chelates of iron with simple sugars such as fructose to overcome iron chlorosis is discussed. Preliminary results revealed that a 5/1 molar ratio of fructose/iron was effective in bringing about iron uptake by bean leaves (P. vulgaris var. Canadian Wonder). The incorporation of polysorbate 20 increased the rate of uptake.

LIST OF ABBREVIATIONS

a.i.	active ingredient
Å	Angstrom
°C	degrees celsius (formerly centigrade)
cm	centimetre
g	gramme
h	hour
ha	hectare
kg	kilogramme
l	litre
m	metre
m^2	square metre
µ	micron
µg	microgramme
µl	microlitre
mg	milligramme
ml	millilitre
M	mole
p.	page
pp.	pages
%	percentage
lb	pound
p.s.i.	pounds per square inch
r.h.	relative humidity
recryst.	recrystallisation
S.D.	standard deviation
sec	second
sl.	slight
sp.	species
var.	variety
>	greater than
<	less than

Chapter One

INTRODUCTION

1.1 THE USE OF ADDITIVES IN HERBICIDE FORMULATIONS

Herbicides are commonly formulated as water soluble powders and liquids, emulsifiable concentrates, flowable suspension concentrates, flowable emulsions, wettable powder concentrates, dust concentrates, field strength dusts, granular products and fumigants. The major purposes behind formulation are (a) the dilution of the toxicant to a level which is toxic to the target species but which minimises toxicity to other organisms in its surrounding environment, (b) to enable a small amount of highly concentrated toxicant to be spread uniformly over a relatively large area, (c) to bring about maximum efficiency of contact between the toxicant and the target species.

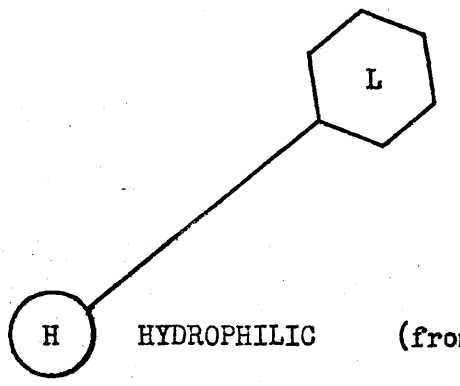
Any formulation normally contains the toxicant (s) and one or more of the following : solvents, emulsifiers and carriers. An additive is a substance or formulation of substances which when added to a herbicide formulation increases its effectiveness. Such effects can often also be found if some component of the formulation other than the toxicant is replaced by another, e.g. if one solvent system is replaced by another (Lawrence and Blackburn, 1963; Hyzak et al., 1969). In this case, the replacement solvent could be said to have additive properties. Thus, the definition of an additive is not a distinct one. Even the classification of the different types of additives is not well defined, e.g. dimethyl sulphoxide (DMSO) is often classed as a penetrant (Hull, 1970; Kanellopoulos, 1974) although it posesses considerable humectant properties (Anon, 1968). Surfactants may act secondarily as humectants (Currier and Dybing, 1959) while many have considerable penetrating properties (Price, 1976).

Since this thesis is basically concerned with the use of additives to improve both the foliar uptake and translocation of pesticides, the purpose of this section of the chapter is to outline the classes of additives used to increase these processes and to indicate their possible modes of action. This is in no way an attempt to review the literature on the use of additives but merely to outline the types of compounds used and how they exert their effects. For the purpose of this discussion, additives have been classified as follows : (a) surfactants, (b) humectants, (c) penetrants (principally DMSO), (d) inorganic salts, (e) oils, (f) anti-drift agents, (g) film forming substances, (h) growth regulators and (i) miscellaneous and formulated products. Of these, a-d are directly relevant to this thesis and hence will be dealt with in more detail.

(a) Surfactants (surface - active agents)

An understanding of the use of surfactants in herbicide formulations must begin with an appreciation of the properties of the surfactant molecule. These have been described in considerable detail by van Valkenburg (1967) and Becher (1973) and may be summarised as follows :

In general, the surfactant molecule consists of two more or less distinct moieties, one of which is hydrophilic, the other being hydrophobic (normally lipophilic). This concept can be illustrated graphically as follows :



(from Behrens, 1964)

Because of the hydrophilic-lipophilic nature of the molecule, it possesses only limited solubility in both water and organic solvents. At low concentrations, the surfactant is in true molecular solution, but because of its hydrophilic-lipophilic nature, the molecules tend to migrate to surfaces or interfaces forming orientated monolayers with the lipophilic portion in the non aqueous phase. This has the effect of reducing the high surface energy of the water surface. When a surfactant exceeds its solubility in a particular solvent, rather than precipitating out, it forms aggregations called micelles in which that portion of the surfactant which is compatible with the solvent is orientated outwards. The surfactant concentration at which micelle formation begins is termed the critical micelle concentration (c.m.c.).

A quantitative measure of this hydrophilic-lipophilic nature was developed by Griffin (1949) and called the HLB system (hydrophilic-lipiphilic balance). Each surfactant is assigned its own HLB value with the more hydrophilic ones having the higher values. A rough approximation to the actual HLB value may be obtained by observing the behaviour of the surfactant in water.

Surfactants can be divided into five basic groups (i) nonionic - so called because they do not form ions in solution, (ii) anionic - form anions in solution, (iii) cationic- form cations in solution, (iv) amphoteric - show anionic or cationic properties in solution depending on pH, (v) water insolubles - they may or may not contain ionic groups. The first three groups are the most widely used in herbicide formulations.

The ability to reduce surface and interfacial tensions, the formation of micelles and the HLB are all of central importance in the enhancement of herbicidal activity.

Reductions in surface tension may bring about increased activity by (i) increasing spray retention, although, retention may be decreased by the addition of a surfactant where the plants are of naturally high wettability. Such differential responses may lead to changes in selectivity., (ii) increasing penetration as a result of increased droplet spreading and the elimination of air films between the spray droplets and the leaf surface,

thereby increasing the area of direct contact of the spray solution with the leaf surface. These points have been well discussed by Holly (1976)., (iii) inducing stomatal penetration. The significance of this is still hotly disputed, Babiker (1976) has discussed the relevant factors with particular reference to its significance under practical field conditions. However, as pointed out by several authors (Jansen et al., 1961; Foy and Smith, 1965; Bayer, 1972), the major influences of surfactants on herbicide activity are to be found in the range of concentrations beyond which the greatest changes in surface and interfacial tensions occur. Freed (1959) showed that different surfactants which caused the same reductions in surface tension of aminotriazole solutions promoted absorption to widely differing degrees, Smith and Foy (1967) found no correlation between pH or surface tension and toxicity of 10 paraquat-surfactant mixtures. In addition, there were indications that interactions between the paraquat and the anionic surfactants were occurring. Foy and Smith (1965) concluded that although minimum surface tension and contact angle occurred at 0.1-0.5% for all surfactants, maximum herbicidal activity was observed at more than 10xthat level. Thus, when surfactant concentrations were greater than 0.1-0.5% herbicidal enhancement was not correlated with surface tension lowering, contact angle, observed wettability or initial surfactant toxicity.

There is evidence that the herbicide - surfactant - plant interaction may be of a highly specific nature. Morton and Combs (1969) demonstrated that surfactants with ether linkages were more effective than those with ester linkages in increasing the activity of picloram - 2,4,5-T applied to Prosopis juliflora but both types were equally effective on Quercus virginiana and Smilax bona-nox. Wills (1971) demonstrated that MSMA activity could be enhanced by several polyoxyalkylene glycol ester surfactants but not by other non-ionic, most anionic and all cationic surfactants examined. Similarly, Jansen (1965) demonstrated very specific surfactant structural requirements for maximum herbicidal activity.

Various authors (Smith et al., 1966; Takeno and Foy, 1974a; Wyrill and Burnside, 1977) have demonstrated the importance of

HLB in the differential response obtained from surfactants while more specifically, Linder (1975) proposed that the biological activity of a herbicide is affected by the oil/water partition function expressed by the Hansch equation. Surfactants may modify this equation thereby improving uptake. Sirois (1967) demonstrated that the relative efficiency of a homologous series of non-ionic ethylene oxide ether surfactants was correlated with HLB and shown to be the result of phase distribution phenomena. A correlation was also found between surfactant phytotoxicity and enhancement in herbicide phytotoxicity which he proposed may explain the role of surfactants, i.e. the more phytotoxic, the greater the cellular disorganisation and alteration of cell permeability to herbicides. Thus, since surfactant phytotoxicity is a function of HLB, changes in membrane permeability could also be expected to be a function of HLB. Bland and Brian (1972) found the uptake of paraquat to be a function of the partitioning of the surfactant between water and the leaf wax + lipid layer. Several authors (Buchanan and Staniforth, 1966; Bottomley, 1970; Haapala, 1970; Healey et al., 1971) have shown that surfactants are capable of disrupting cellular organisation through their effects on membrane integrity. Haapala (1970) has proposed that this may be due to micelles solubilising into the membrane, the formation of mixed micelles or complex formation with globular proteins in the plasmalemma. Other more subtle biochemical effects on the cuticle and underlying tissues may also be possible (Parr and Norman, 1965). Takeno and Foy (1974b) have demonstrated that surfactants may solubilise surface waxes while it has also been proposed that surfactants may induce swelling of the cutin and/or dissolve certain cuticular components (Furmidge, 1959; Jansen, 1964a; 1964b). Swelling of the cutin could increase the area of the hydrophilic and lipophilic channels thereby promoting uptake (Jansen, 1964a; 1964b). Jansen (1964a) has also proposed that cuticle hydration due to water from a droplet containing a surfactant being more available to the cuticle may be a primary function. Becher and Becher (1969) used the theory of capillarity to partly explain surfactant effects in a more mathematical fashion.

Surfactants have also been shown to have direct effects on translocation. Bland and Brian (1972) and Brian (1972) demonstrated that certain surfactants inhibited translocation although they enhanced uptake while Corkins (1970) demonstrated that ^{14}C-maleic hydrazide uptake was not enhanced by surfactant addition, but translocation was.

Holly (1976) lists a number of functions which surfactants may perform resulting in changes in biological activity. His discussion however, was based mainly on wettability and spray retention with respect to selectivity. These points are as follows with certain additions to points 7 and 9

Surfactants may :

1. Increase total spray retention where plant surfaces are naturally of low wettability.

2. Decrease total spray retention where plant surfaces are naturally readily wettable.

3. Increase spray retention at key sites favourable for penetration or subsequent damage to the plant.

4. Increase penetration by increasing the area of contact with the leaf through greater droplet spread.

5. Increase penetration by increasing the area of contact with the leaf as a result of the elimination of air films between spray droplets and leaf surface.

6. Lengthen the period of penetration by acting as a humectant, keeping spray droplets moist almost indefinitely.

7. Increase penetration through the cuticle by (i) acting as a co-solvent, (ii) solubilising surface waxes or other cuticular components, (iii) bringing about hydration of the cuticle, (iv) entering the cuticle and bringing about swelling of the cutin, (v) changing the partition function between the leaf wax + lipid layer and water.

8. Increase penetration by increasing direct entry through stomata by lowering the surface tension of the spray solution.

9. Facilitate movement along cell walls after entry into the foliage by (i) lowering interfacial tensions or (ii) disrupting membrane permeability.

In addition, the following factors have also been put forward by Currier and Dybing (1959):

(a) Reducing interfacial tension between relatively polar and apolar submicroscopic regions of the cuticle.

(b) Interacting directly with the herbicide in some manner.

(b) Humectants

Humectants are much less widely used in herbicide formulations than are surfactants. Generally they are low molecular weight glycols, e.g. glycerol (Hopp and Linder, 1946; Holly, 1956; Hughes, 1968; Babiker and Duncan, 1975a), sorbitol (Smith et al., 1959; Babiker and Duncan, 1975a), ethylene glycol (Hull and Morton, 1971), propylene glycol (Hughes, 1968). In addition, sucrose (Babiker and Duncan, 1975a), molasses (Fogg, 1948) and polymeric materials such as polyethylene glycol, polypropylene glycol (Pfeiffer and Stanley, 1959) and polypropylene diol (Hughes, 1968) have been used.

Crafts et al. (1953) have suggested that humectants may have two major roles :

1. They prevent drying of the spray solution on the leaf surface thus increasing the penetration time. Gray (1956) demonstrated that glycerol kept streptomycin in solution and in close contact with the leaf surface.

2. They become sorbed by the cuticle thus increasing its polar nature and compatability with water soluble chemicals.

In addition, Richter (1968) demonstrated that ethylene glycol could disrupt cytoplasmic membranes, glycerol had no effect. Ennis et al. (1952) attributed increased 2,4-D activity following the addition of 0.5% Carbowax to improved foliar retention.

Conflicting reports have appeared in the literature on the effectiveness of humectants in enhancing biological activity (see 3.1). Crafts et al. (1958) have suggested that toxic dehydration caused by the humectants may hinder absorption and translocation while Babiker and Duncan (1975b) have suggested that at low humidity, simply by remaining on the leaf surface, glycerol may retard penetration while at high humidity, glycerol penetration appeared to take place.

(c) Penetrants (principally DMSO)

The role of penetrants was reviewed relatively recently by Hull (1970). The most commonly used is DMSO although BLO (γ - butyrolactone) and M-Pyrol (N-methyl-2-pyrollidone) have also shown promise (Hull, 1970).

DMSO possesses a remarkable potential as a solvent for many organic and inorganic chemicals including herbicides (Keil, 1965; Brady and Peevy, 1968). In addition, it possesses considerable humectant properties (Anon, 1968). It is reported as being able to enhance both penetration and translocation (Keil, 1965; Lapham, 1966; Mussell et al., 1967; Foy et al., 1972) although the mechanisms by which such effects are achieved are largely unknown. From the evidence available, Hull (1970) proposed that concentration was an important factor in the enhancement of penetration. Although concentrations of about 5% and above have been shown to be phytotoxic, it is at such concentrations that many of the more dramatic effects have been demonstrated, although, he proposes that the destruction of plasma membranes above a threshold concentration could not in itself totally explain its mode of action.

Mussell et al. (1967) suggest that although DMSO kept 2,4-D in solution for considerable periods of time, that this was not the sole factor governing enhanced activity. This would suggest that perhaps solubilisation of cuticular components may also be involved, a factor which appears to have been largely overlooked.

Keil (1965) has suggested that DMSO may promote transport of the unchanged toxic moiety although toxic decomposition products could form, while in addition, it is possible that interactions may occur between DMSO and herbicide with the formation of other biologically active compounds. Thus, the possible modes of action of DMSO are as follows :

1. It could lengthen the period of penetration by virtue of being (i) a co-solvent of low volatility and (ii) a humectant.

2. It may solubilise certain cuticular components and/or plasma membranes.

3. Specific DMSO - herbicide - plant interactions may occur leading to the formation of toxic decomposition

products and/or DMSO - herbicide interactions leading to the formation of new biologically active materials.

The first two points would seem to hold most promise.

(d) Inorganic salts

Inorganic salts have not as yet gained wide acceptance in herbicide formulations. The most commonly used is probably ammonium thiocyanate (NH_4SCN) which is also used as a contact herbicide (Brian, 1976). It is commonly formulated in practice with aminotriazole where it has been shown to enhance both penetration (Babiker and Duncan, 1975a) and translocation (Donnalley and Ries, 1964) although there is also conflicting evidence. Forde (1966) found it retarded translocation while Donnalley and Ries (1964) found it to have no effect on absorption. In addition, it has on occasion been used in combination with fenoprop, 2,4,5-T, dichloroprop, 2,4-D (Robison, 1965; Stritzke, 1972). The mode of action of NH_4SCN will be discussed in detail in Chapter Four.

In addition to NH_4SCN, a number of other ammonium salts have been tested, including ammonium nitrate (NH_4NO_3), ammonium sulphate ($(NH_4)_2SO_4$), ammonium chloride (NH_4Cl) and ammonium citrate but principally the first two.

$(NH_4)_2SO_4$ is used as an activator for the water soluble salts of selective contact sprays such as dinitrocresol, pentachlorphenol and endothal where it buffers the formulation on the acid side of neutrality resulting in the formation of the undissociated parent acid which penetrates the leaf more efficiently (Crafts, 1961). In addition, Aliev (1959) observed improved weed control and increased maize yields from applications of 2,4-D containing superphosphate, NH_4NO_3 and $(NH_4)_2SO_4$. Again these appeared to be pH related effects. More recently, Turner and Loader (1972) demonstrated a synergistic effect of NH_4NO_3 on MCPA and picloram salts. Sodium nitrate ($NaNO_3$) was shown to be inactive while calcium nitrate ($Ca(NO_3)_2$) was antagonistic. Several other ammonium salts also exhibited a synergistic response. Turner (1976) also showed $(NH_4)_2SO_4$ to increase the activity of dichloroprop, mecoprop, 2,3,6-TBA,

benazolin, bentazone and glyphosate. Brady (1970) demonstrated NH_4NO_3 to increase 2,4,5-T absorption. He suggests that the depression in pH caused by adding NH_4NO_3 probably hydrolysed some of the isooctyl ester to the acid thus allowing entry by both aqueous and a lipoidal route. Translocation in post oak (Quercus stellata) was also enhanced. In other species, translocation was unaffected.

Wilson and Nishimoto (1975a; 1975b) found that absorption of ^{14}C-picloram was increased in strawberry guava by 0.5 and 10% $(NH_4)_2SO_4$, translocation was unaffected. Other ammonium salts such as nitrate, chloride and dibasic phosphate also increased absorption while of six sulphates tested, only $(NH_4)_2SO_4$ increased absorption. Suwunnamek and Parker (1975) showed considerable activation of glyphosate on the addition of $(NH_4)_2SO_4$ while almost equal activation was obtained from ammonium dihydrogen phosphate, diammonium hydrogen phosphate, triammonium phosphate and urea. In contrast to these results, Terry (1973) observed no useful enhancements in activity between glyphosate and $(NH_4)_2SO_4$, urea or diammonium hydrogen phosphate on Cyperus rotundus. Babiker and Duncan (1975a), working with aminotriazole, found its uptake to be enhanced by NH_4NO_3 while $(NH_4)_2SO_4$ was without effect. Babiker and Duncan (1975b) found asulam uptake to be enhanced by urea.

With regard to mode of action of ammonium salts, the following points have been put forward :

1. Enhanced uptake has been demonstrated on many occasions (Babiker and Duncan 1975a; 1975b; Wilson and Nishimoto, 1975a; 1975b; Turner, 1976).

2. Enhancement in uptake does not necessarily appear to be due to (i) a drop in pH, (ii) the deliquescent effect of the salts nor (iii) a reduction in surface tension, since most have little or no effect on this parameter (Turner and Loader, 1972; Wilson and Nishimoto, 1975b).

3. Wilson and Nishimoto (1975b) have proposed that enhanced activity may be due to a direct and immediate effect on the absorption pathway while Turner and Loader (1972) have suggested that enhanced activity is not primarily a result of increased uptake but possibly enhanced movement through the mesophyll.

4. Enhanced stomatal penetration does not appear to be the major factor involved since in many cases, enhanced uptake was through astomatal surfaces (Wilson and Nishimoto, 1975a).

5. Enhanced translocation within the conducting tissues does not appear to be a major factor (Turner and Loader, 1972; Wilson and Nishimoto, 1975a).

6. Baur et al. (1974) have proposed that the action of ammonium chloride was in promoting the mixing of internal tissue water and external water droplets.

(e) Oils

Currier and Dybing (1959) reviewed the early literature on the influence of oils on foliar penetration while more recently this has been discussed by Hull (1970) and Kanellopoulos (1974). They are mainly used as solvents for herbicides in emulsifiable concentrates, however, many have been used as additives, either pure or as oil/water emulsions with solutions of herbicides, emulsifiable concentrates, wettable powders, ultra low volume formulations etc. (Kanellopoulos, 1974). The main types used are petroleum, synthetic hydrocarbon and silicone and vegetable oils (Kanellopoulos, 1974).

They have been shown to increase the activity of a range of herbicides (Jones and Anderson, 1964; Wilson et al., 1967; Aya and Ries, 1968; Chenault and Wiese, 1969; Preest, 1975) however, where selectivity is required, increased crop injury has been shown to result from their addition (Wilson et al., 1967; Chenault and Wiese, 1969).

Hull (1970) has stated that most work suggests that oils penetrate principally via the stomata or through minute fissures in the cuticle, thereby increasing uptake. Oils of low toxicity have been shown to enhance activity while those of high toxicity, although they may enhance uptake, have been shown to reduce translocation due to rapid killing of the conducting tissue. A slight toxicity may however be beneficial through increasing protoplasm permeability (van Overbeek and Blondeau, 1954).

Enhanced uptake has also been put down to increased wetting of the leaf surface and the increased lipophilicity of the herbicide/oil formulation (Kirkwood, 1976).

(f) Anti-drift agents

Rather than by altering the spray equipment or the spraying procedure, e.g. by using larger nozzles, higher application rates and altering the height from which spraying is carried out etc, spray drift can be reduced by the addition of certain additives to the spray solution. Such compounds are normally polymeric in nature, e.g. hydroxyethyl cellulose, cross linked polyacrylates and polysaccharide gums. They are variously called anti-drift agents, thickening agents, particulating agents, deposit builders and spray gell agents. They reduce drift by minimising the production of small droplets although Suggitt (1966) points out additional advantages of the unique slow drying and greater leaf retention properties of hydroxyethyl cellulose thickened sprays. Further detail may be obtained from Hull (1970).

(g) Film-forming substances

These are designed to bring about filming of the herbicide formulation on the leaf surface and to increase the rain resistance of the spray deposit, e.g. amine stearates and polymeric polysulphides. Amine stearates have been shown to increase rain fastness and reduce droplet bounce (Amsden, 1962).

(h) Growth regulators

Kinins, gibberellic acid, IAA etc have all been shown to enhance the uptake and movement of inorganic and organic substances. Their influence on herbicides does not however appear to have been accepted in practice. They are discussed in considerable detail by Hull (1970).

(i) Miscellaneous and formulated products

Several authors (Darlington and Barry, 1965; Turner, 1972; Babiker and Duncan, 1975b) have demonstrated the possibility of using chemicals which may injure the cuticle or epidermis in order to increase uptake, e.g. potassium ethyl xanthate, tributyl phosphate, trimethyl phosphate, chloroform etc. A copolymer of vinylpyrrolidinone has been used to prevent the precipitation of water soluble salts of ring-substituted aryloxyaliphatic acids (Barker, 1960) while tribasic amine citrates have been used to prevent the precipitation of amine salt formulations of 2,4-D and 2,4,5-T (Warren, 1960) when such herbicides are being used in hard water areas. Niacin, biotin, ascorbic acid and adenine have been used to enhance the activity of the ester and triethylamine formulations of 2,4,5-T. Such miscellaneous additives are further discussed by Kanellopoulos (1974). In addition, he lists many of the formulated commercial additives, their trade names, composition and recommended uses.

1.2 THESIS OBJECTIVES

The object of this thesis is to investigate the use of additives on the foliar uptake and translocation of herbicides (mainly aminotriazole (3-amino-1,2,4-triazole)) using growth room and *in vitro* studies initially, in order to gain a considerable degree of background information on the behaviour of both aminotriazole and the additives in relation to the environment. This was with a view to incorporating some of these additives into herbicide formulations for the control of bracken. The relevances of aminotriazole and bracken are discussed in Chapter Two. All too often, papers have appeared in the literature on the use of certain additives in enhancing the activity of herbicides with seemingly little logical reasoning behind why the concentration of additive being used is included.

Hence, this is an attempt to put the use of additives on a more rational basis than is the custom at present, thereby hoping to reduce variability of response.

Chapters Two - Four are concerned to a large extent with the uptake (absorption or penetration) of aminotriazole. Uptake is defined in these studies as the amount of aminotriazole which cannot be removed from the leaf surface by a water wash. This will be a maximum value as it will include any of the chemical which is adsorbed firmly enough to resist washing (Holly, 1956).

Chapter Two : Penetration of bean leaves by aminotriazole as influenced by adjuvants and humidity.

This investigation set out to assess the influence of various concentrations of polyoxyethylene 20 sorbitan monolaurate (polysorbate 20) and glycerol and their interaction with each other and with humidity on aminotriazole penetration of bean foliage under conditions controlled in a growth room.

Chapter Three : Foliar uptake of aminotriazole from humectant-surfactant combinations and the influence of humidity on their effects.

This was an investigation of the influence of a range of concentrations of several humectants on aminotriazole uptake by bean leaves, their interaction with polysorbate surfactants and the influence of humidity on such interactions. Again, this investigation was carried out under conditions controlled in a growth room.

Chapter Four : Mode of action of thiocyanate and iodide in aminotriazole formulations.

The purpose of this investigation was to assess the effects of (a) ammonium thiocyanate, other thiocyanate salts, iodide salts and various other compounds on the degradation of

aminotriazole and asulam (methyl (4-aminobenzenesulphonyl) carbamate) in certain free radical generating systems, (b) successful inhibitors on aminotriazole uptake by bean leaves under growth room conditions.

Chapter Five : Field investigations into the effect of various herbicide formulations on bracken control.

This investigation was designed to assess the practical use of aminotriazole formulations developed in Chapters Two - Four. In addition, preliminary investigations into (a) the use of ammonium thiocyanate in asulam formulations and (b) the effects of a wide range of glyphosate (N- (phosphonomethyl) glycine) concentrations were commenced.

Chapter Six : The influence of morphological changes in bracken pinnae on the penetration of foliar applied aminotriazole.

This was designed to investigate the influence of the environment on certain morphological parameters and the influence of such changes on the foliar uptake of aminotriazole. Such differences in uptake, if present, could have a considerable influence on the activity of aminotriazole.

Chapter Seven : Absorption of iron by bean leaves from fructose-iron chelates.

It would appear that there is a considerable requirement for a cheap foliar applied iron chelate which might reduce many of the problems associated with foliar sprays as used at present. This was a preliminary investigation to determine in the first place whether simple sugar/iron chelates, applied to the foliage, could be taken up relatively efficiently, with a view to extending the work by studying their translocation and use in practice.

Chapter Two

PENETRATION OF BEAN LEAVES BY AMINOTRIAZOLE AS INFLUENCED BY ADJUVANTS AND HUMIDITY

2.1 INTRODUCTION

The penetration of plant foliage is particularly relevant in the case of foliar applied systemic herbicides and it is only when this process is fully understood and turned to advantage that the maximum effectiveness of applied chemicals can be achieved (Freed and Montgomery, 1958; Hammerton, 1967). More efficient penetration of herbicides should enable less chemical to be applied, thereby not only reducing costs, but also perhaps improving selectivity, reducing long term soil effects and minimising possible environmental hazards (Foy and Smith, 1969; Turner, 1974).

The cuticle is the first barrier in the penetration process to be crossed (van Overbeek, 1956; Martin and Juniper, 1970; Wathana et al., 1972; Babiker and Duncan 1975 b). The submicroscopic structure of this layer, the physical nature of the wax deposits (Silva Fernandes, 1965) and the turgor of the plant foliage (Fogg, 1947; Hammerton, 1967) are all relevant when considering intimacy of contact between the spray droplets and the leaf surface and hence are important in penetration.

Penetration is also dependent upon the environment to which the plant is subjected, before, during and after spraying (Caseley, 1974), i.e. conditions such as low humidity, high temperature and wind which are known to hasten the drying out of spray solutions can curtail penetration and severely limit the effectiveness of foliar treatments, including those of herbicides (Riepma Kzn, 1960; Herrett and Linck, 1961; Middleton and Sanderson, 1965; Hammerton, 1967; Caseley, 1974).

One approach which can be adopted to improve the

efficiency of penetration of foliar applied herbicides is to select a suitable environment (Brian, 1970). As has been demonstrated on occasion, this restricts the use of herbicides to certain geographical regions (Corkins, 1960; Clor et al., 1964) and also restricts spraying times, e.g. to early morning (Volger, 1969). Such limitations will inevitably increase the cost of the treatment (Colthurst et al., 1966).

A more constructive and versatile approach is to modify the properties of the spray fluid in order to achieve efficient penetration (Holly, 1956; Babiker and Duncan, 1975 b). This can be accomplished by incorporating adjuvants such as surfactants, humectants and other constituents, in an attempt to modify the environmental factors and possibly also the leaf surface barrier itself (Babiker and Duncan, 1975 b). Reports on the benefits of including such additives are on the whole conflicting (Gray, 1956; Holly, 1956; Linser, 1964; Babiker and Duncan, 1975 a). However, as work carried out in this laboratory (Babiker and Duncan, 1975 a) and elsewhere (Holly, 1956) has suggested that the effect of the additives themselves may be influenced by environmental and other variables, this aspect was taken into account in the present study.

The investigation therefore set out to assess the effect of polyoxyethylene 20 sorbitan monolaurate (polysorbate 20; "Tween 20" - registered trade mark of the Atlas Chemical Industry Incorporated) and glycerol and their interaction with humidity on the penetration of bean foliage (Phaseolus vulgaris var. Canadian Wonder) by aminotriazole (3 - amino - 1,2,4, - triazole) under conditions controlled in a growth room.

Aminotriazole was selected for the study because of (a) its general importance as a herbicide, (b) its considerable use on bracken in Britain in the past (Erskine, 1960; Hodgson, 1960; Kirkwood and Fletcher, 1961) - this is of particular relevance to the West of Scotland where there is a considerable bracken population (Hendry, 1958), (c) its known sensitivity to environmental variables such as humidity and rainfall (Volger, 1969; Babiker and Duncan, 1975 a) which again makes

it relevant to the wetter climate in the west. Polysorbate 20 was selected since it is regularly used in similar penetration studies (Smith and Foy, 1967; Sharma et al., 1971). It also has many desirable properties such as co-solvency (Mitchell et al., 1960; Babiker and Duncan, 1975 b), insensitivity to the presence of hard water (Behrens, 1964), its hygroscopic nature (Price, 1976) and low inherent phytotoxicity (St. John et al., 1974). Glycerol was selected since it also is regularly used in penetration studies (Gray, 1956; Holly, 1956) and has such desirable properties as (a) hygroscopic nature and (b) lack of surface active properties (Holly, 1956) enabling its effects to be distinguished from those of surfactants. Phaseolus vulgaris was selected since again it is regularly employed for herbicide penetration studies (Sargent and Blackman, 1962; Jones and Foy, 1972), it grows quickly and is easily handled.

2.2 Experimental

2.2.1 Materials

Aminotriazole (3-amino-1,2,4-triazole) and polysorbate 20 were purchased from Koch-Light Laboratories Ltd. Glycerol was purchased from British Drug Houses Ltd. Aminotriazole was recrystallised from absolute ethanol (Castelfranco et al., 1963).

2.2.2 Methods

2.2.2.1 Penetration

All penetration experiments were performed in a growth room adjusted to a 16h day length and a temperature of $30\pm3^{\circ}C$. The plants were germinated in trays of vermiculite and seedlings selected on the basis of uniformity of size and lack of damage. These were transplanted to pots, grown in the same growth medium and ultimately treated when 9 - 13 days old, when the primary leaves were fully expanded. The aminotriazole solutions were placed randomly as discrete droplets (ca 2 µl) on the upper leaf surface in a total volume of 20 or 40 µl/leaf by means of a 10 µl Eppendorf pipette or in the case of those

experiments concerning the penetration of both the aminotriazole and the polysorbate 20 as ca 5 µl droplets in a total volume of 200 µl/leaf by means of a 100 µl Eppendorf pipette. The plants were then either left exposed in the growth room or covered with polythene bags for the duration of the experiment. The former treatment was referred to as the low humidity level (LHL) and the latter as the high humidity level (HHL). Enclosing plants in polythene bags may result in a number of changes in the atmospheric environment of the plant but the increase in relative humidity is considered to be the major factor involved (Sharma et al., 1971). The relative humidity under bags is expected to approach 100% (Clor et al., 1962). Preliminary experiments indicated that no change in temperature occurs under polythene bags over the duration of the experiments. For each treatment, a minimum of 6 and a maximum of 16 leaves were treated. Unless stated otherwise, the timing of the experiments was started after the droplets had dried off (20-30 minutes after application). On completion of the experiments, the 20 or 40 µl/leaf and 200 µl/leaf treatments were washed with 10 and 25 ml de-ionised water respectively and the washings analysed for polysorbate 20 and/or aminotriazole. Preliminary experiments had confirmed that total recovery of both was possible by this method provided it was carried out immediately after treatment. In all treatments, the solutions persisted as discrete droplets until completion of the experiments, as opposed to spreading over the leaf surface.

2.2.2.2 Influence of humidity on penetration

Four sets of experiments were devised for estimating the role of humidity in penetration. The experimental conditions were selected bearing in mind (a) the humidity conditions which could prevail in the field during and after spraying (Brian, 1966; Fogg, 1947) and (b) field experience with aminotriazole which demonstrated that its penetration is greatly influenced by changes in atmospheric humidity (Volger, 1969; Babiker and Duncan, 1975 a).

In the first set of experiments the following three sets of treatments were carried out:-

(a) Treated plants, after the droplets had dried (20-30 min) were held (i) at the HHL and (ii) at the LHL for the duration of the experiment.

(b) Treated plants were initially held at the LHL followed by varying times at the HHL.

(c) Treated plants, after the droplets had dried were initially held at the HHL followed by varying times at the LHL.

In the second experiment, the following treatments were carried out:-

(i) Plants were held at the LHL.

(ii) Preconditioned bags were placed over the plants immediately after treatment, i.e. when the droplets were wet.

(iii) Unconditioned bags were placed over the plants immediately after treatment, i.e. when the droplets were wet.

(iv) Preconditioned bags were placed over the plants after allowing the droplets to dry (20-30 min).

(v) Unconditioned bags were placed over the plants after allowing the droplets to dry (20-30 min).

For preconditioning, the bags were placed over plants not employed in the experiment in order to build up humidity within the bags prior to treatment. In (ii) and (iii) above, the applied droplets remained wet for the duration of the experiment. In all subsequent experiments at the HHL only unconditioned bags were employed and they were placed over the plants after the droplets had dried.

In the third experiment, the following treatments were carried out:-

(i) Plants were held at the LHL for 4h.

(ii) Plants were held at the LHL and rewetted after 2h (one rewetting).

(iii) Plants were held at the LHL and rewetted at 1h intervals (three rewettings).

(iv) Plants were held at the LHL and rewetted at 30 min intervals (seven rewettings).

(v) Plants were held at the HHL for 4h.

Because the aminotriazole solution was applied to the leaf surface as discrete droplets (ca 2 µl), the rewetting was carried out in a similar manner by placing the water droplets (ca 2 µl) over the area of the original droplets.

In the fourth experiment, differing concentrations of aminotriazole (1.5 - 13.5 µg/µl) in a total volume of 20 µl were applied per leaf. After the droplets had dried out, the plants were placed at the HHL for the duration of the experiment.

2.2.2.3 Influence of polysorbate 20 on penetration

Three sets of experiments were devised to investigate the effects of polysorbate 20 on aminotriazole penetration, its roles in the process and its interaction with humidity.

In the first set of experiments, aminotriazole (60.0 µg in 20 µl) was applied in the presence of a range of polysorbate 20 concentrations. The experiments were carried out at both humidity levels.

In the second set of experiments the following two sets of treatments were carried out:-

(a) The polysorbate 20 concentration was fixed and the aminotriazole concentration varied.

(b) Both the polysorbate 20 and the aminotriazole concentrations were varied. In some treatments, although the concentrations of both chemicals were varied, the aminotriazole/polysorbate 20 w/w ratio was kept approximately constant (1/2).

Both of the above sets of treatments were carried out at the LHL.

In the third set of experiments the following two sets of treatments were carried out:-

(a) Aminotriazole (600.0 µg in 200 µl) was applied in the presence of a fixed polysorbate 20 concentration (6.4g/litre) and the plants held at the LHL. Aminotriazole and polysorbate 20 were determined at hourly intervals for 5h.

(b) Aminotriazole (600.0 µg in 200 µl) was applied in the presence of a polysorbate 20 concentration of 6.4g/litre and the plants held initially at the LHL for 4h. Subsequently,

they were held at both humidity levels for varying periods of time. Both aminotriazole and polysorbate 20 were determined.

In both of the above sets of treatments, the timing of the experiments was from the moment of application of the toxicant to the leaf.

2.2.2.4 Influence of glycerol on penetration

Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a range of glycerol concentrations. The experiments were carried out at both humidity levels.

2.2.2.5 Influence of polysorbate 20 - glycerol combinations on penetration

Aminotriazole (60.0 µg in 20 µl) was applied in the presence of polysorbate 20 and glycerol.

In one experiment a fixed concentration of glycerol (0.3ml/litre) and a range of polysorbate concentrations (<0.1 - 40g/litre) were employed with plants held at the HHL.

In a second experiment, fixed concentrations of glycerol (0.6ml/litre) and polysorbate 20 (6.4g/litre) were employed, both alone and in combination. This experiment was carried out simultaneously at both humidity levels.

In a third experiment, fixed concentrations of glycerol (0.6ml/litre) and polysorbate 20 (3.2g/litre) were employed with plants held at the LHL. Aminotriazole was determined at varying intervals after application. In this experiment, the timing was from the moment of application of the toxicant to the leaf.

2.2.2.6 Polysorbate 20 estimation

Polysorbate 20 estimation was by a modification of the method of Greff et al. (1965) using dichloromethane to extract the coloured polysorbate 20 - cobaltothiocyanate complex from solution.

2.2.2.7 Aminotriazole estimation

Aminotriazole concentrations were estimated by the

method of Storherr and Burke (1961).

2.2.2.8 Statistical analysis

The data was analysed using the Analysis of Variance technique to determine significant differences between treatments. Probability (P)<5% was taken as being significant.

2.3 RESULTS AND DISCUSSION

2.3.1 Influence of humidity on penetration

The effect of humidity on aminotriazole penetration was quite apparent (Table 2.1a). From the results obtained it would appear that in arid regions, low humidity could impose a serious limitation on the use of aminotriazole. Similar conclusions have been drawn for other herbicides (Clor et al., 1964). In humid regions as well, variations in humidity could result in variable penetration, producing erratic performances. This is particularly relevant to high rainfall areas as has been observed with many herbicides (Hammerton, 1967) including aminotriazole (Holroyd et al., 1970).

Since attempts to build up humidity in advance by employing preconditioned bags resulted in only negligible differences in penetration compared with their non-preconditioned equivalents (Table 2.2) this approach was discontinued. It would thus appear that humidity develops fairly rapidly under polythene bags.

With regard to the actual role of humidity in penetration the following points deserve special mention: (a) with plants held at the HHL, penetration from initially dry droplets was approximately twice that from wet droplets (Table 2.2), (b) by transferring plants from the LHL to the HHL, increased penetration occurred even though the droplets had dried out initially (Table 2.1b), (c) rewetting the treated area with de-ionised water (30 min intervals) at the LHL resulted in a substantial increase in penetration (29.6% compared with 12.5% where no rewetting is involved). This is still well below the figure achieved in the same period at the HHL (75.6%) (Table 2.3).

These points, in agreement with other studies (Prasad et al., 1967; Sharma and Vanden Born, 1970) suggest that

Table 2.1 Influence of humidity on aminotriazole penetration. Aminotriazole application rate - 60.0 µg/leaf.

Treatment		MQLS[a] (µg)	Mean% penetration	SD[b] (%)
(a) Plants held at either the HHL[c] or the LHL[d] for the duration of the experiment (assessed after 17h)				
HHL		0.0	100.0	0.0
LHL		54.0	10.0	9.4
(b) Plants held initially at the LHL for varying times, followed by varying times at the HHL (assessed after 2.5h)				
LHL(h)	HHL(h)			
2.5	-	56.2	6.4	5.4
2.0	0.5	54.3	9.5	12.6
1.5	1.0	45.2	24.6	16.6
1.0	1.5	43.1	28.2	3.2
0.5	2.0	24.8	58.7	3.8
-	2.5	17.2	71.4	9.2
(c) Plants held initially at the HHL for varying times, followed by varying times at the LHL (assessed after 2.5h)				
HHL(h)	LHL(h)			
2.5	-	12.4	79.4	10.3
2.0	0.5	17.0	71.6	11.4
1.5	1.0	23.3	61.2	17.4
1.0	1.5	30.1	49.9	6.3
0.5	2.0	31.6	47.4	18.7
-	2.5	57.5	4.2	3.8

a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n-1 degrees of freedom)

c High humidity level

d Low humidity level

the enhancement of herbicide penetration by humidity can be attributed only in part to a slower rate of droplet drying. Other factors such as the effects of humidity on the leaf surface barrier e.g. cuticular hydration (van Overbeek, 1956; Prasad et al., 1967) and its effect on the water continuum of the plant (Lynch and Sweet, 1971) and hence the availability of the aqueous route (Crafts, 1964) could be envisaged as playing a key role in penetration. This would appear to be relevant bearing in mind that aminotriazole is taken up by an aqueous route (Crafts, 1964).

Table 2.2 Influence of the state of droplet drying on aminotriazole penetration. Aminotriazole application rate - 60.0 µg/leaf (assessed after 2h).

Treatment	MQLS[a] (µg)	Mean% penetration	SD[b] (%)
Plants at the LHL[d]	56.2	6.4	6.0
Droplets wet (bags preconditioned)	41.7	30.5	8.5
Droplets wet (bags not preconditioned)	40.2	33.0	11.1
Droplets dry (bags preconditioned)	11.9	80.2	19.3
Droplets dry (bags not preconditioned)	9.0	85.0	12.4

a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n-1 degrees of freedom)

d Low humidity level

An additional factor contributing to aminotriazole penetration is the lowering of concentration which could result from rewetting or the presence of wet droplets (Tables 2.2 and 2.3). The effect of concentrating the solution was most obvious when plants previously held at the LHL and

therefore with dry droplets were transferred to the HHL (Table 2.1b). This presumably favours the formation of relatively highly concentrated films of solution on the leaf surface as has been reported in the case of other chemicals (Middleton and Sanderson, 1965). This argument seems to be supported by the increased uptake noted following an increase in aminotriazole concentration (Figure 2.1). An increase in uptake with increasing concentration suggests that more chemical will be absorbed by the foliage as the aqueous droplets dry out. However, it would appear that the droplets dry out too quickly under low humidity for this effect to be noted in practice or to be of practical value as penetration was shown to cease or become very slow from solid deposits (Table 2.1).

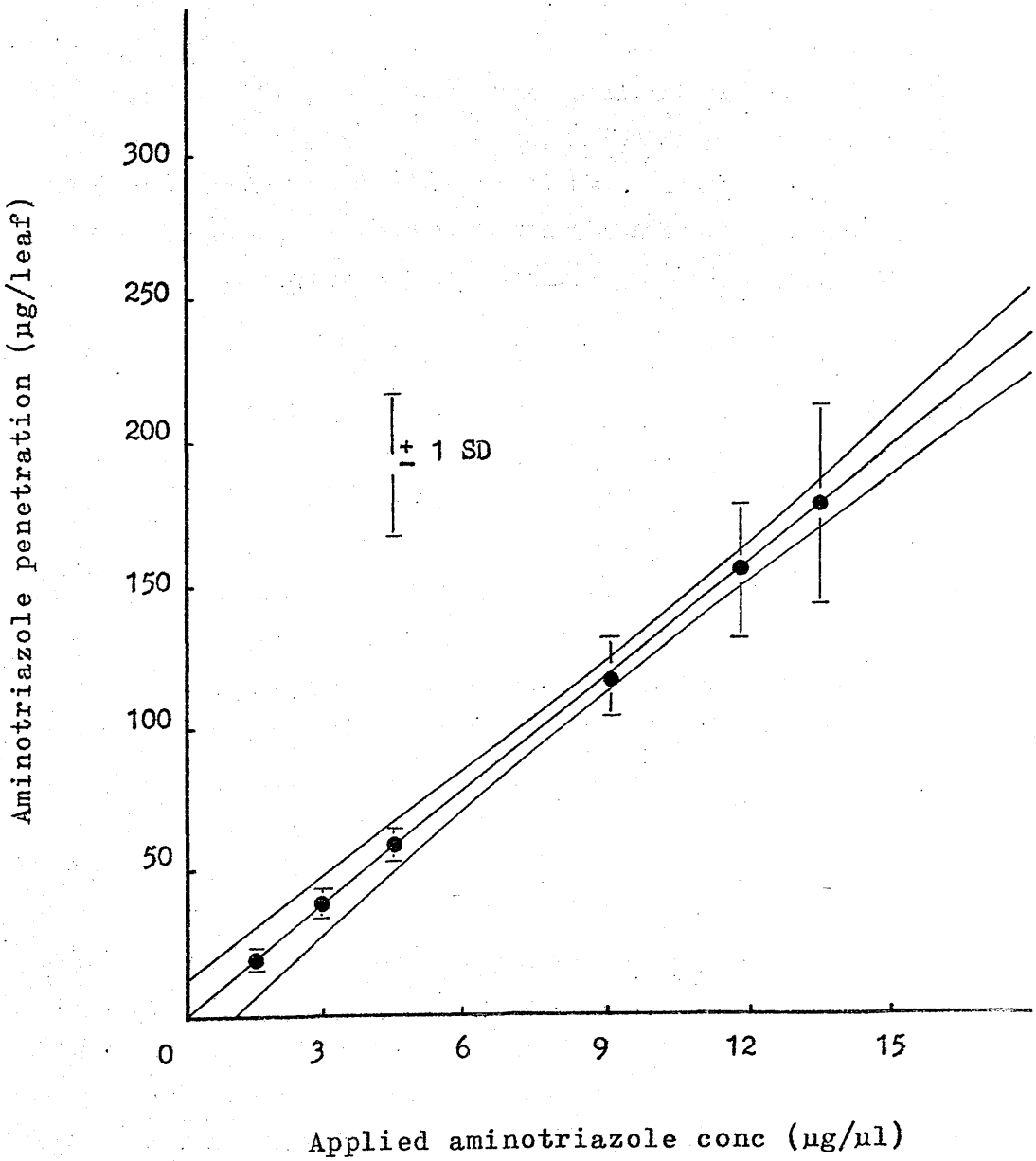
Table 2.3 Influence of rewetting on aminotriazole penetration with plants held at the low humidity level. Aminotriazole application rate - 60.0 µg/leaf (assessed after 4h).

Treatment	MQLS[a] (µg)	Mean% penetration	SD[b] (%)
No rewetting	52.5	12.5	3.4
One rewetting	51.8	13.6	2.4
Three rewettings	49.6	17.3	3.2
Seven rewettings	42.2	29.6	5.5
Plants at the HHL[c]	14.6	75.6	5.5

a Mean quantity of aminotriazole left on the leaf surface
b Standard deviation (n-1 degrees of freedom)
c High humidity level

A point of practical interest which arises from this work is that, other points being equal, if low humidity is followed by high humidity, an increase in the efficiency of aminotriazole penetration results. Therefore, an application between mid-day and evening when the humidity is likely to change from low to

Figure 2.1 The effect of concentration on aminotriazole penetration assessed after 2·5h with plants held at the high humidity level. (The curved lines on either side of the computed best straight line are the 95% confidence limits)



high during the night (Fogg, 1947; Brian, 1966) may be more advantageous than applying the chemical in the early morning (Volger, 1969) when the reverse is likely to occur. It should, however, be pointed out that penetration will always be greater under high humidity. Under low humidity, the chemical will tend to crystallise out on the leaf surface. Recrystallisation will deplete the concentration of herbicide in solution and so curtail penetration.

2.3.2 Influence of polysorbate 20 on penetration

At all concentrations tested, polysorbate 20 enhanced penetration at the LHL (Table 2.4a). The lowest surfactant concentration (0.2g/litre) gave the smallest increase over the aqueous control (9.8%) followed by increases of greater than 70% with polysorbate 20 concentrations covering the range 3.2 - 12.8g/litre. An apparent reduction in the degree of enhancement of penetration was noted at a concentration of 40g/litre.

This differential response to polysorbate 20 concentration was more obvious when the experiments were carried out over a shorter time interval and using slightly different surfactant concentrations (Table 2.4b).

A slightly modified pattern of response to increasing polysorbate 20 concentration was observed at the HHL. An enhancement in penetration (>40%) was noted for the concentration range 0.2 - 3.2g/litre (Table 2.4c). In a subsequent experiment, where the polysorbate 20 concentration was varied over a wider range, the same pattern of response was found to hold at low surfactant concentrations (0.2 and 3.2g/litre) while an apparent decrease in the degree of enhancement (non-significant) was noted at higher surfactant concentrations (6.4 and 12.8g/litre). At a concentration of 40g/litre, penetration was even lower (by 12.2%) than for the aqueous control (Table 2.4d). The decrease in penetration observed with increasing surfactant concentration corresponds to a similar pattern of behaviour which has been noted in practice with other chemicals (Corkins, 1960; Black and Wilson, 1969).

The difference in response to surfactant concentration

Table 2.4 Influence of polysorbate 20 on aminotriazole penetration. Aminotriazole application rate - 60.0 µg/leaf.

Polysorbate 20 (g/litre)	MQLS[a] (µg)	Mean % penetration	SD[b] (%)
(a) Plants held at the LHL[d] (assessed after 4h)			
0.0	50.4	16.0	3.7
0.2	44.5	25.8	8.1
3.2	4.2	93.0	5.4
6.4	3.8	93.7	1.9
12.8	5.8	90.4	1.8
40.0	9.9	83.5	2.3
(b) Plants held at the LHL[d] (assessed after 2h)			
0.0	55.4	7.7	3.5
0.2	53.9	10.2	5.9
0.8	50.3	16.1	3.4
6.4	33.0	45.0	7.0
40.0	24.4	42.6	15.9
80.0	43.6	27.4	7.6
(c) Plants held at the HHL[c] (assessed after 2h)			
0.0	28.9	51.9	17.0
0.2	1.8	97.0	2.9
0.4	0.8	98.6	1.3
0.8	1.7	97.2	1.7
1.6	3.3	94.5	2.8
3.2	3.1	94.8	1.4
(d) Plants held at the HHL[c] (assessed after 2h)			
0.0	15.1	74.9	13.4
0.2	1.7	97.1	2.4
3.2	6.8	88.7	4.7
6.4	9.9	83.5	6.9
12.8	12.5	79.1	9.0
40.0	22.4	62.7	10.0

Polysorbate 20 (g/litre)	MQLS[a] (µg)	Mean% penetration	SD[b] (%)
(e) Selected concentrations of polysorbate 20 (based on results obtained previously at the HHL[c] and the LHL[d]) (assessed after 2h)			
0.0[c]	11.5	80.9	16.3
0.0[d]	55.9	6.8	5.7
0.2[c]	2.3	96.1	0.3
0.2[d]	52.3	12.8	7.2
6.4[c]	4.3	92.8	1.8
6.4[d]	23.9	60.2	13.9

a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n - 1 degrees of freedom)

c High humidity level

d Low humidity level

was quite apparent when the penetration of aminotriazole at two surfactant concentrations (0.2 and 6.4g/litre) was compared at both humidity levels. Both surfactant treatments gave comparable increases in penetration over the aqueous controls at the HHL while at the LHL the degree of enhancement with the higher surfactant concentration was more than eight times the figure obtained with the lower surfactant concentration (Table 2.4e).

When the polysorbate 20 concentration was fixed at 3.2g/litre and the aminotriazole concentration varied between 30.0 and 720.0 µg in 20 µl (Table 2.5a), the percentage penetration of aminotriazole was reduced from 78.3% at the lowest aminotriazole level (aminotriazole/polysorbate 20 ratio of 1/2) to 20.6% at the highest level (aminotriazole/polysorbate 20 ratio of 12/1) and aminotriazole recrystallisation was noted in the 180.0 - 720.0 µg range. However, when the ratio of aminotriazole to polysorbate 20 was kept approximately constant (1/2), similar penetration figures were obtained irrespective of the aminotriazole concentration (Table 2.5b) and no recrystallisation was observed. In addition, in all cases where a 1/2 aminotriazole/surfactant ratio was compared against the equivalent aminotriazole concentration but the original polysorbate 20 concentration (3.2g/litre) the differences were significant.

These results suggest that solubilisation of aminotriazole in the surfactant plays a key role in penetration. This is supported by various reports on other herbicides (Black and Wilson, 1969; Babiker, 1976). In addition, these findings indicate that the ratio of herbicide to surfactant is of central importance to the process of penetration and could be responsible, at least in part, for the conflicting reports which have appeared on herbicide - surfactant interactions.

When aminotriazole (600.0 µg in 200 µl) in 6.4g/litre polysorbate 20 (1/2, aminotriazole/surfactant ratio) was applied to plants at the LHL (Figure 2.2) and both the aminotriazole and polysorbate 20 measured at hourly intervals, the results brought out several points:-

Table 2.5 Influence of the aminotriazole/polysorbate 20 w/w ratio on aminotriazole penetration with plants held at the low humidity level. Aminotriazole applied in a volume of 20 µl/leaf.

ATA[e] concn. (µg)	ATA/Polysorbate 20 ratio (approx.)	MQLS[a] (µg)	Mean % penetration	SD[b] (%)
(a) Polysorbate 20 concentration fixed at 3.2g/litre (assessed after 2.5h)				
30	1/2	6.5	78.3	5.5
60	1/1	25.7	57.1	18.9
120	2/1	54.6	54.5	13.6
180	3/1	138.6	23.0	14.7
240	4/1	171.1	28.7	10.7
360[f]	6/1	322.6	10.4	11.6
720[g]	12/1	571.1	20.6	16.1
(b) Both the aminotriazole and the polysorbate 20 concentration varied (assessed after 2h)				
30	1/2	6.3	79.0	9.9
120	1/2	37.4	68.8	19.5
120	2/1	71.5	40.4	15.8
180	1/2	51.7	71.3	14.8
180	3/1	130.7	27.4	13.4
240	1/2	99.6	58.5	20.1
240	4/1	179.0	25.4	8.7

a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n-1 degrees of freedom)

e Aminotriazole

f Necrosis around sites of application

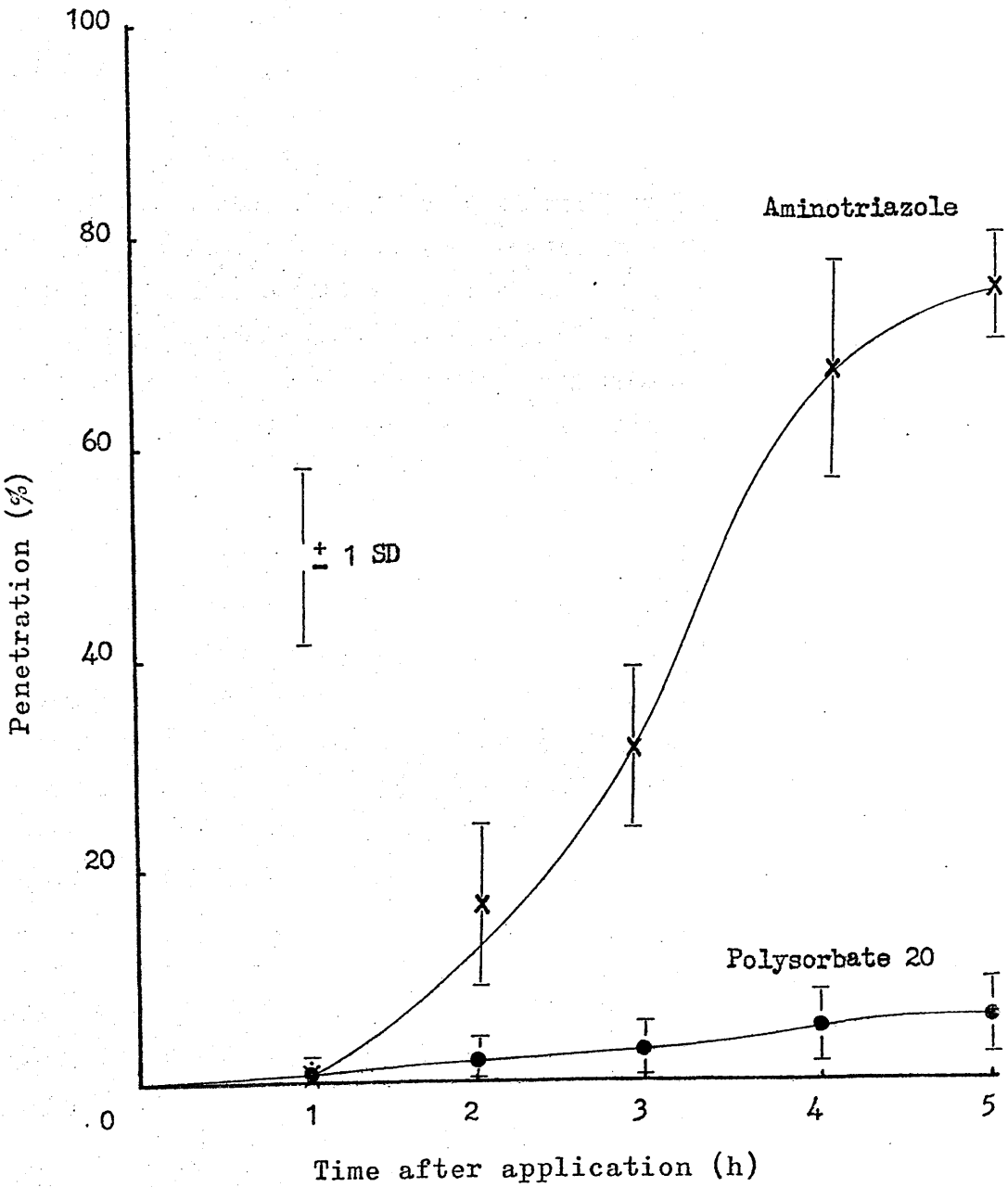
g Severe necrosis around sites of application

The surfactant was not taken up at a comparable rate to the aminotriazole as has been shown to occur for other pesticide/surfactant combinations (Price, 1976). Only 5.4% of the polysorbate 20 was taken up compared with 77.4% of the aminotriazole after 5h. Thus, under the experimental conditions employed here, the polysorbate 20 could not be envisaged as acting as a medium for transporting the aminotriazole across the cuticle. This slow penetration of the polysorbate 20 would seem to support the findings of Smith and Foy (1966) who showed that its penetration into, and subsequent movement within several plant species was low. However, this does not exclude the possibility of the small percentage of polysorbate 20 entering the leaf having a considerable effect on aminotriazole penetration by (a) inducing swelling of the cutin and/or dissolving certain cuticular components (Furmidge, 1959; Jansen 1964a, 1964b) thereby increasing permeability, (b) solubilising into plasma membranes (Sirois, 1967), (c) more subtle biochemical effects on the cuticle or underlying membranes (Parr and Norman, 1965), (d) other, perhaps more specific, herbicide/surfactant interactions (Freed and Montgomery, 1958).

The other possibility does exist, however, that the small amount of surfactant entering the leaf is purely incidental to the aminotriazole penetration process and that the main functions of the polysorbate 20 are to (a) reduce surface tensions (Kirkwood, 1976) and remove air films between the spray droplets and the leaf surface (Currier and Dybing, 1959) thereby helping to establish contact between the droplets and the water continuum of the plant (Babiker and Duncan, 1975b) and possibly promote stomatal penetration (Currier and Dybing, 1959), (b) prevent aminotriazole recrystallisation after the loss of the volatile carrier (water) by acting as a cosolvent and subsequently to enable diffusion of the aminotriazole from the surfactant to the water continuum of the plant.

In addition, it can be seen from the graph of aminotriazole penetration (Figure 2.2) that there is a lag in penetration over the first 3h and in particular over the first 1h after

Figure 2.2 Influence of time on aminotriazole and polysorbate 20 penetration from an aminotriazole/polysorbate 20 formulation with plants held at the LHL. Polysorbate 20 conc 6·4 g/litre. Aminotriazole application rate 600·0 µg/leaf in 200 ul.



treatment. This would seem to be correlated with the observation that over this 3h period the spray droplets were slowly drying out. Between 3 and 4h there is a surge in penetration which again would seem to be correlated with the observation that by this stage the droplets were present as films, presumably mainly of polysorbate 20 and aminotriazole. Between 4 and 5h there is a noticeable decline in the rate of penetration. This could result from the depletion of the aminotriazole concentration as absorption proceeds. Similar results have been reported by Middleton and Sanderson (1965) using inorganic ions.

When the penetration of both polysorbate 20 and aminotriazole were compared at both humidity levels (Table 2.6) it was found that there was no significant difference in polysorbate 20 penetration after 6h at the LHL compared with 4h at the LHL followed by 2h at the HHL. Aminotriazole penetration was, however, significantly greater in the latter treatment. The same pattern of response also applied over 9h at the LHL compared with 4h at the LHL followed by 5h at the HHL. However, after 26h at the LHL, significantly more polysorbate had penetrated the leaf compared with 4h at the LHL followed by 22h at the HHL although the same trend of significantly greater aminotriazole penetration at the HHL remained. This increase in polysorbate 20 penetration was accompanied by considerable damage to the leaf around the application sites. Thus it would appear that the polysorbate 20 was in some way disrupting the leaf tissue and in this way gaining entry. The reason for the greater amount of damage at the LHL could be that the polysorbate was in the form of a more concentrated film on the leaf surface compared with the HHL. It has been shown to be capable of retaining more than 50% of its own weight in water at 95% relative humidity (Price, 1976).

It would therefore appear that the enhanced penetration of aminotriazole at the HHL is not due to any effects of increased polysorbate 20 penetration but more probably due to the effect of humidity on the leaf surface barrier and in particular the water continuum of the plant (Lynch and Sweet, 1971) and hence the availability of the aqueous route (Crafts, 1964).

Table 2.6 Influence of humidity on polysorbate 20 penetration and the subsequent effect on aminotriazole penetration. Polysorbate concentration 6.4g/litre. Aminotriazole application rate - 600.0 µg/leaf.

Treatment	Polysorbate 20 penetration (%)	SD[b] (%)	Aminotriazole penetration (%)	SD[b] (%)
4h LHL[d]	8.5	3.1	53.5	8.2
4h LHL + 2h HHL[c]	7.6	5.8	82.4	3.1
6h LHL	7.4	4.8	46.9	10.6
4h LHL + 5h HHL	13.7	6.0	88.2	3.1
9h LHL	10.2	3.3	63.1	6.7
4h LHL + 22h HHL[h]	17.3	3.4	97.6	0.3
26h LHL[f]	29.5	6.9	90.3	7.7

b Standard deviation (n-1 degrees of freedom)

c High humidity level

d Low humidity level

f Necrosis around sites of application

h Slight necrosis around sites of application

Thus, the differential response in penetration to surfactant concentration and the effects of humidity on this response (Table 2.4 - 2.6) may be attributed to the complexity of the penetration process and the diverse roles of the surfactant in the process. The net result could be considered as a balance of many factors including (a) solubilisation of aminotriazole in the surfactant, (b) a dilution effect which will become more apparent at higher surfactant concentrations, (c) depletion of concentration as absorption proceeds leading to a fall in the rate of penetration, (d) establishment of contact between the spray droplets and the water continuum of the plant, (e) increasing the permeability of the cuticle without necessarily penetrating the leaf, (f) partitioning of the aminotriazole between the surfactant and the leaf surface.

The significance of the contribution of some or all of these factors could vary under low and high humidity conditions thus leading to the differences in penetration response noted here at both humidity levels.

2.3.3 Influence of glycerol on penetration

Aminotriazole penetration was enhanced in the presence of glycerol at the LHL. Increases of between 1.3 and 31.6% compared with the aqueous control were noted in the presence of a range of glycerol concentrations (Table 2.7a). The increase obtained decreased markedly with increasing glycerol concentration. At the HHL, dilute glycerol solutions (0.1 - 1.3ml/litre) resulted in comparable or slightly reduced penetration (Table 2.7b), while in the case of more concentrated glycerol solutions (2.5 - 15.0ml/litre) reduced penetration occurred compared with the aqueous control (Table 2.7c).

2.3.4 Influence of polysorbate 20 - glycerol combinations on penetration

The addition of a range of polysorbate 20 concentrations (0.1 - 6.4g/litre) to a fixed concentration of glycerol (0.3ml/litre) at the HHL resulted in increased penetration (>30%) compared with the aqueous and glycerol controls (Table 2.8a). However, this increase was reduced to 11.3% at a high surfactant concentration (40g/litre).

A study of the interaction between polysorbate 20 (6.4g/litre), glycerol (0.6ml/litre) and their combination with humidity (Table 2.8b) revealed that (a) penetration was always significantly greater at the HHL when compared with the corresponding treatment at the LHL, (b) the greatest difference in penetration between the two humidity levels occurred with glycerol (53.5%), (c) only a minor difference in penetration (4.4%) occurred between the two humidity levels in the case of the polysorbate 20 - glycerol combination. On this occasion penetration appeared to be less affected by humidity than in other treatments.

The findings with glycerol and polysorbate 20 - glycerol combinations suggest that the role of glycerol in aminotriazole

Table 2.7 Influence of glycerol on aminotriazole penetration. Aminotriazole application rate - 60.0 µg/leaf.

Glycerol (ml/litre)	MQLS[a] (µg)	Mean% penetration	SD[b] (%)
(a) Plants held at the LHL[d] (assessed after 2h)			
0.0	59.2	1.3	2.6
0.6	40.3	32.9	18.7
2.5	42.4	29.4	5.8
5.0	44.5	25.9	6.3
10.0	52.3	12.9	10.5
15.0	58.4	2.6	3.0
(b) Plants held at the HHL[c] (assessed after 2h)			
0.0	3.2	94.7	7.0
0.1	4.0	93.4	5.5
0.2	3.5	94.1	6.7
0.3	8.1	86.5	14.7
0.6	8.8	85.4	9.8
1.3	19.4	67.6	13.0
(c) Plants held at the HHL[c] (assessed after 1.5h)			
0.0	39.0	35.0	13.6
2.5	51.2	14.7	3.2
5.0	55.6	7.3	3.7
7.5	54.1	9.8	4.2
10.0	55.7	7.1	5.7
15.0	53.5	10.8	12.2

a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n - 1 degrees of freedom)

c High humidity level

d Low humidity level

penetration is also the resultant of many interacting variables. Glycerol at low humidity will solubilise aminotriazole preventing recrystallisation and should aid penetration compared with the aqueous controls where the loss of the volatile carrier (water) is readily apparent. However, this advantage may be negated by the following (a) a dilution effect (see polysorbate 20 above), (b) glycerol may dehydrate the cuticle thus disrupting the plant water continuum which could affect the aqueous route and thereby hamper penetration (Crafts et al. 1958), (c) simply by remaining on the leaf surface, glycerol may reduce aminotriazole penetration. The continued presence of carrier on the leaf surface has been shown to hold back toxicants (Hartley, 1966). Penetration in this case can be envisaged as being a function of the equilibrium distribution ratio of the toxicant between the carrier and the leaf surface (Hoskins, 1962). Such adverse effects due to glycerol would be expected to increase at higher concentration levels. At high humidity cuticular dehydration and the significance of glycerol as a solubiliser of aminotriazole will both be less important while the dilution effect will increase. However, these arguments need not necessarily hold in the presence of a surfactant as the results here indicate (Table 2.8). The following explanation can be given for this (a) the surfactant will prevent or minimise disruption of the plant water continuum (Ashton and Crafts, 1973), (b) it will reduce the surface energy and thus may facilitate glycerol penetration which could in turn bring about increased aminotriazole penetration through its effect on leaf permeability (Gray, 1956), (c) more subtle interactions between glycerol and polysorbate 20 or between them and other components of the system cannot be ruled out at this stage.

When aminotriazole was applied in the presence of fixed concentrations of both polysorbate 20 and glycerol and measured at varying time intervals (Figure 2.3), the trends were very similar to those in Figure 2.2 with a lag in penetration over the first 0.5h, a surge over the next 0.5h followed by a decline in the rate of penetration over

Table 2.8 Influence of polysorbate 20 - glycerol combinations on aminotriazole penetration. Aminotriazole application rate - 60.0 µg/leaf.

Polysorbate 20 (g/litre)	Glycerol (ml/litre)	MQLS[a] (µg)	Mean% penetration	SD[b] (%)
(a) Plants held at the HHL[c] (assessed after 2h)				
0.0	0.0	23.9	60.1	22.0
0.0	0.3	23.9	60.1	16.4
0.1	0.3	2.5	95.8	9.0
0.2	0.3	1.1	98.1	3.4
6.4	0.3	1.9	96.8	2.9
40.0	0.3	17.2	71.4	5.7
(b) Plants held at both humidity levels and run simultaneously (assessed after 2h)				
6.4[c]	0.0[c]	3.2	94.7	2.8
6.4[d]	0.0[d]	15.7	73.8	11.6
0.0[c]	0.6[c]	16.0	73.4	6.1
0.0[d]	0.6[d]	48.1	19.9	10.1
6.4[c]	0.6[c]	1.0	98.4	1.9
6.4[d]	0.6[d]	3.6	94.0	2.7

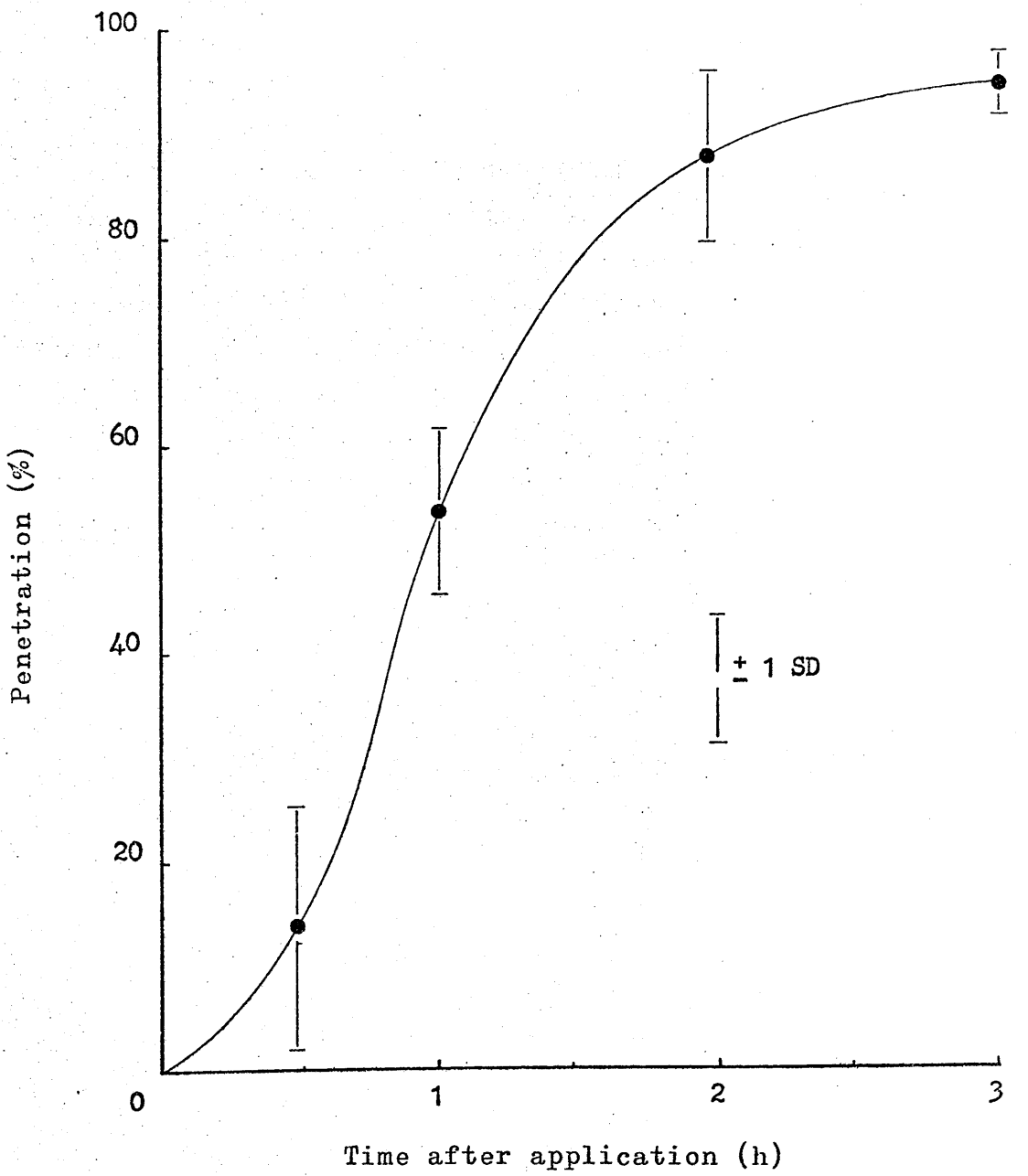
a Mean quantity of aminotriazole left on the leaf surface

b Standard deviation (n - 1 degrees of freedom)

c High humidity level

d Low humidity level

Figure 2.3 Influence of time on aminotriazole penetration in the presence of a polysorbate 20 (3·2 g/litre)-glycerol (0·6 ml/litre) combination, with plants held at the LHL. Aminotriazole application rate 60·0 µg/leaf in 20 µl.



the final 2h. The reasoning behind this would appear to be as for the previous figure (see 2.3.2). In this case, the lag was much shorter than before. This could be due to the difference in droplet size, ca 2 µl in this case compared with ca 5 µl in the previous exercise and/or differences in temperature and humidity.

From the experimental evidence obtained here, it would appear that aminotriazole penetration is a resultant of many interacting factors, some of which can be brought at least partially under control by means of additives. The study suggests that additives can perform several functions, some of which may be antagonistic. In addition, the results point out that the efficiency of additives is affected by their concentrations and their interaction with environmental variables such as humidity. It would seem that the latter factor can be brought at least partially under control by including a combination of additives (Table 2.3b). A polysorbate 20 - glycerol combination would appear to be an outstanding possibility in this regard. The effects of such treatments on translocation, selectivity and field performance of aminotriazole will be evaluated in Chapter Five.

Chapter Three

FOLIAR UPTAKE OF AMINOTRIAZOLE FROM HUMECTANT-SURFACTANT COMBINATIONS AND THE INFLUENCE OF HUMIDITY ON THEIR EFFECTS

3.1 INTRODUCTION

As with other additives, considerable controversy has appeared in the literature over the use of humectants such as glycerol, ethylene glycol and dimethyl sulphoxide (DMSO) as additives to improve the foliar uptake of herbicides (Lapham, 1966; Brady and Peevy, 1968; Hull and Morton, 1971; Shellhorn and Hull, 1971; Jones and Foy, 1972; Jones and Evans, 1973; Babiker and Duncan, 1975a; 1975b). Although DMSO is often classed apart from other humectants as a penetrant (Kanellopoulos, 1974), it does possess considerable humectant properties (Anon, 1968). Hull (1970) has suggested that the conflicting behaviour observed for sprays containing DMSO may be due in part to the concentration of DMSO employed, while Babiker and Duncan (1975b) have suggested that variations in humidity may account for the conflicting results observed for glycerol. The previous chapter revealed that glycerol could enhance aminotriazole penetration although the effect was concentration dependent; at higher concentrations, reduced and sometimes negative effects were observed. In addition, the combination of glycerol with polysorbate 20 was often more beneficial than using solely the humectant. This investigation initially set out to assess the influence on aminotriazole penetration of (a) DMSO, at various concentrations, (b) DMSO - polysorbate 20 combinations and (c) humidity in the presence of DMSO - polysorbate 20 combinations. However, it was later extended to include certain other industrial high boiling point humectants and solvents i.e. dimethyl formamide (DMF), glycerol, ethylene glycol and polypropylene glycol 400 (PPG 400) and other polysorbate surfactants. Consequently, the DMSO-polysorbate 20 combination has been studied in more detail.

All experiments were carried out under growth room conditions using bean plants (Phaseolus vulgaris var. Canadian Wonder) as the test species. Aminotriazole, polysorbate 20, glycerol and Phaseolus vulgaris were selected for the reasons previously described in Chapter Two. DMSO was selected for study because (a) it possesses a remarkable potential as a solvent for many types of inorganic and organic compounds including organic herbicides (Keil, 1965; Brady and Peevy, 1968), (b) it is reported as being able to facilitate both penetration and translocation (Lapham, 1966; Mussell et al., 1967; Foy et al., 1972) and (c) it is very hygroscopic (Anon, 1968). DMF was chosen for study since it has similar properties to DMSO and is often used where a solvent with a slow rate of evaporation is required (Anon 1968). Ethylene glycol was selected because it is commonly used as an industrial solvent and humectant (Anon, 1968) and has been used in penetration studies (Hull and Morton, 1971). PPG 400 was chosen for study because it is non volatile (Anon, 1971) and in the monomeric form is often used as a substitute for ethylene glycol and glycerol (Anon, 1968).

3.2 EXPERIMENTAL

3.2.1 Materials

Aminotriazole, polysorbate 20, glycerol and Phaseolus vulgaris were as previously described (see 2.2.1). Polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate, polyoxyethylene 20 sorbitan mono-oleate and polyoxyethylene 20 sorbitan trioleate (polysorbate 40, 60, 80 and 85 respectively; "Tweens 40, 60, 80 and 85" - registered trade mark of the Atlas Chemical Industry Incorporated) were purchased from Koch-Light Laboratories Ltd. DMSO and PPG 400 were purchased from British Drug Houses Ltd. DMF and ethylene glycol were purchased from Hopkin and Williams Ltd.

3.2.2 Methods

3.2.2.1 Penetration

All penetration experiments were performed in a growth room adjusted to a 16h day length and a temperature of $30 \pm 3^{\circ}c$. The plants were germinated, selected, treated and washed as

previously described (see 2.2.2.1). In all treatments, a total volume of 20 µl was applied per leaf. The solutions again persisted as discrete droplets as opposed to spreading over the leaf surface. Unless otherwise stated, humidity was maintained at 50$^{+}_{-}$ 10% relative humidity (r.h.).

In one experiment, plants were either left exposed in the growth chamber (approx. 30% r.h.) or covered with polythene bags for the reasons previously described (see 2.2.2.1), (100% r.h.).

In the studies concerning the effect of humidity on surfactant-humectant combinations, large desiccators containing phosphorus pentoxide, sodium hydroxide pellets, magnesium nitrate and water were used to maintain the humidity within the desiccators at prescribed levels. This was monitored using Fischer Type 111 hair hygrometers contained within the desiccators. A few hours prior to the commencement of these humidity studies, bean plants were transferred from pots to small conical flasks containing water. These were then sealed off around the stem of the plant at the mouth of the flask using sealing tissue (Gallenkamp Ltd; "Parafilm") to prevent evaporation from the flask as this tended to saturate the atmosphere despite the desiccants. Two plants were placed in each desiccator which was then sealed and left until the humidity had equilibrated. (It was found that using more than two plants per desiccator greatly reduced the extent to which humidity could be controlled).

On equilibration of the humidity, plants to be used in the experiment were treated and two were immediately placed in each of the four desiccators to replace those used to equilibrate the environment i.e. four replicates per treatment. The humidity did tend to fluctuate at change-over, but re-equilibrated within approximately 15 min.

In all other experiments, eight or ten leaves per treatment were used and unless otherwise stated, the timing of the experiments was from the moment of application of the toxicant to the leaf. Termination of the experiments was to some degree arbitrary although it was generally taken as the point when the majority of the droplets in the experiment as a whole appeared to have dried out. In some treatments,

there was considerable variation in penetration, this would appear to be linked with the observation that droplets (even on the same leaf) tended to dry out at considerably differing rates. This was more obvious at some humidity levels than others.

Large differences between results observed following application of the standard aqueous aminotriazole solution can be explained in terms of slight variations (a) within the humidity range employed, (b) in plant age and (c) in growing conditions prior to treatment.

3.2.2.2 Influence of humectants on penetration

Aminotriazole (60.0 or 180.0 µg in 20 µl) was applied in the presence of a range of DMSO, DMF, ethylene glycol and PPG 400 concentrations.

3.2.2.3 Influence of humectant-surfactant combinations on penetration

1. Aminotriazole (60.0 or 180.0 µg in 20 µl) was applied in the presence of DMSO, ethylene glycol, PPG 400 or glycerol (5.0ml/litre) or DMF (50.0ml/litre) and a range of polysorbate 20 concentrations (0.2-1.0g/litre) and 0.2-5.0g/litre for DMSO.

2. Aminotriazole (180.0 µg in 20 µl) was applied in the presence of DMF (50.0ml/litre) or DMSO, ethylene glycol, PPG 400 or glycerol (5.0ml/litre) and/or polysorbate 20 (0.2g/litre).

3. Aminotriazole (180.0 µg in 20 µl) was applied in the presence of DMSO or glycerol (5.0ml/litre) and one of a range of polysorbate surfactants (0.2g/litre).

4. Aminotriazole (30.0-240.0 µg in 20 µl) was applied in the presence of DMSO (5.0ml/litre) and polysorbate 20 (0.2g/litre).

3.2.2.4 Influence of humidity on penetration from humectant-surfactant combinations

1. Aminotriazole (180.0 µg in 20 µl) was applied in the presence of DMSO, ethylene glycol or PPG 400 (5.0ml/litre) and polysorbate 20 (0.2g/litre) to plants held under differing humidity regimes.

2. Aminotriazole (60.0 µg in 20 µl) was applied under the following treatment schemes:-

(i) In the presence of DMSO (5.0ml/litre) and polysorbate 20 (0.2g/litre) and the plants placed under polythene bags when most of the water had evaporated from the droplets.

(ii) In the absence of the above additives and the plants placed under polythene bags when most of the water had evaporated from the droplets.

(iii) In the presence of DMSO (5.0ml/litre) and polysorbate 20 (0.2g/litre) and the plants left exposed in the growth chamber.

3.2.2.5 Influence of a range of DMSO-polysorbate 20 combinations on penetration and the influence of humidity on them

1. Aminotriazole (180.0 µg in 20 µl) was applied in the presence of a range of DMSO concentrations (0.0-50.0ml/litre) and polysorbate 20 (0.2g/litre).

2. Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a range of DMSO concentrations (0.0-50.0ml/litre) and polysorbate 20 (3.2g/litre).

3. Aminotriazole (180.0 µg in 20 µl) was applied in the presence of a range of DMSO concentrations (0.0-100.0ml/litre) and polysorbate 20 (0.2g/litre). Humidity was maintained at 30-40% r.h.).

3.2.2.6 Aminotriazole estimation

Aminotriazole concentrations were determined by the method of Storherr and Burke (1961).

3.2.2.7 Statistical analysis

Analysis of the data was carried out as previously described (see 2.2.2.8).

3.3 RESULTS AND DISCUSSION

3.3.1 Influence of humectants on penetration

3.3.1.1 DMSO

Despite the variability encountered, aminotriazole penetration was significantly enhanced at the 1.0, 5.0 and 10.0ml/litre DMSO levels. There was no significant difference in penetration between the lowest and highest DMSO levels (0.1 and 50.0ml/litre) and the aqueous control (Table 3.1a).

3.3.1.2 Ethylene glycol

At none of the concentrations tested did ethylene glycol significantly enhance aminotriazole penetration. At the 50.0ml/litre level, penetration at 14.9% was significantly less than for the aqueous control (33.8%) (Table 3.1b).

3.3.1.3 DMF

Only at the highest DMF concentration employed (50.0ml/litre) was aminotriazole penetration significantly enhanced, but even then, penetration was only 28.1% compared with 18.4% for the aqueous control. (Table 3.1c).

3.3.1.4 PPG 400

Again, despite the variability encountered, penetration was significantly enhanced from 41.1% to 78.9% at the 10.0ml/litre level while at the 50.0ml/litre level, as with ethylene glycol, penetration was again reduced, to 18.2% (Table 3.1d).

From the above results and observations, the following points can be drawn:-

1. In many of the treatments, the droplets appeared to have dried out and recrystallisation of the aminotriazole was in evidence. Thus, further penetration under these conditions would be minimal as penetration has been shown to cease or proceed very slowly from solid deposits (see 2.3.1).

2. Although the droplets were still wet and no recrystallisation of the aminotriazole appeared to have occurred at the 50ml/litre levels of DMSO, ethylene glycol and PPG 400, there was no significant difference between this level of DMSO

Table 3.1 Influence of humectants on aminotriazole penetration.

	Humectant (ml/litre)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a)	DMSO	ATA[d] application rate 180.0µg/leaf (assessed after 1.5h)			
	0.0	160.2	11.0	7.6	dry, recryst.
	0.1	159.2	11.4	6.6	dry, recryst.
	1.0	111.4	38.1	26.0	dry, recryst.
	5.0	95.9	46.7	14.8	dry, recryst.
	10.0	110.2	38.8	19.3	dry, recryst.
	50.0	148.1	17.7	11.7	wet, no recryst.
(b)	Ethylene glycol	ATA application rate 180.0µg/leaf (assessed after 5h)			
	0.0	119.2	33.8	11.9	dry, recryst.
	1.0	116.6	35.2	18.0	dry, recryst.
	5.0	115.6	35.8	13.6	dry, recryst.
	10.0	103.7	42.4	13.0	dry, recryst.
	50.0	153.2	14.9	7.8	wet, no recryst.
(c)	DMF	ATA application rate 60.0µg/leaf (assessed after 2.5h)			
	0.0	49.0	18.4	8.5	dry, recryst.
	1.0	46.8	22.0	10.0	dry, recryst.
	5.0	48.2	19.7	6.3	dry, recryst.
	10.0	43.6	27.4	11.0	dry, recryst.
	50.0	43.1	28.1	6.3	dry, recryst.
(d)	PPG 400	ATA application rate 180.0µg/leaf (assessed after 3.5h)			
	0.0	106.0	41.1	28.8	dry, recryst.
	1.0	66.2	63.2	37.0	dry, sl. recryst.
	5.0	59.9	66.7	21.1	dry, sl. recryst.
	10.0	38.0	78.9	8.8	dry, sl. recryst.
	50.0	147.2	18.2	7.3	wet, no recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n - 1 degrees of freedom)

d Aminotriazole

and the aqueous control while in the case of ethylene glycol and PPG 400, there was a significant reduction in penetration compared with the aqueous controls. The following points were previously put forward to explain this in the case of glycerol (see 2.3.4) and would appear to hold in this situation. The solubilisation of the toxicant by the humectant is negated by the following (a) a dilution effect which will become more obvious at higher humectant concentrations, (b) the humectant may dehydrate the cuticle, thus disrupting the plant water continuum which could affect the aqueous route and thereby hamper penetration and (c) simply by remaining on the leaf surface the humectant may hold back aminotriazole penetration. Thus, penetration can be envisaged as being a function of the equilibrium distribution ratio of the toxicant between the carrier (humectant) and the leaf surface.

However, these experiments were conducted under controlled environmental conditions and over short time intervals. Therefore, these adverse effects might be lessened if the experiments were carried out over much longer time intervals; furthermore, under natural conditions, variations in humidity could lead to the possibility of humectant penetration at high humidity or loss of water at low humidity, thus altering the equilibrium distribution ratio between the carrier and the leaf surface.

3.3.2 Influence of humectant-surfactant combinations on penetration

Similar patterns of response were found for all humectants tested when applied in the presence of a range of polysorbate 20 combinations (Table 3.2).

In the case of DMF, the addition of 50.0ml/litre brought about no enhancement in penetration over the aqueous control while the addition of the surfactant to the DMF brought about an approximate six-fold increase in the percentage penetration over both the aqueous and humectant controls (Table 3.2a). The addition of ethylene glycol (5.0ml/litre) brought about no significant enhancement in penetration over the aqueous control, however, the inclusion of the polysorbate 20 brought about a

Table 3.2 Influence of combinations of humectants and polysorbate 20 on aminotriazole penetration.

Humectant (ml/litre)	P20[e] (g/litre)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a) DMF	ATA[d] application rate 60.0µg/leaf (assessed after 2h)				
0.0	0.0	51.0	15.0	12.5	dry, recryst.
50.0	0.0	51.5	14.2	5.2	dry, recryst.
50.0	0.2	5.4	91.0	5.4	dry, no recryst.
50.0	0.6	7.0	88.3	10.2	dry, no recryst.
50.0	1.0	7.1	88.1	2.7	dry, no recryst.
(b) Ethylene glycol	ATA application rate 180.0µg/leaf (assessed after 3.5h)				
0.0	0.0	121.3	32.6	12.0	dry, recryst.
5.0	0.0	116.3	35.4	10.8	dry, recryst.
5.0	0.2	8.6	95.2	8.2	dry, no recryst.
5.0	0.6	6.5	96.4	4.6	dry, no recryst.
5.0	1.0	4.5	97.5	0.6	dry, no recryst.
(c) PPG 400	ATA application rate 180.0µg/leaf (assessed after 2.5h)				
0.0	0.0	139.5	22.5	9.9	dry, recryst.
5.0	0.0	83.0	53.9	17.0	dry, recryst.
5.0	0.2	27.2	84.9	7.3	dry, no recryst.
5.0	0.6	22.1	87.7	15.8	dry, no recryst.
5.0	1.0	18.0	90.0	6.6	dry, no recryst.
(d) Glycerol	ATA application rate 180.0µg/leaf (assessed after 2.5h)				
0.0	0.0	164.7	8.5	6.6	dry, recryst.
5.0	0.0	159.1	11.6	2.4	dry, recryst.
5.0	0.2	6.7	96.3	3.1	dry, no recryst.
5.0	0.6	18.7	89.6	11.6	dry, no recryst.
5.0	1.0	21.2	88.2	9.5	dry, no recryst.
(e) DMSO	ATA application rate 180.0µg/leaf (assessed after 1h)				
5.0	0.0	139.0	22.8	6.3	dry, recryst.
5.0	0.2	8.6	95.2	2.5	dry, no recryst.
5.0	0.6	4.3	97.6	2.0	dry, no recryst.
5.0	1.0	5.0	97.2	4.7	dry, no recryst.
5.0	3.0	6.3	96.5	2.3	surfactant film, no recryst.
5.0	5.0	15.7	91.3	9.8	surfactant film, no recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n - 1 degrees of freedom)

d Aminotriazole

e Polysorbate 20

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three-fold enhancement over both the aqueous and humectant controls (Table 3.2b). The addition of PPG 400 (5.0ml/litre) to the solution significantly enhanced penetration while the addition of the surfactant further increased its effect significantly (Table 3.2c). The addition of glycerol (5.0ml/litre) to the solution did not significantly enhance aminotriazole penetration, however, the further addition of polysorbate 20 brought about an approximate eleven-fold increase in percentage penetration over the aqueous control and an eight-fold increase over the glycerol control (Table 3.2d). In the case of DMSO where a slightly different approach was adopted in that the DMSO control was compared with DMSO combined with a wider polysorbate 20 range it was found that all DMSO-polysorbate 20 combinations brought about an approximate four-fold enhancement in percentage penetration over the DMSO control (Table 3.2e).

In each case (Table 3.2a - e), penetration from these humectant-surfactant combinations was always within the 80 - 100% range while the level of surfactant employed did not appear to be critical as there were no significant differences over the surfactant ranges employed.

When combinations of polysorbate 20 (0.2g/litre) with DMF (50.0ml/litre), ethylene glycol, PPG 400, glycerol or DMSO (5.0ml/litre) were compared with the effects of the additives individually (Table 3.3a - e) it was found that the effects of the combinations were more than additive over the individual effects of the surfactant and the humectant in all cases except DMSO-polysorbate 20 where the effect was approximately additive.

When a range of polysorbate surfactants (polysorbate 20, 40, 60, 80 and 85) (0.2g/litre) were combined with DMSO (5.0ml/litre) (Table 3.4a), each DMSO-polysorbate combination significantly enhanced penetration over the DMSO control by a factor of approximately four. In all cases, penetration for these combinations was in the 90-100% range and there were no significant differences between the combinations. A similar pattern of response was found for glycerol-polysorbate 20 combinations (5.0ml + 0.2g/litre) (Table 3.4b) where an

Table 3.3 Influence of humectants and polysorbate 20, alone and in combination, on aminotriazole penetration. Aminotriazole application rate 180.0µg/leaf.

Humectant (ml/litre)	P20[e] (g/litre)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a) DMF (assessed after 2.5h)					
0.0	0.0	170.6	5.2	6.1	dry, recryst.
0.0	0.2	135.5	24.7	18.5	dry, recryst.
50.0	0.0	163.4	9.2	6.5	dry, recryst.
50.0	0.2	45.7	74.6	18.4	dry, sl. recryst.
(b) Ethylene glycol (assessed after 2h)					
0.0	0.0	180.0	0.0	0.0	dry, recryst.
0.0	0.2	173.9	3.4	4.9	dry, recryst.
5.0	0.0	136.8	24.0	12.5	dry, recryst.
5.0	0.2	55.3	69.3	16.8	dry, sl. recryst.
(c) PPG 400 (assessed after 3h)					
0.0	0.0	180.0	0.0	0.0	dry, recryst.
0.0	0.2	130.3	27.6	24.2	dry, recryst.
5.0	0.0	150.8	16.2	21.8	dry, recryst.
5.0	0.2	36.2	79.9	21.4	dry, sl. recryst.
(d) Glycerol (assessed after 2h)					
0.0	0.0	175.3	2.6	3.6	dry, recryst.
0.0	0.2	178.6	0.8	2.4	dry, recryst.
5.0	0.0	153.0	15.0	5.4	dry, recryst.
5.0	0.2	51.5	71.4	11.1	dry, sl. recryst.
(e) DMSO (assessed after 1.5h)					
0.0	0.0	162.7	9.6	7.2	dry, recryst.
0.0	0.2	125.5	30.3	21.2	dry, recryst.
5.0	0.0	103.3	42.6	16.7	dry, recryst.
5.0	0.2	38.3	78.7	22.3	dry, sl. recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n - 1 degrees of freedom)

e Polysorbate 20

Table 3.4 Influence of combinations of humectants and various polysorbate surfactants on aminotriazole penetration. Aminotriazole application rate 180.0µg/leaf.

Humectant (ml/litre)	Surfactant (0.2g/litre)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a) DMSO	(assessed after 2h)				
5.0	-	139.7	22.4	11.6	dry, recryst.
5.0	polysorbate 20	5.4	97.0	2.0	dry, no recryst.
5.0	polysorbate 40	10.4	94.2	3.7	dry, no recryst.
5.0	polysorbate 60	9.2	94.9	3.9	dry, no recryst.
5.0	polysorbate 80	5.2	97.1	1.8	dry, no recryst.
5.0	polysorbate 85	3.4	98.1	0.8	dry, no recryst.
(b) Glycerol	(assessed after 3h)				
5.0	-	130.9	27.3	12.9	dry, recryst.
5.0	polysorbate 20	7.4	95.9	0.7	dry, no recryst.
5.0	polysorbate 40	19.3	89.3	6.7	dry, no recryst.
5.0	polysorbate 60	18.9	89.5	3.0	dry, no recryst.
5.0	polysorbate 80	22.3	87.6	12.8	dry, no recryst.
5.0	polysorbate 85	6.7	96.3	1.1	dry, no recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n - 1 degrees of freedom)

approximate three-fold increase in penetration over the glycerol control was achieved. In all cases, penetration from these combinations was in the 85-100% range.

An interesting response was noted when aminotriazole (30.0-240.0 µg in 20 µl) was applied in the presence of fixed concentrations of DMSO (5.0ml/litre) and polysorbate 20 (0.2g/litre) (Table 3.5). Percentage penetration rose significantly from 56.8% at the lowest aminotriazole level to >90% at the two highest levels (180.0 and 240.0 µg in 20 µl). In turn, this meant that the actual amount of aminotriazole being taken up by the leaf rose constantly throughout the range of concentrations applied. However, the amount remaining on the leaf surface remained quite constant.

Table 3.5 Influence of aminotriazole concentration on penetration from a DMSO-polysorbate 20 combination (5.0ml + 0.2g/litre). Aminotriazole applied in a volume of 20µl/leaf. (Assessed after 2.5h).

ATA[d] concn(µg)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
30	13.0	56.8	9.3	dry, no recryst.
60	12.4	79.3	8.0	dry, no recryst.
120	16.0	86.7	7.6	dry, no recryst.
180	12.2	93.2	5.0	dry, no recryst.
240	17.0	92.9	6.9	dry, no recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n-1 degrees of freedom)

d Aminotriazole

3.3.3 Influence of humidity on penetration from humectant-surfactant combinations

The effect of humidity on aminotriazole uptake from humectant-surfactant combinations was, despite a certain amount of variability, quite obvious (Table 3.6). In the case of DMSO-polysorbate 20 (Table 3.6a), at low humidity (<30% r.h.) the droplets appeared to dry out quite quickly (0.5h approx.) and substantial recrystallisation occurred. On completion of the experiment, penetration at this humidity level was very low (3.1%). At a slightly higher humidity level ($45^{+}_{-}10$% r.h.) the droplets remained moist for a much longer period and there was no indication of recrystallisation on drying, and on completion of the experiment. Penetration at this level was considerably greater (86.8%). At 55-65% r.h. penetration was again reduced (48.2%). In this case, some droplets had dried out (no recrystallisation) while others still appeared quite moist. This, as was suggested earlier (see 3.2.2.1), may help to explain the variability encountered. At 100% r.h. all droplets appeared quite moist and penetration was negligible (0.3%).

Similar trends in penetration and observations on the state of droplet drying and recrystallisation to the above were also found for ethylene glycol (Table 3.6b) and PPG 400 (Table 3.6c).

In the above three cases, it was shown that at the highest humidity levels employed (80-100% r.h. approx.) the spray droplets remained quite moist and penetration was negligible. However, it was shown for DMSO-polysorbate 20 (Table 3.6d) that, if most of the water was allowed to evaporate off from the droplets and the plants were then quickly enclosed within polythene bags where humidity would be expected to approach 100% relatively quickly (see 2.3.1), penetration was quite substantial (85.4%) whereas a similarly treated aqueous control showed considerably less uptake (32.1%). In addition, plants treated with the same DMSO-polysorbate 20 combination and left exposed in the growth room (30% r.h. approx.) showed considerably less uptake (30.0%) than their equivalents under bags.

Table 3.6 Influence of humidity on the effect of certain combinations of humectants (5.0ml/litre) and polysorbate 20 (0.2g/litre) on aminotriazole penetration. Aminotriazole application rate 180.0µg/leaf.

Humidity (% r.h.)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a) DMSO	(assessed after 2.5h)			
< 30	174.4	3.1	4.4	dry, recryst.
35–55	23.8	86.8	12.1	dry, no recryst.
55–65	93.2	48.2	35.5	dry/wet, no recryst.
100	179.5	0.3	0.5	wet, no recryst.
(b) Ethylene glycol	(assessed after 3.5h)			
< 30	165.4	8.1	6.6	dry, recryst.
40–60	30.8	82.9	22.6	dry, no recryst.
50–70	62.3	65.4	26.5	dry/wet, no recryst.
80–90	175.3	2.6	3.9	wet, no recryst.
(c) PPG 400	(assessed after 4h)			
30–40	173.0	3.9	7.8	dry, recryst.
40–50	13.5	92.5	3.0	dry, no recryst.
60–80	21.4	88.1	5.6	dry, no recryst.
90–100	175.9	2.3	4.0	wet, no recryst.

(d) DMSO (assessed 1.5h after most of the water had evaporated from the droplets) Aminotriazole application rate 60.0µg/leaf.

DMSO (ml/litre)	P20[e] (g/litre)	MQLS (µg)	MP (%)	SD (%)	Droplet appearance
5.0	0.2(100% r.h.)	8.8	85.4	10.0	dry, no recryst.
0.0	0.0(100% r.h.)	40.7	32.1	13.5	sl. wet, sl. recryst.
5.0	0.2(~30% r.h.)	42.0	30.0	12.1	dry, recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n - 1 degrees of freedom)

e Polysorbate 20

3.3.4 Influence of a range of DMSO-polysorbate 20 combinations on penetration as influenced by humidity

The results in Table 3.7(a-c) indicate that the adverse effects of humidity can to some extent be overcome by varying the DMSO concentration. The results depicted in Table 3.7(a) and (b) working at 50±10% r.h. revealed several points:-

1. The addition of a fixed level of polysorbate 20 to a range of DMSO concentrations (1.0-50.0ml/litre) demonstrated that although the concentration of polysorbate 20 within the range studied was not critical (see 3.3.2), the DMSO concentration was.

2. The results in Tables 3.7(a) and (b) showed similar trends in increased aminotriazole penetration, optimising at DMSO levels of 5.0-10.0ml/litre, despite using different polysorbate 20 and aminotriazole concentrations - the DMSO concentration range being the only constant factor.

3. A significant reduction in penetration was observed at the 50.0ml DMSO/litre level over the 2h period employed (droplets appeared moist).

The results of Table 3.7(c) on the other hand, obtained working at a lower humidity level (30-40% r.h.) and over a longer period of time (5h) revealed that the combination containing 50.0ml DMSO/litre brought about significantly higher penetration than the 5.0 and 10.0ml/litre levels, the optima in the previous two tables. At the 100ml/litre level, penetration had decreased slightly and there was considerable contact necrosis.

A point which arose from these results was that the optimum enhancement in aminotriazole penetration was less obvious at the higher polysorbate 20 concentration when DMSO combinations were compared with the surfactant controls (Table 3.7(a) and (b). At the low polysorbate 20 concentration (0.2g/litre) considerable recrystallisation took place. This was not the case at the higher level (3.2g/litre). This would tend to support previous findings (see 2.3.2) which suggested that solubilisation of the aminotriazole in the surfactant plays a key role in penetration.

Table 3.7 Influence of a range of DMSO-polysorbate 20 combinations on aminotriazole penetration and the effect of humidity on them.

DMSO (ml/litre)	P20[e] (g/litre)	MQLS[a] (µg)	MP[b] (%)	SD[c] (%)	Droplet appearance
(a) Aminotriazole application rate 180.0µg/leaf. (assessed after 2h), 50±10% r.h.					
0.0	0.2	144.9	19.5	19.1	dry, recryst.
0.1	0.2	81.1	50.5	31.1	dry, recryst.
1.0	0.2	110.9	38.4	30.0	dry, recryst.
5.0	0.2	36.4	79.8	11.8	dry, sl. recryst.
10.0	0.2	15.7	91.3	3.8	dry, no recryst.
50.0	0.2	65.5	63.6	18.6	dry/wet, no recryst.
(b) Aminotriazole application rate 60.0µg/leaf. (assessed after 2h), 50±10% r.h.					
0.0	3.2	21.7	63.8	31.5	surfactant film, no recryst.
0.1	3.2	12.7	78.8	18.1	surfactant film, no recryst.
1.0	3.2	9.7	83.9	14.4	surfactant film, no recryst.
5.0	3.2	2.4	96.0	1.7	surfactant film, no recryst.
10.0	3.2	5.0	91.7	5.5	surfactant film, no recryst.
50.0	3.2	13.8	77.0	10.0	dry/wet, no recryst.
(c) Aminotriazole application rate 180.0µg/leaf. (assessed after 5h), 30-40% r.h.					
0.0	0.0	176.9	1.7	1.9	dry, recryst.
5.0	0.2	46.8	74.0	18.4	dry, sl. recryst.
10.0	0.2	33.5	81.4	9.4	dry, sl. recryst.
50.0	0.2	10.3	94.3	1.9	dry, no recryst.
100.0[f]	0.2	21.8	87.9	5.7	dry, no recryst.

a Mean quantity of aminotriazole left on the leaf surface

b Mean penetration

c Standard deviation (n-1 degrees of freedom)

e Polysorbate 20

f Severe contact necrosis

3.4 CONCLUSIONS

From the experimental evidence obtained here, it would appear that under the conditions employed in this study, penetration is not greatly influenced by the addition of humectants to spray solutions of aminotriazole. However, the additional inclusion of a surfactant brought about substantial increases in penetration over both the aqueous and humectant controls. This effect seemed to apply equally to a range of humectant-polysorbate surfactant mixtures. The increased penetration following the addition of the surfactant could be due to a reduction in surface tension. This could influence the penetration of the humectants themselves which could, in turn, increase cell membrane permeability (Gray, 1956; Hull, 1970). The alternative hypothesis is that the humectants do not penetrate the leaf but merely act as co-solvents, maintaining the aminotriazole in solution while the surfactant encourages better contact between the spray droplets and the water continuum of the plant, thereby encouraging partitioning of the aminotriazole into the leaf (see 2.3.2). However, in many cases, in particular where DMF and DMSO were involved, it was observed that those droplets containing humectant-surfactant combinations appeared to have dried out before those containing only humectants. This suggests that penetration of the humectant as well as volatilisation may be involved.

The influence of humidity on uptake from these humectant-surfactant combinations was found to be quite critical. This could be explained in terms of the following; at low humidity, volatilisation of those humectants which are volatile may take place more readily than at higher humidity levels thus leading to recrystallisation of the toxicant, while in the case of those which are non volatile, the droplets may dry down to a highly viscous residue which may behave essentially like a crystalline deposit. At high humidity, the toxicant solution will be relatively dilute and hence penetration may be curtailed. Further work at low humidity which involved varying the humectant concentration suggested that this adverse effect might be at least partially overcome by increasing the humectant

concentration although this would require further investigation. Similarly, under high humidity conditions it might be possible to enhance uptake by reducing the spray volume and/or introducing a more volatile carrier than water. Reducing the spray volume would appear to have certain possibilities since the data in Table 3.5 showed that efficiency of penetration could be increased by increasing the concentration of aminotriazole in solution or equally reducing the volume of carrier in which the aminotriazole is dissolved, while at the same time keeping the percentage concentration of the humectant and surfactant the same.

Thus, if one regards the data as a whole the following suggestions might be made:-

1. At low humidity a relatively high humectant concentration may be required for efficient penetration.

2. At medium and high humidity a low carrier volume may be required while the humectant concentration can be relatively low.

The effects of such treatments on translocation, selectivity and field performance of aminotriazole will again be evaluated in Chapter Five.

Chapter Four

MODE OF ACTION OF THIOCYANATE AND IODIDE IN AMINOTRIAZOLE FORMULATIONS

4.1 INTRODUCTION

Aminotriazole is commonly used in combination with the contact herbicide ammonium thiocyanate (NH_4SCN). This is probably one of the few truly synergistic herbicide combinations known (Crafts, 1961). On occasion, NH_4SCN has been used in combination with other herbicides but with perhaps less dramatic results (Robison, 1965; Greenham, 1970; Stritzke, 1972). Various reasons have been put forward attempting to explain this synergistic combination of aminotriazole and NH_4SCN although some of the evidence is conflicting. Babiker and Duncan (1975a) have shown that NH_4SCN increases aminotriazole absorption by bracken, while Donnalley and Ries (1964) found it had no effect on aminotriazole absorption by quackgrass regardless of the time of application (i.e. one day before, one day after or in combination with the aminotriazole). However, when applied one day before or in combination with the aminotriazole, it increased translocation over aminotriazole alone and NH_4SCN applied one day after. They proposed that the NH_4SCN lessened rapid damage to cells at the absorption site, thereby permitting the foliage to absorb and translocate over a longer period of time. In contrast to this, Forde (1966) showed that aminotriazole translocation from quackgrass leaves was retarded over 12h by NH_4SCN. In agreement with Donnalley and Ries (1964) however, he suggests that since over a 96h period NH_4SCN moved very little compared with the considerable translocation of aminotriazole, that the synergistic effect of NH_4SCN was at the treatment area, rather than the ultimate site

of action. This is further confirmed by van der Zweep (1965) who in addition showed that a more pronounced synergistic response could be obtained using 6-benzyladenine instead of NH_4SCN.

The formation of conjugates between aminotriazole and endogenous plant constituents such as amino acids and sugars appears to be the major metabolic alteration of aminotriazole in higher plants (Ashton and Crafts, 1973). Most of these are less toxic than aminotriazole and their formation can be considered to be a detoxication mechanism (Ashton and Crafts, 1973). However, Carter (1965) has proposed that the speed and extent of aminotriazole transformation indicates that metabolic alteration probably contributes to the phytotoxicity of the compound and any change in the rate or extent of metabolic breakdown could lead to changes in phytotoxicity. One of the major metabolic products formed has been identified as 3-(3-amino-1,2,4-triazole-1-yl)-2-aminopropionic acid (3-ATAL) (Massini, 1963; Carter, 1965). Its formation is largely accepted as representing detoxication since it does not appear to be nearly as toxic as aminotriazole or as mobile (Massini, 1963). Furthermore, Carter (1965) has shown that its formation is greatly retarded by the presence of NH_4SCN. Other divalent sulphur compounds were tested, but none were as effective as NH_4SCN (Carter, 1969).

Several authors (Sund et al., 1960; Hilton, 1962; Castelfranco et al., 1963) have shown that the inhibition of growth and chlorophyll development caused by aminotriazole can be reversed if riboflavin or some of its derivatives are supplied to the plant simultaneously with the aminotriazole. Two hypotheses have been put forward to explain this :- 1. aminotriazole inhibits riboflavin synthesis (Sund et al., 1960; Sund and Little, 1960). 2. riboflavin brings about the destruction of aminotriazole (Castelfranco et al., 1963).

It has also been shown that aminotriazole can be incorporated into protein upon incubation with riboflavin in the presence of light (Castelfranco and Brown, 1963; Brown and Carter, 1968) and that the photochemical riboflavin - light system can be replaced by chemical free radical generating systems such as ascorbic acid, cupric sulphate and molecular oxygen (Castelfranco and Brown, 1963).

In addition, Carter et al. (1969) found that serine and cysteine had no effect on this alkylation of aminotriazole onto bovine serum albumin, whereas cystine and methionine slightly inhibited the reaction and NH_4SCN was strongly inhibitory.

Taken collectively, these points could be envisaged as pointing towards the role of NH_4SCN as preventing rapid formation of conjugates at the absorption site, probably by inhibiting free radical reactions. Riboflavin could be important in this respect although any number of endogenous free radical generating systems could be involved. Thus, this could permit more efficient distribution of the toxic moiety from the site of absorption to the conducting tissues and hence allow more efficient translocation to the root system.

Thus, the purpose of this investigation was to assess the effect of

(a) NH_4SCN on free radical generating systems which have been shown to be capable of oxidising aminotriazole, namely (1) the photochemical riboflavin - light system (Castelfranco and Brown, 1963; Brown and Carter, 1968) (ii) a chemical system :- ferrous sulphate ($FeSO_4$) - hydrogen peroxide (H_2O_2) (Fenton's Reagent) (Plimmer et al., 1967).

(b) other compounds on these systems with a view to incorporating successful ones into aminotriazole formulations for spraying bracken since results have shown that NH_4SCN increases aminotriazole translocation in bracken and reduces its necrotic foliar toxicity (see Chapter Five). These compounds include (i) ammonium (NH_4^+) salts - to assess the effect of the NH_4^+ ion, (ii) thiocyanate (SCN^-) salts - to assess the effect of the SCN^- ion, (iii) thiocyanate related compounds such as cyanide, ferricyanide and ferrocyanide salts, (iv) various halide salts, including iodide (I^-) salts which are known to inhibit free radical reactions and in particular, have been shown to inhibit certain steps in the photodegradation of riboflavin involving the flavin triplet state (Weimar and Neims, 1975), (v) EDTA - which should promote oxidation by acting as an electron donor for the riboflavin (Merkel and Nickerson, 1954), (vi) 6-benzylaminopurine (6-benzyladenine) which van der Zweep (1965) showed to have a more pronounced synergistic effect on aminotriazole formulations

than did NH_4SCN, and several organic sulphur compounds which Carter (1969) showed to have various effects on 3-ATAL formation in the trifoliate leaves of bean plants.

(c) the aforementioned free radical generating systems on asulam (methyl (4 - aminobenzenesulphonyl) carbamate) oxidation and the influence of a range of compounds on them. These include ammonium salts, thiocyanate and related salts and various halide salts. In addition, its rate of oxidation in the riboflavin - light system was compared with that of aminotriazole with and without the addition of NH_4SCN.

(d) NH_4SCN and other compounds which successfully inhibit aminotriazole oxidation, on aminotriazole uptake by bean leaves (Phaseolus vulgaris var. Canadian Wonder), to compare their effects and to measure their rates of foliar uptake. Initially, this study was confined to KI and NH_4SCN but was later extended to include a range of iodide and thiocyanate salts.

4.2 EXPERIMENTAL

4.2.1 Materials

Aminotriazole was as previously described (see 2.2.1). Asulam (methyl (4 - aminobenzenesulphonyl) carbamate) was purchased from National Physical Laboratory. Riboflavin was purchased from Sigma London Chemical Company Ltd. All other reagents were purchased from British Drug Houses Ltd or Hopkin and Williams Ltd.

4.2.2.Methods

4.2.2.1 Oxidation studies

(a) Riboflavin photosensitised oxidation

In all studies the reaction mixtures were constantly shaken in 50ml volumetric flasks under fluorescent lighting for the duration of the experiment (24-72h). These reaction mixtures consisted of 5.0ml sodium phosphate buffer (0.1M, pH 7.2), 2.0ml riboflavin solution (100.0µg/ml) and the concentrations of aminotriazole or asulam and additives as required.

Additive concentrations were expressed as molar ratios of additive/aminotriazole and additive/asulam although the actual concentrations of additives used were the same for both herbicides. This system was adopted for convenience of presentation and because the commercial formulation of aminotriazole is based on a 1/1 molar ratio of NH_4SCN/aminotriazole. However, it is important to bear in mind that it is the ratio of the free radical generating capacity of the system to the concentration of free radical inhibitor which is the governing factor in the overall oxidation process (Weimar and Neims, 1975).

After the addition of the additives, all reaction mixtures were made up to the same but minimum possible volume with de-ionised water. On completion of the experiments, the reaction mixtures were made up to 50.0ml. Aliquots were withdrawn and determined for aminotriazole by the method of Storherr and Burke (1961) or for asulam by the method of Bratton and Marshall (1939) adapted for asulam by Brockelsby and Muggleton (1973).

(b) Oxidation by hydroxyl radicals - $FeSO_4$-H_2O_2 (Fenton's Reagent).

Reaction mixtures consisted of 1.5ml of 0.2M $FeSO_4$ in 0.1M H_2SO_4 and 3.0ml of 1% w/v H_2O_2. To this was added 2,400.0 µg of aminotriazole or asulam and the required additive. Again, all reaction mixtures were made up to the same minimum possible volume with de-ionised water. These were then shaken for the duration of the experiment, normally 1-2h. On completion of the experiment, 75.0 or 100.0 µl aliquots were withdrawn from the mixture, made up to 5.0ml and analysed for asulam or aminotriazole respectively. Analysis was as previously described (see 4.2.2.1(a)).

Neither the riboflavin, Fenton's reagent or any of the additives except 6-benzyladenine interfered with the asulam and aminotriazole determinations.

Each reaction mixture was carried out in duplicate.

4.2.2.2 Influence of NH_4SCN on the riboflavin photosensitised oxidation of aminotriazole.

Aminotriazole (600.0 µg) was shaken in a reaction mixture as per 4.2.2.1(a) in the presence of various concentrations of NH_4SCN (0-5/1 M/M NH_4SCN/aminotriazole).

4.2.2.3 Influence of NH_4SCN on the oxidation of aminotriazole by Fenton's Reagent.

Aminotriazole (2,400.0 µg) was shaken in a reaction mixture as per 4.2.2.1(b) in the presence of various concentrations of NH_4SCN (0-23/1 M/M NH_4SCN/aminotriazole).

4.2.2.4 Influence of various additives on the riboflavin photosensitised oxidation of aminotriazole

Aminotriazole (600.0 µg) was shaken as per 4.2.2.1(a) in the presence of various additives at a 5/1 molar ratio of additive/aminotriazole.

4.2.2.5 Influence of various additives on the oxidation of aminotriazole by Fenton's Reagent.

Aminotriazole (2,400.0 µg) was shaken as per 4.2.2.1(b) in the presence of various additives at a 14/1 molar ratio of additive/aminotriazole.

4.2.2.6 Comparison of the influence of iodide and thiocyanate salts on the riboflavin photosensitised oxidation of aminotriazole.

Aminotriazole (600.0 µg) was shaken as per 4.2.2.1(a) in the presence of NaI, KI, NH_4SCN and KSCN at a 5/1 molar ratio of salt/aminotriazole.

4.2.2.7 Influence of various additives on the riboflavin photosensitised oxidation of asulam.

Asulam (600.0 µg) was shaken as per 4.2.2.1(a) in the presence of various additives at a 13.5/1 molar ratio of additive/asulam.

4.2.2.8 Influence of various additives on the oxidation of asulam by Fenton's Reagent.

Asulam (2,400.0 µg) was shaken as per 4.2.2.1(b) in the presence of NH_4SCN, NaI and NH_4NO_3 at a 38/1 molar ratio of additive/asulam.

4.2.2.9 Comparison of the rates of riboflavin photosensitised oxidation of aminotriazole and asulam.

Aminotriazole and asulam (7.14×10^{-6} moles, 600.0 and 1643.0 µg respectively) were shaken as per 4.2.2.1(a) in the presence and absence of a 5/1 molar ratio of NH_4SCN/aminotriazole or asulam.

4.2.2.10 Penetration studies

Initial studies (those results depicted in Tables 4.8 and 4.9) were carried out in a growth room adjusted to a 16h day length and a temperature of $30 \pm 3^{\circ}C$. Humidity within the room was not controlled and was found to vary between 35-65%r.h. All other studies were carried out in a Fisons Series III Growth Cabinet (Model 600G3/THTL) adjusted to a 16h day length and a temperature of $30 \pm 0.75^{\circ}C$. These were carried out at two humidity levels, 48 ± 5%r.h. and 64 ± 5%r.h. All plants were grown in the growth room at $30 \pm 3^{\circ}C$ as previously described (see 2.2.2.1). When the studies involved using the Fisons growth cabinet, the plants were transferred to it from the growth room and treated on the same day. Treating and washing was as previously described (see 2.2.2.1). In the studies concerned with the effects of iodide and thiocyanate salts on aminotriazole uptake, the solutions were placed randomly as discrete droplets (ca 2 µl) on the upper leaf surface in a total

volume of 20 µl/leaf by means of a 10 µl Eppendorf pipette. In those studies concerned with the uptake of iodide and thiocyanate salts the solutions were placed randomly as discrete droplets (ca 5 µl) on the upper leaf surface in a total volume of 200 µl/leaf by means of a 100 µl Eppendorf pipette. All treatment solutions persisted as discrete droplets as opposed to spreading over the leaf surface. In all experiments, eight leaves per treatment were used. The timing of the experiments was from the moment of application of the toxicant to the leaf. On completion of the experiments, the 20 µl/leaf and 200 µl/leaf treatments were washed with 10 and 25ml respectively of de-ionised water and the washings analysed for aminotriazole by the method of Storherr and Burke (1961) and for iodide and thiocyanate by Volhard titration (Vogel, 1961). Preliminary experiments revealed that 100% recovery of all chemicals was possible if washing was performed immediately after treatment.

4.2.2.11 Influence of NH_4SCN and KI on aminotriazole penetration.

(a) Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a range of NH_4SCN concentrations (0 - 5/1 molar ratio of NH_4SCN/aminotriazole).

(b) Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a range of KI concentrations (0 - 5/1 molar ratio of KI/aminotriazole).

(c) Aminotriazole (60.0 µg in 20 µl) was applied in the presence of 1/1 and 3/1 molar ratios of NH_4SCN and KI/aminotriazole.

4.2.2.12 Penetration of aminotriazole and NH_4SCN or KI

Aminotriazole (600.0 µg in 200 µl) was applied in the presence of a 3/1 molar ratio of NH_4SCN or KI/aminotriazole.

4.2.2.13 Influence of various iodide salts on aminotriazole penetration.

Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a 3/1 molar ratio of sodium iodide (NaI), calcium iodide (CaI_2), magnesium iodide (MgI_2) or KI/aminotriazole. This experiment was

carried out at two humidity levels, 48±5% r.h. and 64±5% r.h.

4.2.2.14 Influence of various thiocyanate salts on aminotriazole penetration

Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a 3/1 molar ratio of potassium thiocyanate (KSCN), sodium thiocyanate (NaSCN), calcium thiocyanate ($Ca(SCN)_2$) or NH_4SCN/aminotriazole. Again, this experiment was carried out at 48±5% r.h. and 64±5% r.h.

4.2.2.15 Influence of polysorbate 20 on aminotriazole penetration from a MgI_2 formulation.

Aminotriazole (60.0 µg in 20 µl) was applied in the presence of a 3/1 molar ratio of MgI_2/aminotriazole and a range of polysorbate 20 concentrations (0.0-5.0g/litre). This experiment was carried out at 64±5% r.h.

4.2.2.16 Penetration of aminotriazole and thiocyanate or iodide salts.

Aminotriazole (600.0 µg in 200 µl) was applied in the presence of a 3/1 molar ratio of NH_4SCN, KSCN, KI or NaI/ aminotriazole. This experiment was carried out at both humidity levels.

4.2.2.17 Influence of droplet size on aminotriazole penetration from NH_4SCN and KSCN formulations

Aminotriazole (60.0 µg in 20 µl and 600.0 µg in 200 µl) was applied in the presence of a 3/1 molar ratio of NH_4SCN or KSCN/ aminotriazole. The experiment was carried out at both humidity levels.

4.2.2.18 Statistical analysis

This was carried out as previously described (see 2.2.2.8).

4.3 RESULTS

4.3.1 Oxidation studies

4.3.1.1a Influence of NH_4SCN on the riboflavin photosensitised oxidation of aminotriazole

In the absence of riboflavin, no oxidation of the aminotriazole took place under fluorescent lighting over the 72h reaction period. However, in the presence of riboflavin, almost 80% of the aminotriazole was oxidised. In the molar ratio range 0.01 - 1/1, the NH_4SCN had no significant effect on oxidation. However, at 2/1, oxidation was reduced to 47.5% and at 5/1 to 8.9% (Table 4.1a).

4.3.1.1b Influence of NH_4SCN on the oxidation of aminotriazole by Fenton's Reagent

In the absence of NH_4SCN from the reaction mixture, 93.7% of the aminotriazole was oxidised in the 2h reaction period. The addition of NH_4SCN reduced this oxidation at all levels tested in a continuous trend from 85.2% with a 0.5/1 molar ratio to 0.7% with a ratio of 23/1. In those reaction mixtures containing NH_4SCN, there was considerable production of hydrogen cyanide (HCN) (Table 4.1b).

4.3.1.2 Influence of various additives on the riboflavin photosensitised oxidation of aminotriazole

Aminotriazole oxidation was greatly decreased in the presence of all thiocyanate salts tested i.e. ammonium, potassium, sodium and calcium. It was also strongly decreased by potassium cyanide and to a lesser degree by potassium ferrocyanide. Potassium ferricyanide had no significant effect on oxidation. Ammonium salts such as acetate, nitrate and tri-ammonium citrate had little or no effect. Of the halides tested, only iodide salts strongly inhibited aminotriazole oxidation. Bromide, chloride and

Table 4.1 Influence of ammonium thiocyanate (NH_4SCN) on aminotriazole oxidation in free radical generating systems.

NH_4SCN/ATA[a] M/M	MQAU[b] (µg)	Mean % oxidation	SD[c] (%)
(a) Riboflavin photosensitised oxidation. Aminotriazole 600.0 µg/reaction mixture (assessed after 72h).			
0/1 (no riboflavin)	600.0	0.0	0.0
0/1	133.2	77.8	2.7
0.01/1	151.8	74.7	1.8
0.1/1	132.6	77.9	6.3
1.0/1	170.4	71.4	4.5
2.0/1	315.0	47.5	4.5
5.0/1	546.6	8.9	3.6
(b) Oxidation by hydroxyl radicals (Fenton's Reagent). Aminotriazole 2,400.0 µg/reaction mixture (assessed after 2h).			
0/1	151.2	93.7	3.0
0.5/1	355.2	85.2	1.0
2.5/1	523.2	78.2	1.0
4.5/1	794.4	66.9	1.0
14/1	2349.6	2.1	1.0
23/1	2333.2	0.7	1.0

a Aminotriazole

b Mean quantity of aminotriazole unoxidised

c Standard deviation (n-1 degrees of freedom)

Table 4.2 Influence of various additives on the riboflavin photosensitised oxidation of aminotriazole. Additive/aminotriazole 5/1 M/M. Aminotriazole 600.0 µg/reaction mixture (assessed after 72h).

Additive	MQAU[b] (µg)	Mean % oxidation	SD[c] (%)
(a)			
None (no riboflavin)	600.0	0.0	0.0
None	157.2	73.8	1.8
Ammonium thiocyanate	547.2	8.8	1.8
Potassium thiocyanate	543.6	9.4	2.7
Potassium ferricyanide	172.2	71.3	1.8
Potassium ferrocyanide	430.8	28.2	0.9
Ammonium acetate	127.2	78.8	0.0
Ammonium nitrate	127.2	78.8	5.3
(b)			
None	184.8	69.2	1.8
Sodium fluoride	159.0	73.5	6.2
Sodium chloride	148.8	75.2	1.6
Sodium bromide	99.6	83.4	2.6
Sodium iodide	474.0	21.0	1.7
Potassium bromide	133.2	77.8	5.2
Potassium iodide	529.8	11.7	4.4
Potassium cyanide	577.8	3.7	0.0
(c)			
None	138.0	77.0	5.4
EDTA	0.0	100.0	0.0
Urea	145.8	75.7	7.3
tri-Ammonium citrate	92.4	84.6	9.1
Calcium thiocyanate	453.6	24.4	10.9
Sodium thiocyanate	507.6	15.4	1.8

Table 4.2 (contd).

Additive	MQAU[b] (µg)	Mean % oxidation	SD[c] (%)
(d)			
None (no riboflavin)	600.0	0.0	0.0
None	168.6	71.9	0.8
Ammonium thiocyanate	528.6	11.9	4.4
Thioacetamide	228.6	61.9	4.4
L-Cysteine hydrochloride	160.8	73.2	11.5
Thiourea	337.2	43.8	0.0
6-Benzyladenine	265.8	55.7	2.6
6-Benzyladenine riboside	325.8	45.7	0.9

b Mean quantity of aminotriazole unoxidised

c Standard deviation (n-1 degrees of freedom)

fluoride salts either had no effect or slightly increased oxidation. Urea had no effect, while EDTA strongly increased oxidation. Of the organic sulphur compounds tested, only thiourea had any great effect on oxidation, but its effect was still far less than that of NH_4SCN. The effect of 6-benzyladenine riboside was comparable to that of thiourea. In the case of the less soluble 6-benzyladenine which did not totally dissolve, the solution was filtered and an aliquot taken for determination. It showed less effect on oxidation than its riboside (Table 4.2).

4.3.1.3 Influence of various additives on the oxidation of aminotriazole by Fenton's Reagent.

Ammonium and potassium thiocyanate both strongly inhibited aminotriazole oxidation while other ammonium salts such as acetate, nitrate and tri-ammonium citrate either had no effect or slightly inhibited oxidation. (Table 4.3a).

Of the halide salts tested, bromide and iodide strongly inhibited oxidation while fluoride and chloride had much less effect. When an iodide salt was present, molecular iodine was formed during the reaction (Table 4.3b).

Of the organic sulphur compounds tested (Table 4.3c) both L - cysteine hydrochloride and thiourea had effects comparable to that of NH_4SCN, thioacetamide was less effective.

Ferricyanide, ferrocyanide and cyanide were not tested since they complexed with the iron from the Fenton's Reagent, producing a blue precipitate. 6-Benzyladenine and 6-benzyladenine riboside both interfered with the aminotriazole determination at the levels being tested. However, it appeared that they did not have such a pronounced effect on oxidation as thiocyanate salts. For this reason, further investigation of these compounds was not pursued.

4.3.1.4 Comparison of the influence of iodide and thiocyanate salts on the riboflavin photosensitised oxidation of aminotriazole.

In the absence of riboflavin, no aminotriazole was oxidised, while in its presence, 73.5% oxidation occurred over the 48h reaction

Table 4.3 Influence of various additives on the oxidation of aminotriazole by hydroxyl radicals (Fenton's Reagent). Additive/aminotriazole 14/1 M/M. Aminotriazole 2,400.0 µg/reaction mixture (assessed after 1h).

Additive	MQAU[b] (µg)	Mean % oxidation	SD[c] (%)
(a)			
None	276.0	88.5	1.0
Ammonium thiocyanate	2270.4	5.4	0.0
Potassium thiocyanate	2284.8	4.8	6.7
Ammonium acetate	1020.0	57.5	0.9
Ammonium nitrate	276.0	88.5	1.0
tri-Ammonium citrate	859.2	64.2	1.0
(b)			
None	489.6	79.6	0.8
Sodium fluoride	1274.4	46.9	0.0
Sodium chloride	976.8	59.3	0.0
Sodium bromide	2030.4	15.4	0.8
Sodium iodide	2073.6	13.6	0.0
Potassium bromide	2133.6	11.1	0.0
Potassium iodide	2119.2	11.7	0.8
(c)			
None	537.6	77.6	3.2
Ammonium thiocyanate	2102.4	12.4	1.6
Thioacetamide	1483.2	38.2	1.6
L-Cysteine hydrochloride	2224.8	7.3	10.3
Thiourea	2102.4	12.4	0.0

b Mean quantity of aminotriazole unoxidised

c Standard deviation (n-1 degrees of freedom)

period. The addition of the following four salts: NH_4SCN, KSCN, KI or NaI reduced aminotriazole oxidation to less than 10% in all cases, and there were no significant differences between any of the four oxidation levels (Table 4.4).

4.3.1.5 Influence of various additives on the riboflavin photosensitised oxidation of asulam.

Similar trends in oxidation to those found for aminotriazole were observed for asulam. NH_4SCN, KSCN and potassium ferrocyanide all greatly reduced asulam oxidation. Potassium ferricyanide had much less effect while ammonium acetate and ammonium nitrate had no effect (Table 4.5a).

Iodide salts also greatly reduced oxidation while potassium cyanide reduced it significantly but to a lesser degree. Fluoride chloride and bromide salts had little or no effect (Table 4.5b).

4.3.1.6 Influence of various additives on the oxidation of asulam by Fenton's Reagent.

Again, a similar pattern of response to that obtained for aminotriazole was observed. Virtually all the asulam (97.7%) was destroyed within 1h. Ammonium nitrate had no significant effect on this while NH_4SCN and NaI reduced the level of oxidation significantly although the degree of reduction was not nearly so great as was found for aminotriazole (Table 4.6). This may be due to the ease of oxidation of the asulam or the state of activity of the Fenton's reagent. This factor was not deemed important enough at that stage to warrant further investigation.

4.3.1.7 Comparison of the rates of riboflavin photosensitised oxidation of aminotriazole and asulam.

The results of Table 4.7 indicate that aminotriazole is more readily oxidised than asulam (74.3% compared with 58.3%) while the addition of NH_4SCN reduced asulam oxidation to 9.6% and aminotriazole oxidation to 17.6%. This would possibly suggest

Table 4.4 A comparison of the influence of iodide and thiocyanate salts on the riboflavin photosensitised oxidation of aminotriazole. Additive/aminotriazole 5/1 M/M. Aminotriazole 600.0 µg/reaction mixture (assessed after 43h).

Additive	MQAU[b] (µg)	Mean % oxidation	SD[c] (%)
None (no riboflavin)	600.0	0.0	0.0
None	159.0	73.5	0.9
Ammonium thiocyanate	551.4	8.1	2.6
Potassium thiocyanate	555.6	7.4	3.5
Potassium iodide	555.6	7.4	1.7
Sodium iodide	540.6	9.9	3.5

b Mean quantity of aminotriazole unoxidised

c Standard deviation (n-1 degrees of freedom)

Table 4.5 Influence of various additives on the riboflavin photosensitised oxidation of asulam. Additive/asulam 13.5/1 M/M. Asulam 600.0 µg/reaction mixture (assessed after 24h).

Additive	MQAsU[d] (µg)	Mean % oxidation	SD[c] (%)
(a)			
None (no riboflavin)	600.0	0.0	0.0
None	300.0	50.0	1.3
Ammonium thiocyanate	579.0	3.5	4.9
Potassium thiocyanate	568.2	5.3	2.5
Potassium ferricyanide	357.6	40.4	5.0
Potassium ferrocyanide	562.8	6.2	3.7
Ammonium acetate	279.0	53.5	1.3
Ammonium nitrate	279.0	53.5	3.7
(b)			
None	273.6	54.4	4.9
Sodium fluoride	252.6	57.9	9.9
Sodium chloride	304.8	49.2	2.5
Sodium bromide	321.0	46.5	6.2
Sodium iodide	562.8	6.2	1.2
Potassium bromide	315.6	47.4	2.5
Potassium iodide	562.8	6.2	1.2
Potassium cyanide	447.0	25.5	1.2

c Standard deviation (n-1 degrees of freedom)

d Mean quantity of asulam unoxidised

Table 4.6 Influence of various additives on the oxidation of asulam by hydroxyl radicals (Fenton's Reagent). Additive/asulam 33/1 M/M. Asulam 2,400.0 µg/reaction mixture (assessed after 1h).

Additive	MQAsU[d] (µg)	Mean % oxidation	SD[c] (%)
None	55.2	97.7	1.1
Ammonium nitrate	36.0	98.5	0.0
Ammonium thiocyanate	811.2	66.2	0.0
Sodium iodide	902.4	62.4	5.4

c Standard deviation (n-1 degrees of freedom)

d Mean quantity of asulam unoxidised

Table 4.7 Comparison of the rates of riboflavin photosensitised oxidation of aminotriazole and asulam. NH_4SCN/aminotriazole or asulam 5/1 M/M. Aminotriazole 600.0 µg/reaction mixture, asulam 1643.0 µg/reaction mixture (assessed after 48h).

Reaction mixture	MQHU[e] (µg)	Mean % oxidation	SD[c] (%)
Asulam alone	685.1	58.3	3.7
Asulam + NH_4SCN	1485.3	9.6	0.0
Aminotriazole alone	154.2	74.3	3.3
Aminotriazole + NH_4SCN	494.4	17.6	2.5

c Standard deviation (n-1 degrees of freedom)

e Mean quantity of herbicide unoxidised

that the state of activity of the Fenton's reagent was responsible for the results noted in 4.3.1.6.

4.3.2 Penetration studies

4.3.2.1 Influence of NH_4SCN and KI on aminotriazole penetration.

(a) NH_4SCN Aminotriazole penetration was significantly enhanced at all NH_4SCN levels tested. Penetration was increased from 13.5% in the aqueous control to 65.0% at the 1/1 molar ratio level. At the 2/1 level, penetration was enhanced further (80.1%). However, subsequent increases in NH_4SCN concentration had no further effect (Table 4.8a).

(b) KI Penetration was again considerably enhanced at all KI levels tested, increasing from 5.9% for the aqueous control to 70.7% at the 1/1 molar ratio level. The 2/1 and 3/1 molar ratio levels brought about further significant increases in penetration, to 76.8% and 87.2% respectively, while at the highest concentration tested (5/1), the trend began to reverse significantly (79.3%) (Table 4.8b).

(c) When the effects of KI and NH_4SCN on penetration were compared at two selected levels (1/1 and 3/1 molar ratios) the following points were observed. At the 1/1 level both KI and NH_4SCN significantly enhanced penetration over the aqueous control. However, penetration from the KI formulation was significantly greater than from the NH_4SCN (54.2% as against 36.6%).

A similar trend was found at the 3/1 level. Again, penetration was significantly enhanced over the aqueous control and also over the respective 1/1 ratio levels. Again, penetration from the KI formulation (81.0%) was significantly greater than from the NH_4SCN (63.5%). It was also noted that at the 1/1 level NH_4SCN caused slight leaf dehydration whereas KI caused none while at the 3/1 level although KI caused some dehydration, NH_4SCN caused considerably more (Table 4.8c).

Table 4.8 Influence of NH_4SCN and KI on aminotriazole penetration. Aminotriazole application rate 60.0 µg/leaf (assessed after 3h).

Add.[f]/ATA[a] M/M	MQLS[g] (µg)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(a) NH_4SCN				
0/1	51.9	13.5	10.6	dry, ATA recryst, no effect on leaf
1/1	20.7	65.5	5.0	dry, no ATA or NH_4SCN recryst, some leaf dehydration
2/1	11.9	80.1	1.9	dry, no ATA or NH_4SCN recryst, some leaf dehydration
3/1	12.7	78.8	3.3	dry, no ATA or NH_4SCN recryst, much leaf dehydration
5/1	12.8	73.6	7.1	dry, no ATA or NH_4SCN recryst, much leaf dehydration
(b) KI				
0/1	56.5	5.9	4.2	dry, ATA recryst, no effect on leaf
1/1	17.6	70.7	4.7	dry, no ATA or KI recryst, no effect on leaf
2/1	13.9	76.8	4.9	dry, no ATA or KI recryst, no effect on leaf
3/1	7.7	87.2	3.6	dry, no ATA or KI recryst, slight leaf dehydration
5/1	12.4	79.3	7.0	some wet, no ATA or KI recryst, some leaf dehydration

Table 4.8 (continued)

Add.[f]/ATA[a] M/M	MQLS[g] (µg)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(c) KI or NH_4SCN				
0/1	58.9	1.8	2.5	dry, ATA recryst, no effect on leaf
1/1 (NH_4SCN)	38.0	36.6	8.9	dry, no ATA recryst, some leaf dehydration
1/1 (KI)	27.5	54.2	6.7	dry, no ATA recryst, no effect on leaf
3/1 (NH_4SCN)	21.9	63.5	8.4	dry, no ATA recryst, much leaf dehydration
3/1 (KI)	11.4	81.0	3.5	dry, no ATA recryst, slight leaf dehydration

a Aminotriazole

c Standard deviation (n-1 degrees of freedom)

f Additive

g Mean quantity of aminotriazole left on the leaf surface

h Mean penetration of aminotriazole

4.3.2.2 Penetration of aminotriazole and NH_4SCN or KI

Aminotriazole penetration was significantly enhanced by both NH_4SCN and KI. However, although penetration from the KI formulation was again greater than from the NH_4SCN, the difference was not significant. The opposite was true of the NH_4SCN and KI penetration. NH_4SCN penetration was greater than KI although again the difference was not significant (Table 4.9).

Table 4.9 Penetration of aminotriazole and NH_4SCN or KI. Aminotriazole application rate 600.0 µg/leaf. NH_4SCN or KI/aminotriazole 3/1 M/M (assessed after 2.5h)

Treatment	M Add.P[j] (%)	SD[c] (%)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
Aqueous control	-	-	0.0	0.0	ATA[a] recryst, no effect on leaf
KI	65.7	13.1	43.7	13.6	some recryst probably KI and ATA, some leaf dehydration
NH_4SCN	72.3	7.1	34.5	7.9	no recryst, some droplets wet, considerable dehydration

a Aminotriazole
c Standard deviation (n-1 degrees of freedom)
h Mean penetration of aminotriazole
j Mean penetration of additive (KI or NH_4SCN)

In addition to the non - significant difference in aminotriazole penetration between the two formulations on this occasion, the actual extent of the penetration was lower than might have been expected if the figures obtained are compared with those in Table 4.8. Some KI had re-crystallised on the leaf surface while in the NH_4SCN formulation no re-crystallisation was observed. This would suggest that humidity was lower than in previous experiments and this factor could limit the effectiveness of KI.

4.3.2.3 Influence of various iodide salts on aminotriazole penetration

At the lower humidity level ($48^{+}_{-}5\%$ r.h.) there was virtually no penetration from the aqueous control or the CaI_2 and MgI_2 formulations (1.0% or less) while KI and NaI significantly enhanced penetration (34.6% and 52.1% respectively) although there was no significant difference between the two (Table 4.10a).

At the higher humidity level ($64^{+}_{-}5\%$ r.h.), penetration from the aqueous control and the CaI_2 and MgI_2 formulations was of the order of less than 10% while KI and NaI again enhanced penetration considerably (66.3% and 66.4% respectively) (Table 4.10b).

4.3.2.4 Influence of various thiocyanate salts on aminotriazole penetration

At the lower humidity level NH_4SCN, NaSCN and KSCN (in order of increasing effect) all significantly enhanced aminotriazole penetration compared with the aqueous control. $Ca(SCN)_2$ had no significant effect on penetration. The effect of KSCN was significantly greater than that of NaSCN which in turn was significantly greater than that of NH_4SCN (Table 4.11a).

At the higher humidity level $Ca(SCN)_2$ significantly enhanced penetration but the actual increase in percentage penetration was small (4.1%). NH_4SCN, NaSCN and KSCN all brought about a considerable enhancement in penetration and there were no significant differences in penetration between the three. (Table4.11b)

Table 4.10 Influence of various iodide salts on aminotriazole penetration. Aminotriazole application rate 60.0 µg/leaf. Iodide salt/aminotriazole 3/1 M/M (assessed after 3h).

Iodide salt	MQLS[g] (µg)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(a) 48±5% r.h.				
None	59.4	1.0	2.8	ATA[a] recryst, no effect on leaf
KI	39.2	34.6	21.5	recryst, probably ATA and KI, no effect on leaf
NaI	23.7	52.1	10.3	no visible recryst, droplets dry, no effect on leaf
CaI_2	60.0	0.0	0.0	no recryst, droplets wet, no effect on leaf
MgI_2	59.8	0.4	0.8	no recryst, droplets wet, no effect on leaf
(b) 64±5% r.h.				
None	57.5	4.2	2.3	ATA recryst, no effect on leaf
KI	20.2	66.3	9.6	no recryst, droplets dry, slight leaf dehydration
NaI	20.2	66.4	3.6	no recryst, droplets dry, no leaf dehydration
CaI_2	57.7	3.8	2.1	no recryst, droplets wet, no leaf dehydration
MgI_2	54.1	9.9	3.5	no recryst, droplets wet, slight leaf necrosis - punctured appearance

a Aminotriazole

c Standard deviation (n-1 degrees of freedom)

g Mean quantity of aminotriazole left on the leaf surface

h Mean penetration of aminotriazole

Table 4.11 Influence of various thiocyanate salts on aminotriazole penetration. Aminotriazole application rate 60.0 µg/leaf. Thiocyanate salt/aminotriazole 3/1 M/M (assessed after 3h).

Thiocyanate salt	MQLS[g] (µg)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(a) 48±5% r.h.				
None	59.0	1.7	2.0	ATA[a] recryst, no effect on leaf
NH_4SCN	38.5	35.9	6.8	no recryst, some droplets wet, some leaf dehydration
KSCN	25.0	58.4	5.4	no recryst, few droplets wet, some leaf dehydration
NaSCN	33.1	44.9	4.9	no recryst, few droplets wet, some leaf dehydration
$Ca(SCN)_2$	58.3	2.8	3.0	no recryst, droplets wet, no effect on leaf
(b) 64±5% r.h.				
None	58.4	2.6	3.0	ATA recryst, no effect on leaf
NH_4SCN	21.3	64.5	7.1	no recryst, droplets dry, some leaf dehydration
KSCN	20.6	65.7	8.8	no recryst, droplets dry, slight leaf dehydration
NaSCN	18.6	69.0	4.4	no recryst, droplets dry, no effect on leaf
$Ca(SCN)_2$	56.0	6.7	3.1	no recryst, droplets wet, no effect on leaf

a Aminotriazole

c Standard deviation (n-1 degrees of freedom)

g Mean quantity of aminotriazole left on the leaf surface

h Mean penetration of aminotriazole

4.3.2.5 Influence of polysorbate 20 on aminotriazole penetration from a MgI_2 formulation

At none of the concentrations tested did the addition of polysorbate 20 increase penetration compared with MgI_2 alone (Table 4.12).

Table 4.12 Influence of polysorbate 20 (0.0-5.0g/litre) on aminotriazole penetration from a magnesium iodide formulation. Aminotriazole application rate 60.0 µg/leaf. MgI_2/aminotriazole 3/1 M/M (assessed after 3h). Humidity 64±5% r.h.

Polysorbate 20 (g/litre)	MQLS[g] (µg)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
0.0	51.9	13.5	2.6	no recryst, droplets wet, leaf has punctured appearance
0.2	52.7	12.2	4.2	no recryst, droplets wet, leaf has punctured appearance
1.0	54.8	8.7	2.9	no recryst, droplets wet, leaf has punctured appearance
5.0	52.7	12.2	6.5	no recryst, droplets wet, leaf has punctured appearance

c Standard deviation (n-1 degrees of freedom)
g Mean quantity of aminotriazole left on the leaf surface
h Mean penetration of aminotriazole

4.3.2.6 Penetration of aminotriazole and thiocyanate or iodide salts

At the lower humidity level NH_4SCN, KSCN and NaI all significantly enhanced aminotriazole penetration compared with the aqueous control and there were no significant differences between the three treatments. There appeared to be considerable re-crystallisation of KI on the leaf surface and penetration was not greatly influenced by this treatment. There was no significant difference in thiocyanate penetration between the two salts tested and although NaI penetration was greater than thiocyanate penetration, the difference between NaI and NH_4SCN was not significant. KI penetration was much lower than that of the other three (Table 4.13a).

At the higher humidity level all four salts significantly enhanced aminotriazole penetration although the effect of NH_4SCN was significantly less than that of the other three, between which there were no significant differences. In addition, there were no significant differences between all four treatments in the level of penetration of the salt (Table 4.13b).

4.3.2.7 Influence of droplet size on aminotriazole penetration from NH_4SCN and KSCN formulations

At the lower humidity level and with the smaller droplet size there was no significant difference in aminotriazole penetration between the two formulations. However, there was significantly greater penetration from the KSCN formulation with the larger droplets. This appeared to be due to a reduction in penetration from the NH_4SCN formulation rather than an increase in penetration from the KSCN formulation since there were no significant differences between the two KSCN treatments and the NH_4SCN small droplet treatment while the degree of penetration from the NH_4SCN large droplet treatment was significantly less than from the other three (Table 4.14a).

At the higher humidity level and with the smaller droplets there was significantly greater penetration from the KSCN formulation while with the larger droplets there was no

Table 4.13 Penetration of aminotriazole and thiocyanate or iodide salts. Aminotriazole application rate 600.0 µg/leaf. Thiocyanate or iodide salt/ aminotriazole 3/1 M/M (assessed after 3h).

Treatment	M Add.P[j] (%)	SD[c] (%)	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(a) 48±5% r.h.					
Aqueous control	---	---	4.6	1.4	ATA[a] recryst, no effect on leaf
NH_4SCN	58.1	13.4	26.1	12.2	no recryst, some droplets wet, some leaf dehydration
KSCN	50.3	2.7	26.6	3.9	no recryst, some droplets wet, some leaf dehydration
KI	19.6	3.8	8.8	5.7	recryst, probably KI and ATA, no effect on leaf
NaI	69.5	7.4	24.3	8.6	no recryst, some leaf dehydration, some droplets wet
(b) 64±5% r.h.					
Aqueous control	---	---	10.5	3.3	ATA recryst, no effect on leaf
NH_4SCN	64.6	14.0	38.0	12.8	no recryst, some droplets wet, some leaf dehydration
KSCN	72.0	2.8	52.1	3.6	no recryst, droplets dry, some leaf dehydration
KI	67.3	11.0	51.6	8.0	no recryst, droplets dry, slight leaf dehydration
NaI	75.0	14.0	51.4	10.0	no recryst, droplets dry, slight leaf dehydration

a Aminotriazole

c Standard deviation (n-1 degrees of freedom)

h Mean penetration of aminotriazole

j Mean penetration of additive (thiocyanate or iodide salt)

Table 4.14 Influence of droplet size on aminotriazole penetration from NH_4SCN and KSCN formulations. Thiocyanate/aminotriazole 3/1 M/M. Application rates 60.0 µg/leaf in 20 µl as ca 2 µl droplets and 600.0 µg/leaf in 200 µl as ca 5 µl droplets (assessed after 3h).

Treatment	MP[h] (%)	SD[c] (%)	Droplet and leaf appearance
(a) 48±5% r.h.			
NH_4SCN(20 µl as ca 2 µl droplets)	29.4	11.3	no recryst, some droplets wet, some leaf dehydration
KSCN(20 µl as ca 2 µl droplets)	32.2	6.5	no recryst, some droplets wet, slight leaf dehydration
NH_4SCN(200 ul as ca 5 µl droplets)	14.5	4.4	no recryst, some droplets wet, some leaf dehydration
KSCN(200 µl as ca 5 µl droplets)	26.3	6.9	no recryst, some droplets wet, some leaf dehydration
(b) 64±5% r.h.			
NH_4SCN(20 µl as ca 2 µl droplets)	49.0	10.9	no recryst, droplets dry, some leaf dehydration
KSCN(20 µl as ca 2 µl droplets)	67.2	6.2	no recryst, droplets dry, slight leaf dehydration
NH_4SCN(200 µl as ca 5 µl droplets)	47.3	4.6	no recryst, droplets mainly dry, some dehydration
KSCN(200 µl as ca 5 µl droplets)	49.3	9.1	no recryst, droplets mainly dry, some dehydration

c Standard deviation (n-1 degrees of freedom)

h Mean penetration of aminotriazole

significant difference between the two. The greater penetration from the KSCN formulation with the smaller droplets appeared to be due to an enhancement in penetration from the KSCN treatment rather than a decrease from the NH_4SCN treatment since there were no significant differences between the two NH_4SCN treatments and the KSCN large droplet treatment (Table 4.14b).

4.4 DISCUSSION AND CONCLUSIONS

From the experimental evidence obtained here, it can be seen that NH_4SCN is capable of inhibiting both the riboflavin photosensitised oxidation of aminotriazole and oxidation by hydroxyl radicals. Considering together the fact that Castelfranco and Brown (1963) have hypothesised that aminotriazole in contact with certain enzyme systems reacts with amino acids and various other compounds via a free radical intermediate and the findings of Carter (1965;1969) that NH_4SCN reduces the formation of the less toxic and less mobile 3-ATAL, this would strongly suggest that the mode of action of NH_4SCN within the plant is that of a free radical inhibitor. The results here substantiate this point (Tables 4.1-4.4) and of course open up the possibility of a whole new range of additives which may enhance translocation and inhibit free radical reactions. A comparison of possible inhibitors revealed that iodide and perhaps bromide and cyanide salts may be of use in this respect.

6-Benzyladenine which van der Zweep (1965) showed to have a more pronounced synergistic effect on aminotriazole formulations than NH_4SCN, and 6-benzyladenine riboside were tested but were found to be much less effective than NH_4SCN. Thus, further investigations into the role of these compounds were forsaken in preference to a more detailed investigation of the roles of iodide and thiocyanate salts in aminotriazole formulations. Two possible explanations which might be put forward to explain this are (a) the 6-benzyladenine may be converted within the plant to a more active free radical inhibitor or (b) 6-benzyladenine may have some quite separate function from free radical inhibition.

Of the organic sulphur compounds tested, thiourea and L-cysteine hydrochloride were found to inhibit aminotriazole oxidation by Fenton's reagent to the same extent as NH_4SCN. Thioacetamide was less effective. None however were as effective as NH_4SCN in the riboflavin photosensitised system.

When the efficiency of iodide and thiocyanate salts were compared in the riboflavin photosensitised system, they were found to be equally effective inhibitors. The production of HCN (and

possibly sulphate) during the Fenton's reagent reaction, in the presence of thiocyanate salts would suggest that the thiocyanate is being oxidised in preference to the aminotriazole. HCN was also detected in the riboflavin system but in much smaller amounts, the initial concentration of thiocyanate in this reaction mixture being much smaller. Oxidation of NH_4SCN in enzymic systems has been shown to result in the production of cyanide and sulphate (Sörbo and Ljunggren, 1953). Similarly, the formation of molecular iodine would suggest that iodide is also being preferentially oxidised.

It should be stressed that the important relationship in such reactions is that of the concentration of free radical inhibitor relative to the free radical generating power of the system. Under actual field conditions such ratios as 5/1 of inhibitor/aminotriazole may not be required to bring about maximum inhibition of aminotriazole oxidation and conjugation formation etc.

Asulam was also shown to be oxidised by the two free radical generating systems employed although in the case of the riboflavin system at least (Table 4.7) not as rapidly as aminotriazole. Again, oxidation was inhibited by thiocyanate and iodide salts and additionally by ferrocyanide (Tables 4.5-4.7). This therefore opens up the possibility that NH_4SCN and other free radical inhibitors may be used to increase the effiency of a wide range of herbicides. Sweetser (1963) has shown a photochemical inactivation of monuron and other substituted phenylurea herbicides by flavin mononucleotide (FMN) and leaves of bean plants which were sprayed with FMN following application of monuron to the roots prevented the herbicidal action of the latter. This work shows strikingly similar trends to that of Sund et al.(1960), Hilton(1962) and Castelfranco et al.(1963) working with aminotriazole. In addition, Stritzke(1972) has shown that the efficiency of dichlorprop, 2,4-D and 2,4,5-T can be increased by the use of an NH_4SCN pre-treatment while Crosby and Wong(1973) have shown 2,4,5-T to be quickly broken down in a riboflavin photosensitised system.

In addition to their effects on free radical reactions, both NH_4SCN and KI were shown to have a considerable effect on aminotriazole penetration of bean leaves. They appear to be acting

at least partly as humectants since they prevented droplet drying and aminotriazole recrystallisation. However, other functions would also appear to be likely since previous work done on humectants such as ethylene glycol and DMF under similar conditions revealed that they had no great influence on aminotriazole penetration unless a small amount of polysorbate 20 was present (see Chapter Three) while a later experiment (Table 4.12) using MgI_2 which alone had no effect on aminotriazole penetration showed the further addition of polysorbate 20 to have no effect either. Of the two salts initially tested, KI appeared to be more efficient although it was thought that humidity might be more of a limiting factor on its efficiency since in one exercise (Table 4.9) its recrystallisation on the leaf surface became quite apparent and droplet drying occurred. This would tend to reduce both its penetration and that of the aminotriazole since penetration from crystalline deposits proceeds slowly, or may cease (see 2.3.1; Mynett and Wain, 1971).

Further investigations (Tables 4.10, 4.11, 4.13 and 4.14) using a series of iodide and thiocyanate salts revealed several points :- (a) penetration of aminotriazole from all iodide or thiocyanate/aminotriazole formulations was reduced by low humidity and in particular from the KI formulation where droplet drying and recrystallisation occurred, (b) CaI_2, MgI_2 and $Ca(SCN)_2$ appeared to be of little value in enhancing aminotriazole penetration at either humidity level under the experimental conditions employed in this study, (c) NaSCN, KSCN and NaI were at least as efficient as NH_4SCN in increasing aminotriazole penetration, (d) the penetration of the salts tested was also reduced by low humidity and in particular that of KI which again recrystallised on the leaf surface. NaI penetration was least affected (Table 4.13).

An interesting point which arose in this study was the degree of aminotriazole penetration from the KSCN formulation relative to that from the NH_4SCN formulation. In Table 4.11, at the lower humidity level, aminotriazole penetration was significantly greater from the KSCN formulation, at the higher humidity level, there was no significant difference between the two. In Table 4.13 the opposite was true, at the lower humidity level there

was no significant difference in penetration while at the higher humidity level penetration from the KSCN formulation was significantly greater. Since the only obvious difference was in droplet size (ca 2 µl droplets in Table 4.11 and ca 5 µl droplets in Table 4.13), this factor was investigated to determine whether it might explain these trends. The results of this experiment (Table 4.14) indicated virtually opposite trends to those expected:- At the lower humidity level (ca 2 µl droplets) there was no significant difference in penetration between the two formulations while at the higher humidity level (ca 2 µl droplets) penetration from the KSCN formulation was significantly greater. With the larger droplets (ca 5 µl), penetration from the KSCN formulation was significantly greater at the lower humidity level while at the higher humidity level there was no significant difference in penetration. However, an important point to bear in mind was that at no time was penetration from the KSCN formulation less than that from the NH_4SCN formulation.

Thus, it would appear that although droplet size has an effect on penetration and that this is regulated by humidity, these trends cannot be explained totally on this basis. Other factors such as variability in plant age, water status of the plant during the course of the experiment and growing conditions prior to treatment may also have an effect on the efficiency of penetration and the trends found. In addition, variability in humidity may help to explain the differences in penetration between results for the same ratios of NH_4SCN/aminotriazole or KI/aminotriazole in Tables 4.8 and 4.9.

Field investigations into the effect of (a) the concentration of NH_4SCN in aminotriazole formulations, (b) NaI on aminotriazole effiency and (c) NH_4SCN on asulam effiency in bracken are under way and initial observations on the effects of these formulations will be made in Chapter Five.

Chapter Five

FIELD INVESTIGATIONS INTO THE EFFECT OF VARIOUS HERBICIDE FORMULATIONS ON BRACKEN CONTROL

5.1 INTRODUCTION

Chapters Two, Three and indeed parts of Chapter Four were designed principally in an attempt to develop aminotriazole formulations which might bring about efficient penetration, the relevance of which was discussed in Chapter Two in relation to the West of Scotland situation. In addition, it was hoped that a little more insight into the mode of action of herbicide additives might be gained. Obviously, such formulations must be tested under field conditions before any definite conclusions as to their use in practice can be drawn. With this point in mind, field trials were set up during the period 1976 - 1978 in parallel to the laboratory studies, to investigate the effectiveness of certain of these formulations on bracken. Again, the relevance of bracken has already been discussed (see 2.1). In addition to aminotriazole, asulam and glyphosate (N- (phosphonomethyl) glycine) have been used in certain preliminary investigations, the purpose behind these will be discussed in detail later in this chapter.

There would be little to be gained from a discussion of the bracken plant and the problems associated with its control since its biology, ecology and early research into its control (mainly cultural, mechanical and control by contact herbicides) have been well reviewed by Braid (1959) while Babiker (1976) has reviewed its biology and control with particular emphasis on the use of systemic herbicides. Also of particular relevance is the Botanical Journal of the Linnean Society 1976, 73, numbers 1-3 which is the published proceedings of a symposium entitled "The biology of bracken". This contains articles covering many aspects of its biology, ecology, taxonomy, phytogeography and control.

A brief review of the history of the three chemicals employed in these studies, in relation to their use on bracken,

would however seem justified.

Aminotriazole

Aminotriazole was first introduced as a herbicide by Amchem Products Inc. (U.S. Patent No. 2,670,282) in 1954 (Ashton and Crafts, 1973). Bylterud (1958) in Scandinavia was one of the first to demonstrate its effectiveness on bracken. In the West of Scotland however, Conway (1959) concluded that at 20 and 40lb/acre aminotriazole showed little promise. Assessment in June, the year after spraying indicated a considerable suppression in growth (75 and 92% reductions in frond density respectively) but by August of the same year the respective figures were 18 and 10%. In 1959, the Bracken Committee of the International Research Group on Weed Control proposed the setting up of trials of standard design throughout Europe, Scandinavia and the United Kingdom. Aminotriazole was one of the herbicides employed in these studies. Fifteen such trials were set up in Britain, thirteen by the A.R.C. unit of Experimental Agronomy at Oxford, the results of which were reported by Hodgson (1960) and two in Argyll by the West of Scotland Agricultural College, the results of which were reported by Kirkwood and Fletcher (1961). All trials were carried out at rates of 10 and 20lb aminotriazole/acre and the activated formulation (containing ammonium thiocyanate) was used for the first time. Meanwhile, trials were carried out in the East of Scotland by Erskine (1960) who also used the activated formulation.

The results of the trials in the West of Scotland revealed that on one site, a maximum reduction in frond density of 50% was achieved at the 20lb/acre application rate. The activated formulation was less effective. On the second site, aminotriazole increased frond density the following season. This increase was most obvious at the 10lb/acre application rate, at the later spraying date and with the activated formulation. These results were poor in comparison to those of Erskine's in the East of Scotland and Hodgson's in England and Wales where consistently higher levels of control were obtained. Erskine's results were

particularly encouraging since frond number reductions of more than 90% were achieved in August the year after spraying from application rates of 5-15lb/acre. From other results obtained by Erskine (1960), using 4-CPA, which indicated a period of maximum susceptibility, Kirkwood and Fletcher (1961) concluded that their poorer results were due to carrying out their spraying programme too late in the season. Their only comment as to weather conditions was that it was warm, dry and sunny at the time of spraying and for at least 24 hours later. Brown and MacKenzie (1972) proposed that the poorer results obtained for aminotriazole in the west were due to the wetter climate while Holroyd et al. (1970) considered that heavy drizzle immediately after spraying may have been an important factor in the failure of aminotriazole to suppress growth the following season.

Erskine (1968) summarising field trials carried out between 1958 and 1968 concluded for aminotriazole that rates of 5 and 7.5lb/acre produced long lasting control entirely acceptable in practice. Plots treated in 1959 and assessed in 1966 gave a degree of control averaging 75% which he said was low due to re-infestation from the edges of the plots. One acre plots treated in 1959 with 7.5lb/acre were in 1968 virtually free from bracken in the centre. From a series of timing trials he found that optimum results were obtained from spraying just before the fronds fully unfurled while Volger and Rosger (1972) using ^{14}C-aminotriazole showed that efficient movement of aminotriazole would only occur late in the season when transport of assimilates through the phloem and into the rhizomes was in an active phase.

Kirkwood and Fletcher (1961) indicated that the activated formulation brought about a poorer response the year after spraying, however, work done in Belgium (Anon, 1963) showed that the treatment of fully developed fronds with aminotriazole (non activated) at 10-20kg/ha or aminotriazole (activated) 5-10kg/ha virtually eliminated bracken for 2 years. Further work (Anon,1965) revealed some regrowth had occurred in the fourth year after spraying with aminotriazole (non activated) 10-20kg/ha while hardly any regrowth was observed following the use of the activated formulation at 5-20kg/ha. Little work has been done on the choice of activator, Varlet et al. (1964) replaced

ammonium thiocyanate with sodium thiocyanate, however, this yielded poorer results the following season.

Thus, from the literature cited it can be seen that acceptable control of bracken can be achieved although poor results have also been recorded.

These are generally, although not always attributed to rainfall shortly after spraying or spraying outwith that period when active transport, of assimilates and presumably aminotriazole, from frond to rhizome is occurring. Other less obvious factors leading to differential levels of control between sites also seem to be of importance (Erskine, 1960; Kirkwood and Fletcher, 1961; McLeod, 1961).

Mode of action in bracken does not seem to have been investigated, however, the following factors have been implicated for other plants : (i) disruption of purine synthesis resulting perhaps in inhibition of cell division (Schweizer and Rogers, 1964; Bartels and Wolf, 1965; Castelfranco and Bisalputra, 1965; Gomaa, 1973), (ii) non specific enzyme inhibition after undergoing a one - electron oxidation to a free radical (Castelfranco and Brown; 1963) although Carter (1969) presents evidence which strongly suggests a specific site of action, (iii) inhibition of chlorophyll synthesis and chloroplast formation (Ashton and Crafts, 1973) however the results of Burns et al. (1971) suggest that the mode of action is more specifically linked with carotenoid synthesis which is necessary to prevent chlorophyll destruction and for chloroplast stabilisation, (iv) inhibition of riboflavin synthesis (see 4.1), (v) inhibition of amino acid and protein synthesis. Considerable controversy exists in the literature over the influence of aminotriazole on histidine biosynthesis (McWhorter and Hilton, 1967; Davies, 1971; Vivekanandhan and Gnanam, 1972; Baumann and Günther, 1976). Bartels and Wolf (1965) and McWhorter and Hilton (1967) have demonstrated that total free amino acid content increases in light grown plants treated with aminotriazole whereas protein content decreases. Bartels and Wolf (1965) concluded that this was an indirect effect resulting from inhibition of purine metabolism while Brown and Carter (1968) concluded from their studies that aminotriazole had no direct effect on protein synthesis.

Obviously, a comprehensive review of the literature concerned with the mode of action of aminotriazole is outwith the scope of this chapter and indeed its relevance might be questioned anyway, however, the literature cited above outlines the basic theories which are being investigated. Carter (1969) discusses the earlier literature on most of these points and others in considerable detail.

Asulam

Asulam was first introduced as a herbicide in 1965 by May and Baker Ltd (British Patents Nos 1,040,541 and 1,052,881), its properties being first described by Ball et al. (1965) and Cottrell and Heywood (1965). Its activity on bracken was first recorded by Holroyd et al. (1970) who observed excellent results at rates of 4-8 lb/acre. It is now Government approved for bracken control and carries a 50% grant towards the cost of control and fertilizer application, at present it is the only herbicide which warrants this grant. The recommended application rate for asulam is 1 gal/acre of a 40% $^{W}/_{v}$ a.i. solution of the sodium salt (Asulox) which is equivalent to 4 lba.i./acre (4.5 kg/ha) although promising results have been obtained at 2.2 and 1.1 kg/ha (Scragg et al., 1972). They also found that results between sites and under varying environmental conditions were very consistent. This type of result was not confirmed by the East of Scotland College of Agriculture (Anon, 1976) who found that results varied with site. Dover (1975) found application rates of less than 3 lb/acre to be less satisfactory than 3 and 4 lb/acre although it has recently been proposed (Anon, 1977) that lower rates sprayed under favourable conditions can give results equivalent to the recommended rate. Soper (1972) concluded that within limits, the timing of application is probably more important than the dosage rate. Optimum results appear to be achieved mainly from July/August sprayings, i.e. when the fronds are more or less fully unfurled (Soper, 1972; Anon, 1974a; Veerasekaran et al., 1976) and studies using ^{14}C-asulam have shown that the optimum time for movement to the rhizomes is when the fronds are almost fully

unfurled (Veerasekaran and Kirkwood, 1972; Veerasekaran et al., 1976), i.e. when assimilate movement from the phloem to the rhizomes is in the active phase. These results are very similar to those of Volger and Rösger (1972) working with ^{14}C-aminotriazole. At this stage, accumulation of the asulam is in the frond buds and rhizome apices (Veerasekaran and Kirkwood, 1972; Veerasekaran et al., 1976; Yukinaga et al., 1976) i.e. regions of meristematic activity.

Martin (1976) summing up experience of asulam in Scotland maintains that if applied at the correct time it will bring about good short term control. Metcalfe and Davies (1977) and Anon (1974a) have proposed that the rate of regeneration is inversely proportional to the initial degree of control while the importance of follow up treatments by intensive grazing, particularly by cattle to reduce this rate has been demonstrated (Dover, 1975; Anon, 1977).

With regard to its mode of action, Cottrell and Heywood (1965) reported that the effects of the benzenesulphonylcarbamates are similar to those of N- phenylcarbamates, causing chlorosis and inhibitory effects in apical and axial meristems e.g. rhizome buds of Agropyron repens by interfering with cell division and expansion. Sterrett and Fretz (1975) reported mitotic irregularities in onion root - tip cells including arrested metaphases and anaphase and chromosome bridges.

In bracken, Martin et al. (1972) showed a possible reduction in the weight of frond bearing rhizome and abnormal development and death of frond buds. More specifically, Veerasekaran (1975) demonstrated that it inhibited oxidative phosphorylation in bud tissue while Veerasekaran et al. (1976; 1977) have shown that it interferes with RNA and protein synthesis in frond buds, eventually leading to distortion, lignification and fissuring of the tissues, thus, these may be major sites of action.

Glyphosate

Glyphosate was first introduced as a herbicide by Monsanto Ltd. Its properties were first discussed by Baird et al. (1971).

On bracken, it has been highly successful in suppressing growth using application rates of between 1.0 and 6.7 kg/ha(Anon,1974b;

Scragg et al., 1974; Williams and Foley, 1975; Lea, 1977) but especially at rates of 2 kg/ha and above. As with aminotriazole and asulam, spraying in July and August when the fronds are fully unfurled appears to be most effective (Anon, 1974b). In terms of reducing frond density, glyphosate has shown slightly superior results to asulam (Williams and Foley, 1975; Lea, 1977). However, it has been shown to affect the underlying grass sward more severly than asulam (Williams, 1977) and in addition, treatment is more expensive (Lea, 1977). The effect on the underlying sward has been shown to depend on the amount of bracken cover at the time of spraying (Anon, 1974b). When spraying is done before the end of July, when the bracken canopy is dense, little damage is done to the underlying sward but with progressively later spraying, more damage occurs.

One encouraging aspect of its effects on bracken is that it not only supporesses frond density but also appears to reduce the carbohydrate content of the storage rhizomes and the weight of frond bearing rhizomes while asulam appears to have no effect on these (Williams and Foley, 1975).

Outwith the bracken situation, other work has revealed that the following may be factors contributing to its mode of action :-

(i) disruption of chloroplasts, other organelles and certain cells (Campbell et al., 1976; Hull et al., 1977),

(ii) inhibition of aromatic amino acid biosynthesis (Jaworski, 1972; Roisch and Lingens, 1974; Davis and Harvey, 1976; Haderlie, 1976),

(iii) delaying the onset of dominance in decapitated rhizome fragments of Agropyron repens (Chancellor and Leakey, 1972),

(iv) starch grain disappearance (Hull et al., 1977).

5.2 EXPERIMENTAL

5.2.1. Materials

Weedazol TL (contains 2lb of aminotriazole and 1.85lb of ammonium thiocyanate per gallon) was purchased from A.H. Marks and Co. Ltd. Asulox (sodium salt of asulam 40%^{W}/va.i.) was purchased from May and Baker Ltd. Roundup (contains 480 g/litre isopropylamine

salt of N - phosphonomethyl glycine - equivalent to 360 g/litre glyphosate) was purchased from Monsanto Ltd. All other chemicals were as previously described (see 2.2.1; 3.2.1; 4.2.1).

5.2.2 Methods

All field experiments were carried out at Carbeth, Stirlingshire (Grid reference NS 527798). Spraying was done with an ICI Mark 3 Knapsack Sprayer at an output pressure of 30 p.s.i. As far as possible, spraying was done around the period of full frond development.

1976 Field Experiments

Three experiments were set up in 1976, these were as follows: 1. Influence of polysorbate 20, glycerol and their combination on control of bracken by aminotriazole and a comparison of the control by these formulations with that of the commercially used formulation, Weedazol TL. 2. Influence of a DMSO/polysorbate 20 additive combination on control of bracken by aminotriazole. 3. The effect of spraying with Weedazol TL followed by cutting of the fronds at ground level at varying time intervals after spraying. A measure of regrowth the following season should therefore give some indication of the rate of translocation. This should have the following advantages over the use of radio-labelled aminotriazole : -

(a) it is cheaper,

(b) there is not the restriction of application to a few fronds which the use of radio-labelled chemicals imposes,

(c) effectiveness of treatments can be directly assessed the following season. In radio-labelled studies it is normally only possible to measure label accumulation at various growing points etc.

In all three experiments, plot size was 5x5m with a 2m path between plots. A randomised block design with 4 replicates per treatment was used in each case. Spraying was done at a volume rate of 1 l/plot (400 l/ha) (see Appendix I) and aminotriazole was applied at a rate of 21 g/plot (7.5 lb/acre or 8.4 kg/ha approx). For details of the method of treatment assessment refer to Appendix I.

Experiment 1

On the basis of the results obtained in Tables 2.5 and 2.8b which revealed that (i) a 1/2 w/w ratio of aminotriazole/ polysorbate 20 brought about optimum penetration and (ii) penetration could be increased by the addition of a small amount of glycerol to the spray solution. The polysorbate 20 concentration used in the formulation was calculated to be 44.8 g/litre of spray solution and the glycerol concentration to be 0.6 ml/litre. Including controls there were 9 treatments. These were as follows:- (i) aminotriazole, (ii) Weedazol TL, (iii) aminotriazole + glycerol, (iv) aminotriazole + polysorbate 20, (v) aminotriazole + polysorbate 20 + glycerol, (vi) glycerol, (vii) polysorbate 20, (viii) polysorbate 20 + glycerol, (ix) no treatment.

The plots were laid down on 21.7.76 and sprayed on 24.7.76. Frond densities and heights were assessed the following year on 8.7.77. Re-assessment of heights was carried out on 22.9.77. Preliminary investigations revealed that frond densities had not increased and hence these were not re-assessed. Frond densities in those plots treated with Weedazol TL were assessed 2 years after spraying, on 11.8.78.

Experiment 2

At the time of setting up this experiment, the only results available were those in Tables 3.1 and 3.2 on DMSO and polysorbate 20, other humectants and the influence of low humidity on their effects had not been tested. Thus, it is for this reason only that DMSO was used. Judging from later results and observations, perhaps the non volatile polypropylene glycol 400 might have been a better candidate, or else higher concentrations of DMSO to combat the possibility of low humidity having an influence on penetration.

The concentrations of additives used in the formulation were : DMSO - 5 ml/litre of spray solution, polysorbate 20 - 1 g/litre. The experiment consisted of 4 treatments, these were as follows : - (i) aminotriazole, (ii) aminotriazole + DMSO + polysorbate 20, (iii) DMSO + polysorbate 20, (iv) no treatment.

The plots were laid down on 26.7.76 and sprayed the

following day. Frond densities and heights were assessed the following year on 29.6.77 and heights re-assessed on 22.9.77.

Experiment 3

This experiment consisted of 8 treatments, these were as follows : -

(i) Plots sprayed and cut immediately afterwards (0days).
(ii) Plots sprayed and cut after 1 day.
(iii) Plots sprayed and cut after 3 days.
(iv) Plots sprayed and cut after 6 days.
(v) Plots sprayed and cut after 12 days.
(vi) Plots sprayed and left uncut.
(vii) Plots unsprayed and left uncut.
(viii) Plots unsprayed and cut 0 days.

The plots were laid down on 3.8.76 and sprayed on 5.8.76. Frond densities and heights were assessed the following year on 6.7.77. Again, further density assessment was not required, however, heights were re-assessed on 22.9.77.

It was intended that penetration studies similar to those carried out in Chapters Two, Three and Four should be carried out in the field in order that the times taken for penetration and for translocation might be distinguished when considering the overall effect of cutting. However, slight showers of drizzle commenced soon after spraying was begun. It was felt that these would be unlikely to affect the spraying as a whole, but could make any penetration studies meaningless while studies which were not run concurrently with the spraying programme could be very misleading.

1977 Field Experiment

This experiment was devised bearing in mind results and observations obtained from Experiment 1 the previous year, it consisted of 4 treatments as follows : -

(i) Aminotriazole + polysorbate 20 + glycerol.
(ii) Weedazol TL.
(iii) Aminotriazole + polysorbate 20 + glycerol + ammonium thiocyanate (NH_4SCN).
(iv) Control - untreated.

Experimental design and all application rates were as previously described for Experiment 1 (1976). The concentration of NH_4SCN in treatment (iii) was the same as that in the Weedazol TL formulation. The plots were laid down on 23.7.77 and sprayed on 8.8.77. Frond densities were assessed the following year on 20.7.78 and heights measured on 8.9.78. Details of the method of assessment are given in Appendix I.

In addition to this exercise, a small penetration experiment as per Chapters Two - Four was set up in the field on 23.8.77 to compare the rates of penetration of the Weedazol TL formulation with that of the aminotriazole + polysorbate 20 + NH_4SCN + glycerol formulation at varying intervals up to 24h. The aminotriazole a.i. rate was 9 µg/ µl. 10 µl of each formulation was applied to leaflet pairs 2-6 from the base of the third and fourth pair of pinnules from the base of the second lowermost pair of pinnae of 10 fronds. One pinna had the 4 pinnules treated with one formulation and the other pinna with the other formulation. Two fronds were harvested at each time interval (1.5, 3, 6, 12 or 24h), the pinnules washed in 25ml deionised water and analysis was carried out as previously described (see 2.2.2.7). Lanolin barriers were used to prevent spreading beyond the treated area.

1978 Field Experiments

Two experiments have been set up as follows :- 1. The effect of certain salts used in the free radical inhibition studies in Chapter Four on the scorching, translocation and overall performance of aminotriazole and asulam. 2. The effect of various glyphosate application rates on bracken control. This experiment was set up in conjunction with Norman Stephen and the results will form part of his thesis.

On the basis of results and observations from the previous two years' work it was decided to increase plot size to 7x7m with a 1m path between plots. A random block design was again used, but with 3 replicates instead of 4. Spraying was done at a volume rate of 2 l/plot (400 l/ha approx.) (see Appendix I).

Experiment 1

On the basis of results obtained in Chapter Four which revealed that iodide and thiocyanate salts were capable of inhibiting the breakdown of both aminotriazole and asulam in free radical generating systems and field observations over the previous two years which revealed that the omission of NH_4SCN from aminotriazole formulations resulted in considerable scorching of the bracken soon after spraying and poorer control the following season, it was decided to incorporate NH_4SCN and sodium iodide (NaI) into aminotriazole formulations and NH_4SCN into an asulam formulation in an attempt to cut down scorching by both herbicides. Veerasekaran et al. (1976) have reported that early spraying with asulam results in severe scorching of the fronds probably reducing basipetal translocation. In addition, ammonium nitrate (NH_4NO_3) was incorporated into an aminotriazole formulation, this compound had shown little or no activity in the free radical generating systems tested. Aminotriazole was applied at a rate of 35.7g/plot (6.5lb/acre or 7.3kg/ha approx.), asulam was applied as Asulox at a rate of 4 pints/acre (2lb a.i./acre or 2.2kg a.i./ha approx.).

Two ratios of aminotriazole/NH_4SCN were formulated, these differed from Weedazol TL in that the molar ratio of aminotriazole/NH_4SCN was not 1/1. The concentration of NH_4SCN present was based on the concentration which should be in a Weedazol TL solution being applied at a rate of 8.4kg/ha approx. (7.5lb/acre) since this concentration had reduced scorching considerably and control the following year was reasonably acceptable. Hence, if the theory put forward in Chapter Four is correct, it will not be the ratio of aminotriazole/NH_4SCN which is important, but the actual concentration of NH_4SCN. Therefore, the two ratios of aminotriazole/NH_4SCN which were applied were 1/1.15 and 1/3.45. The latter ratio was included to determine whether a further increase in NH_4SCN concentration might reduce scorching further and perhaps enhance control. The aminotriazole/NaI and NH_4NO_3 formulations were both applied at a 1/1.15 ratio. The asulam/NH_4SCN formulation contained the same concentration of NH_4SCN as present in the 1/1.15 aminotriazole/NH_4SCN formulation (38.1g/plot or 7.8kg/ha). Aminotriazole was used at a rate of 7.3kg/ha since at 8.4kg/ha

as Weedazol TL, frond density reductions of >80% had consistently been observed previously and therefore, differences in the degree of control between the two thiocyanate formulations, if present, might be difficult to distinguish. It was felt that a reduction of about 1kg/ha in the application rate might be sufficient to enable any differences to be assessed. Similarly, Asulox was applied at a rate of 2.2kg a.i./ha since at this level promising results have been obtained (Scragg et al., 1972), at 4.4kg a.i./ha, frond density reductions of >90% could be expected (Dover, 1975) which would again make it difficult to distinguish any differences between the Asulox and the Asulox/NH_4SCN formulations.

Including controls, this experiment consisted of 12 treatments. These were as follows :- (i) aminotriazole, (ii) aminotriazole + NH_4SCN 1/1.15, (iii) aminotriazole + NH_4SCN 1/3.45, (iv) aminotriazole + NaI 1/1.15, (v) aminotriazole + NH_4NO_3 1/1.15, (vi) Asulox, (vii) Asulox + NH_4SCN, (viii) NH_4SCN alone (38.1g/plot), (ix) NH_4SCN alone (114.3g/plot), (x) NaI alone (74.9g/plot), (xi) NH_4NO_3 alone (40.0g/plot), (xii) control-untreated. All formulations except the Asulox and Asulox + NH_4SCN contained 1g/litre of polysorbate 20 to improve wetting and reduce surface tensions etc. The plots were laid down on 8.8.73 and sprayed on 22.8.78.

Experiment 2

This experiment was devised (a) in an attempt to break bud dormancy and stimulate growth. As pointed out by Veerasekaran et al. (1976), bracken owes much of its success to the abundance of active and dormant buds and since it is believed the latter escape effects from herbicide treatments then any treatment which breaks dormancy would be of obvious advantage if followed by a "lethal" herbicide dosage. Glyphosate has been shown to break bud dormancy in other plants (Parker, 1976) at low levels, (b) to assess the effects of higher application rates on frond density, the underlying grass sward, carbohydrate content of the storage rhizomes and bud viability. Williams and Foley (1975) have shown that glyphosate has a considerable effect on the total carbohydrate content of the storage rhizomes. In addition,

NH_4SCN (38.1g/plot) was incorporated into a glyphosate formulation being sprayed at a rate of 0.56kg/ha (0.5lb/acre approx.) to determine whether control could be enhanced. This glyphosate rate was chosen since a preliminary experiment in 1977 at 0.56kg/ha had brought about frond density reductions of 85% approx. although the emerging fronds were quite vigorous (mean height 73cm approx., controls 132cm approx.) and most showed no chlorosis and much less necrosis than would be typical of emerging fronds from plots treated with aminotriazole. These measurements were taken towards the end of the season on 8.9.78.

The experiment consisted of 8 treatments, these were as follows :- (i) control- no treatment, (ii) 0.02kg/ha, (iii) 0.07kg/ha, (iv) 0.56kg/ha, (v) 2.22kg/ha, (vi) 4.45kg/ha, (vii) 8.89kg/ha, (viii) 0.56kg/ha + NH_4SCN (38.1g/plot or 7.8kg/ha). The plots were laid down on 2.8.78 and sprayed on 10.8.78.

5.3 RESULTS, DISCUSSION AND CONCLUSIONS

1976 Field Experiments

Experiment 1

Spraying was done on a relatively hot dry day. Visual inspection of the plots carried out 48h after spraying revealed extensive aminotriazole recrystallisation on the frond surfaces of those plots sprayed with aminotriazole alone. There was also some indication of scorching of the fronds in some of the plots which had been sprayed with formulations containing aminotriazole, but not those sprayed with Weedazol TL. Further inspection 72h after spraying revealed that there was still aminotriazole recrystallisation on those plots treated with aminotriazole alone but not in such great quantities. Those plots treated with the Weedazol TL formulation showed slight scorching while all other aminotriazole treated plots showed heavy scorching. The first rain was observed 6 days after spraying and by this time there was no evidence of aminotriazole recrystallisation on the frond surfaces. An inspection 11 days after spraying revealed the

following points :- (i) all aminotriazole sprayed plots except those sprayed with Weedazol TL were heavily scorched while in addition there was a distinct lack of chlorophyll in some of the new growth, (ii) the untreated plots showed some very slight scorching, most probably from spray drift, (iii) those plots treated with polysorbate 20, glycerol or polysorbate 20 + glycerol showed slightly more scorching and slight chlorosis of the new growth, (iv) the Weedazol TL treated plots showed a degree of scorching similar to that found in (iii) above but no chlorosis of the new growth.

The considerable scorching of the aminotriazole treated plots (not Weedazol TL) brought about premature dying back of the fronds which resulted in considerable litter accumulation which persisted throughout the following season and may have been at least partly responsible for the suppression of grass growth since the more severe suppression nearly always coincided with areas of considerable litter accumulation. This accumulation was not so obvious in Weedazol TL treated plots.

Assessment of control the year after spraying (Table 5.1) revealed no significant differences in the degree of control obtained for aminotriazole alone, aminotriazole + polysorbate 20, aminotriazole + glycerol and aminotriazole + polysorbate 20 + glycerol while the Weedazol TL formulation had brought about significantly better control. These results corresponded to a 97% reduction in frond density for the Weedazol TL formulation and a mean of 65% reduction for the other aminotriazole formulations. None of the additives had any effect on growth.

Thus, it would appear that reduced scorching is accompanied by increased control the following season (Plate 5.1a) and that NH_4SCN is the governing factor in this process. It would appear unlikely that rapid penetration was causing scorching since in those plots sprayed with aminotriazole alone there was still a considerable quantity of aminotriazole on the frond surfaces 3 days after being sprayed and slight traces after 4-5 days. This was closely followed by considerable rain after 6 days which would likely have been sufficient to remove any residue, if present, from the fronds.

From these observations and the literature available (see 4.1)

Table 5.1 Influence of polysorbate 20, glycerol and their combination on control of bracken by aminotriazole and a comparison of these treatments with Weedazol TL

Treatment	MNF ± LSD[a] 8.7.77	MH ± LSD[b] 8.7.77	MH ± LSD 22.9.77
ATA[c]	25.9 ± 11.1	50.3 ± 11.4	67.3 ± 9.9
Weedazol TL	2.2 ± 1.2	35.8 ± 2.3	45.6 ± 6.9
ATA + P20[d]	26.7 ± 10.0	46.7 ± 10.7	61.0 ± 9.5
ATA + glyc.[e]	22.9 ± 2.9	47.9 ± 4.1	63.3 ± 13.3
ATA+P20+glyc.	27.2 ± 6.6	47.9 ± 11.6	64.5 ± 13.5
P20	68.2 ± 5.2	95.2 ± 5.3	129.9 ± 6.9
glyc.	68.9 ± 5.0	96.3 ± 8.3	128.8 ± 3.4
P20 + glyc.	70.5 ± 7.3	93.7 ± 7.4	129.6 ± 6.5
control	73.1 ± 7.0	98.1 ± 3.5	133.8 ± 3.8

a Mean number of fronds/m^2 ± standard deviation (n-1 degrees of freedom)

b Mean frond height ± standard deviation (n-1 degrees of freedom)

c Aminotriazole

d Polysorbate 20

e Glycerol

(the experimental work in Chapter Four had not been commenced at this point) it would appear that in this instance at least, penetration was not the limiting factor, but in fact translocation.

Re-assessment of the plots in 1978 revealed considerable regrowth on those plots sprayed with aminotriazole, aminotriazole + polysorbate 20, aminotriazole + glycerol and aminotriazole + glycerol + polysorbate 20. Frond densities were measured on those plots which had been sprayed with Weedazol TL, the results indicating a mean frond density of 4.9 fronds/m^2 $\pm$1 SD of 2.0 fronds/m^2. (On the basis of the number of fronds/m^2 in the control plots in 1976 this would represent a reduction in frond density of 93%). The grass sward appeared to have almost totally recovered (Plate 5.1b) and sheep were grazing freely on these and many of the other plots which showed poorer control, but not on the control plots.

Experiment 2

Spraying was again done on a warm dry afternoon. Scorching of the fronds began to appear 48-72h after spraying on all the aminotriazole treated plots. There was some slight recrystallisation of aminotriazole visible on those plots treated with aminotriazole + polysorbate 20 + DMSO but considerably more on those treated with aminotriazole alone. The first rain was observed 72h approx. after spraying. 10 Days after spraying, the following points were observed :- (i) on all plots treated with aminotriazole there was considerable scorching and chlorosis of the new growth, (ii) on those plots treated with DMSO + polysorbate 20 only slight chlorosis and scorching was observed, (iii) there was very slight scorching on 2 of the control plots, probably due to spray drift.

Again, aminotriazole treated plots died back prematurely with a resulting accumulation of litter the following season.

Assessment the following season (Table 5.2) revealed that the aminotriazole + DMSO + polysorbate 20 formulation was significantly more effective in terms of both frond density and height reductions than aminotriazole alone. However, in both cases control was not really satisfactory. Those plots sprayed with DMSO + polysorbate 20 alone showed no significant effects.

Plate 5.1 Influence of various aminotriazole formulations on bracken control.

(a) Background-control; mid picture - typical of aminotriazole without NH_4SCN; foreground - Weedazol TL treated. (One year after treatment.)

(b) Weedazol TL treated. (Two years after treatment.)

Table 5.2 Influence of a DMSO/polysorbate20 additive combination on control of bracken by aminotriazole

Treatment	MNF ± 1SD[a] 29.6.77	MH ± 1SD[b] 29.6.77	MH ± 1SD 22.9.77
ATA[c]+DMSO+P20[d]	22.6 ± 4.0	45.5 ± 2.2	73.3 ± 11.9
ATA	31.8 ± 4.4	56.7 ± 8.6	92.8 ± 3.9
DMSO + P20	64.5 ± 6.3	88.6 ± 4.3	129.4 ± 3.3
Control	71.3 ± 9.7	88.3 ± 7.6	135.1 ± 5.1

a Mean number of fronds/m^2 ± standard deviation (n-1 degrees of freedom)

b Mean frond height ± standard deviation (n-1 degrees of freedom)

c Aminotriazole

d Polysorbate 20

It would therefore appear that increased control from the aminotriazole + DMSO + polysorbate 20 formulation was due to an increase in the rate of penetration. The rainfall 72h after spraying would probably remove any surface residues, this would be more important in the case of those plots sprayed with aminotriazole alone where there were still considerable deposits, hence the difference in control the following season. Grass growth under all plots treated with aminotriazole was affected to some extent, however, it had totally recovered by the following year although, as expected there was also considerable regeneration of the bracken.

Experiment 3

Spraying was done on a cool, overcast day. Soon after spraying was begun, two or three slight showers of drizzle were experienced, however, after this there was no further rain for 6 days. The slight scorching of the fronds in those plots left uncut for the longer periods (6 days, 12 days and uncut) after spraying followed a similar pattern to that observed for Weedazol TL in Experiment 1.

Assessment the following year revealed that only from those plots sprayed and cut 6 days later and onwards was there any sign of effect and even at 6 days this effect was not significant. Cutting after 12 days was followed by significant reductions in both frond density and height although density was still quite high, 47.8 fronds/m^2 compared with 72.5 fronds/m^2 for those plots unsprayed and cut at 0 days. The cutting process itself had no significant effect on frond density as can be seen from a comparison of plots which were unsprayed and uncut with those unsprayed and cut at 0 days although frond height was affected. In those plots sprayed and left uncut, frond density was reduced to 9.6 fronds/m^2 (Table 5.3).

The cutting of fronds will not only remove the aminotriazole source but due to interuption of assimilate movement from the fronds may also affect the movement of aminotriazole within the rhizomes preventing or at least reducing accumulation in the rhizome buds which have been shown to be the main sinks for the herbicide (Volger and Rösger, 1972).

Table 5.3 The effect of post - spraying cutting of the bracken at ground level on subsequent control by Weedazol TL

Treatment	MNF ± LSD[a] 6.7.77	MH ± LSD[b] 6.7.77	MH ± LSD 22.9.77
Unsprayed, uncut	67.4 ± 11.3	86.0 ± 2.7	127.1 ± 10.3
Unsprayed, cut 0 days	72.5 ± 6.5	72.9 ± 4.8	99.7 ± 5.7
Sprayed, cut 0 days	73.5 ± 9.2	71.2 ± 0.4	95.3 ± 6.0
Sprayed, cut 1 day	78.8 ± 6.1	70.2 ± 4.2	99.0 ± 11.2
Sprayed, cut 3 days	70.3 ± 8.3	68.7 ± 1.5	98.3 ± 3.3
Sprayed, cut 6 days	60.2 ± 10.9	63.9 ± 4.7	90.7 ± 14.2
Sprayed, cut 12 days	47.8 ± 13.3	49.0 ± 8.6	70.8 ± 16.7
Sprayed, uncut	9.6 ± 5.0	38.9 ± 5.2	46.2 ± 7.5

a Mean number of fronds/m^2 ± standard deviation (n-1 degrees of freedom)

b Mean frond height ± standard deviation (n-1 degrees of freedom)

Presumably, once penetration had occurred, translocation should have been more or less at a maximum since the fronds had just become fully unfurled. Therefore, if one considers that application must be made to fronds which are fully or almost fully unfurled for effective translocation to the rhizomes to take place and that rhizome apex and bud growth, which will presumably favour herbicide accumulation by acting as active sinks at this stage, ceases in early autumn (Watt, 1950; Conway and Stephens, 1954) then the timing of application is obviously very critical, especially since penetration increasingly limits effectiveness in older fronds (McIntyre, 1962; Veerasekaran et al., 1976) and translocation will decline as a result of decreasing day length and death of the fronds. The results depicted here would suggest that the entire penetration/translocation process from penetration of the fronds to accumulation in the frond buds and rhizome apices may take a matter of weeks rather than days. Hence, application should be as early as possible, therefore calling for much more detailed studies of the movement of herbicides in relation to carbohydrate movement in order that this may be accomplished without loss of effectiveness due to acropetal translocation.

In Experiments 1 and 2 almost all emerging fronds were necrotic and chlorotic, in Experiment 3, only those plots which were sprayed and uncut or sprayed and cut after 12 days were seriously affected.

1977 Field Experiment

It is obvious from the results of the 1976 experiments that the influence of NH_4SCN on the effectiveness of aminotriazole formulations was grossly underestimated. Hence, the investigations set out in Chapter Four were initiated. In addition, this single experiment was set up to determine the influence of NH_4SCN on the aminotriazole + polysorbate 20 + glycerol formulation used in Experiment 1 (1976).

Spraying was carried out on a warm dry afternoon. This dry weather continued for a period of many days after spraying. Observations made 10 days after spraying revealed the following points :- (i) the aminotriazole + polysorbate 20 + glycerol

formulation had caused considerable scorching of the fronds typical of that found the previous year (Plate 5.2a), (ii) the Weedazol TL and aminotriazole + polysorbate 20 + glycerol + NH_4SCN formulations caused very much less scorching (Plate 5.2b), there appeared to be no difference between the two, (iii) the control plots showed virtually no scorching.

From visual assessment of the plots the following season it was felt that there was an increasing order of effectiveness in the three treatments ; aminotriazole + polysorbate 20 + glycerol being least effective and aminotriazole + polysorbate 20 + glycerol + NH_4SCN being most effective. However, statistically, these differences were not significant (Table 5.4).

From Figure 5.1 it can be seen that there is little difference in the rates of penetration of the Weedazol TL and aminotriazole + polysorbate 20 + glycerol + NH_4SCN formulations up to 12h. However, after 24h a significantly greater amount had been taken up from the latter (28.4% as against 47.9%). In Experiment 1 (1976) where no NH_4SCN was used, translocation appeared to be the limiting factor, in this case, efficiency of penetration could account for the observed differences between the two formulations although it is doubtful whether this formulation would be cost effective. However, in the light of these results and those in Chapter Six which indicated more efficient penetration of the lower surface of bracken pinnae from an aminotriazole formulation containing the same concentrations of polysorbate 20 and glycerol as above compared with Weedazol TL within quite a short time interval (4.5h), further work on the use of surfactants in aminotriazole/NH_4SCN formulations could be of considerable value. This would be particularly so if application was by a mist blower to mainly the lower surface or even by helicopter where the downwash from the blades could expose more of the lower surface of the pinnae.

One factor which was overlooked when this experiment was set up was the inclusion of an NH_4SCN control. However, since it is a contact herbicide (Brian, 1976) and since contact damage was reduced by its inclusion in formulations, any significant effects would seem unlikely. NH_4SCN controls have however been built into the 1978 experiments.

Table 5.4 A comparison of the effectiveness of three aminotriazole formulations : Weedazol TL, aminotriazole + polysorbate 20 + glycerol and aminotriazole + polysorbate 20 + glycerol + NH_4SCN

Treatment	MNF ± LSD[a] 20.7.78	MH ± LSD[b] 8.9.78
ATA[c] + P20[d] + glyc[e] + NH_4SCN	4.2 ± 1.2	49.8 ± 17.3
Weedazol TL	7.3 ± 2.6	48.4 ± 15.9
ATA + P20 + glyc	12.8 ± 8.5	62.1 ± 16.2
control	42.1 ± 5.9	153.7 ± 16.3

a Mean numbers of fronds/m^2 ± standard deviation (n-1 degrees of freedom)

b Mean frond height ± standard deviation (n-1 degrees of freedom)

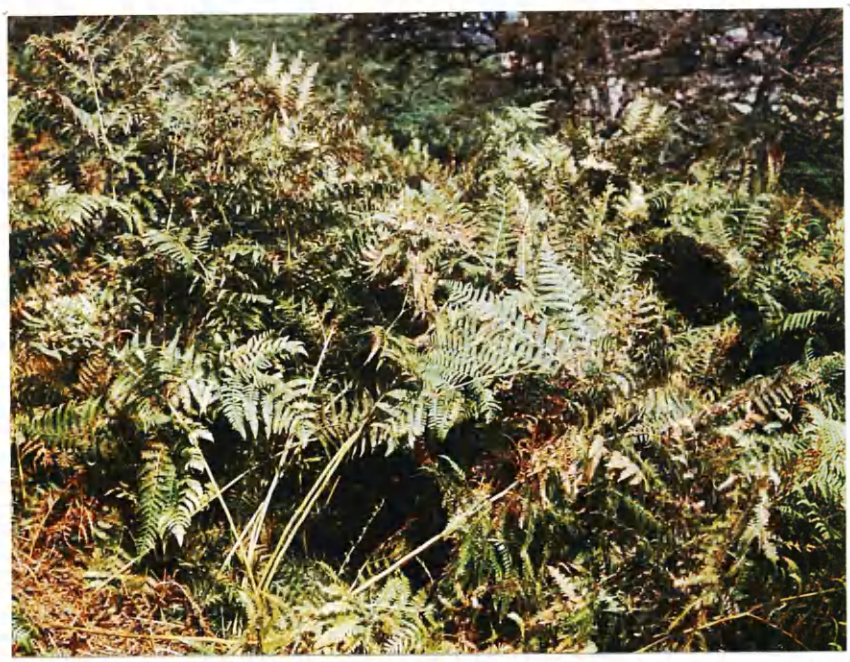
c Aminotriazole

d Polysorbate 20

e Glycerol

Plate 5.2 Influence of various aminotriazole formulations on the scorching of bracken fronds in the year of spraying.

(a) Aminotriazole + polysorbate 20 + glycerol (no NH_4SCN)



(b) NH_4SCN - containing formulations
(Weedazol TL or aminotriazole + polysorbate 20 + glycerol + NH_4SCN)

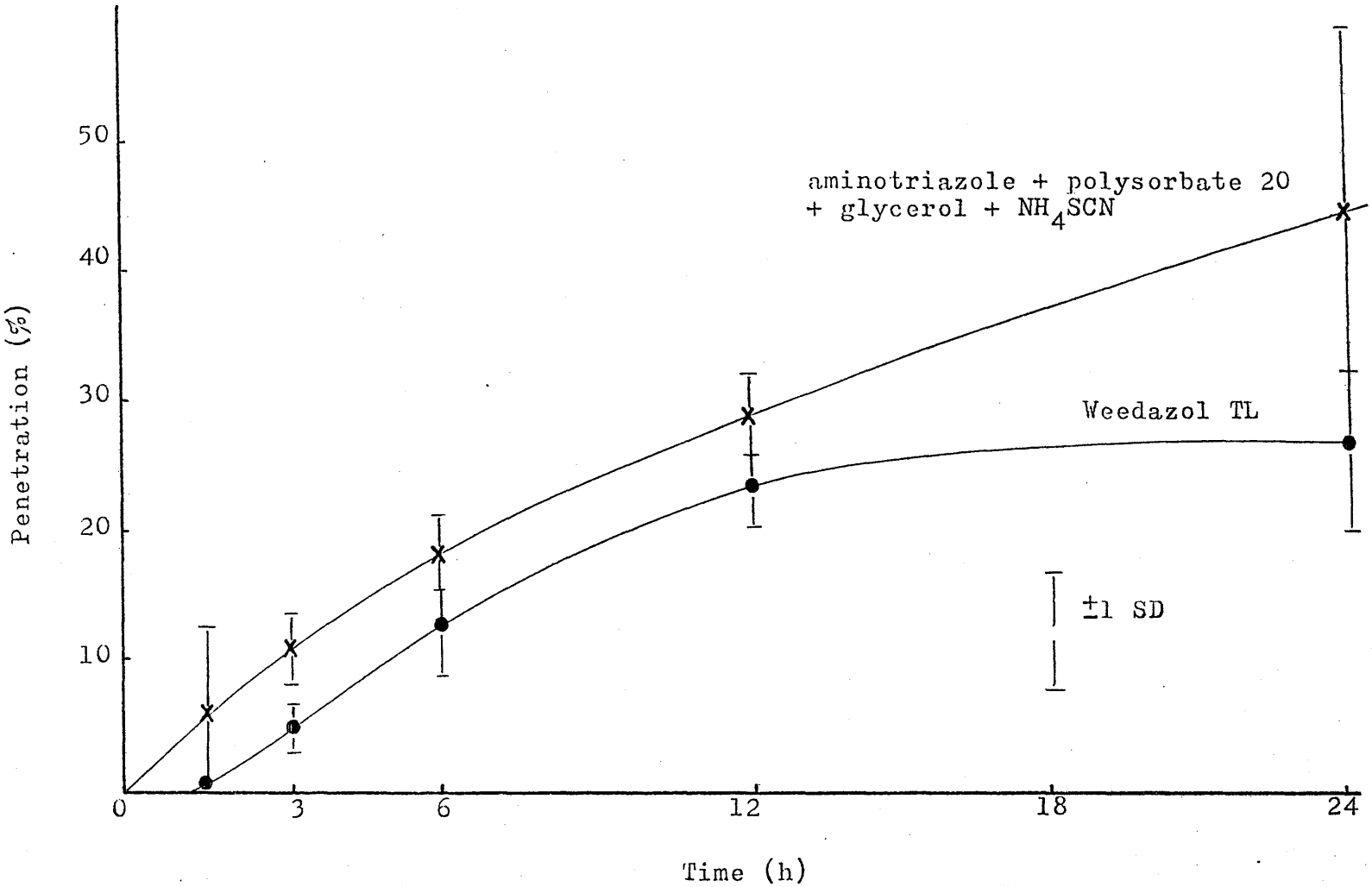


Figure 5.1 Influence of formulation on aminotriazole uptake by bracken pinnules.

The grass sward under all plots treated with aminotriazole was found to have been seriously affected when assessed on 20.7.78. There was considerable litter accumulation on all treated plots which was probably partly responsible although frond numbers on this site were not so dense as those in the 1976 experiments and hence direct contact of the spray with the underlying sward would be more likely. By the middle of September there was considerable recovery. Those fronds which did emerge were typically necrotic and chlorotic.

1978 Field Experiments

Obviously, only observations on the degree of scorching of the fronds and the underlying grass sward are available at this time, however, these do raise some interesting points.

Experiment 1

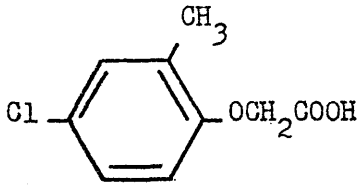
Spraying was done on an overcast cool day, however, no rain was observed for a period of several days after spraying. It would have been preferable if spraying could have been done 3-4 weeks earlier, however, weather conditions made this impossible.

The following points on the degree of scorching of the fronds were noted 8 days after spraying :-

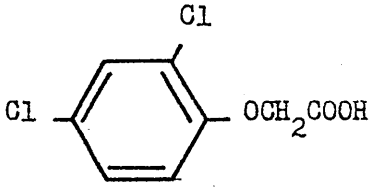
(i) Plots sprayed with aminotriazole alone and aminotriazole + NH_4NO_3 showed moderate scorching (Plate 5.3a) while NH_4NO_3 alone had no effect. This scorching was much less severe than that observed in the previous two years and this is probably attributable to the lateness of spraying. Similar observations have been made for other chemicals (Norris, 1960; Veerasekaran et al., 1976). Such results have in the past been explained on the basis of poorer penetration later in the season (Norris, 1960) and indeed decreasing penetration with increasing frond age has been demonstrated (Veerasekaran et al., 1976). However, if scorching with aminotriazole in the absence of NH_4SCN is due to conjugate formation then differential scorching could be at least partly explained on the basis of the levels of certain plant constituents involved in these reactions at different stages in the growth cycle e.g. serine which is involved in 3-ATAL formation (Carter,1965) or phenolic compounds which could act as reaction inhibitors.

(ii) Plots sprayed with aminotriazole + NaI and NaI alone caused considerable scorching. The NaI would therefore appear to

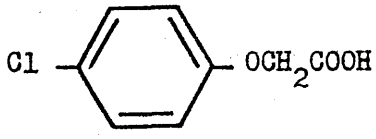
Figure 5.2 Structures of some herbicides used in the control of bracken.



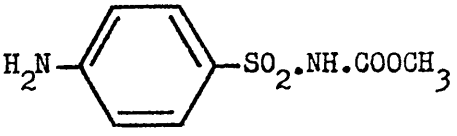
MCPA



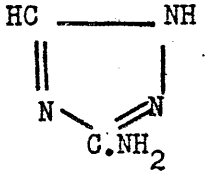
2,4-D



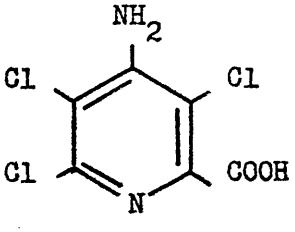
4-CPA



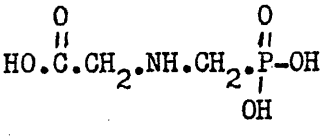
ASULAM



AMINOTRIAZOLE



PICLORAM



GLYPHOSATE

be responsible for this. The effect of such rapid scorching is difficult to predict, however the addition of diquat to an aminotriazole/NH_4SCN formulation has been shown to have a similar effect and ultimately to reduce effectiveness (Arbonnier, 1964; Varlet et al., 1964).

(iii) Plots sprayed with aminotriazole/NH_4SCN 1/1.15 or NH_4SCN alone at the same concentration (38.1g/plot or 7.78kg/ha) showed slight orange-brown scorching at some of the pinnule tips. This type of scorching was totally unlike that from plots sprayed with aminotriazole alone and aminotriazole + NH_4NO_3 and much less severe (Plate 5.3b).

(iv) Plots sprayed with aminotriazole/NH_4SCN 1/3.45 or NH_4SCN alone at the same concentration (114.3g/ plot or 23.3kg/ha) caused similar but more severe scorching to that in (iii).

(v) Plots sprayed with Asulox caused similar scorching to that found for aminotriazole (Plate 5.3c).

(vi) Plots sprayed with Asulox + NH_4SCN caused similar scorching to that found for aminotriazole/NH_4SCN 1/1.15 and NH_4SCN alone at that concentration. This scorching was unlike that caused by Asulox alone and much less severe (Plate 5.3d).

The observations of the behaviour of Asulox + NH_4SCN are particularly encouraging and it would appear that there is a strong possibility of NH_4SCN being of use in Asulox formulations. The addition of NH_4SCN may cut down the severe scorching which can appear after early season spraying and may encourage more efficient translocation, provided assimilate movement is in the correct phase.

Experiment 2

Spraying was done on a reasonably warm sunny afternoon. The first rainfall occurred approximately 36h after spraying. Observations made 13 days later revealed the following :-

(i) at 0.02kg/ha the fronds were virtually unaffected,

(ii) at 0.07kg/ha the fronds were very slightly scorched at the tips of the pinnules,

(iii) at 0.56kg/ha there was some scorching of the pinnule tips, more so than for 0.07kg/ha,

(iv) at 2.22, 4.45 and 8.89kg/ha there was scorching of the pinnule tips. There was no obvious difference between these

Plate 5.3 Influence of NH_4SCN on the scorching of bracken fronds by aminotriazole and asulam in the year of spraying.

(a) Aminotriazole

(b) Aminotriazole + NH_4SCN

Plate 5.3 (contd.)

(c) Asulam

(d) Asulam + NH_4SCN

levels in the degree of scorch, which was not really severe.

(v) at 0.56kg/ha + NH_4SCN the scorching took the form of a more overall lightening in the colour of the foliage as well as the scorching of the pinnule tips. This gave the appearance of a more severe scorching than any of the other treatments.

Further observations on both experiments regarding the degree of damage to the sward were made on 10.11.78. These revealed the following points :-

(i) Plots sprayed with glyphosate at 0.02-0.56kg/ha $\pm$ NH_4SCN had little effect,

(ii) The effects of glyphosate at 2.22-8.89kg/ha varied from moderate to almost complete kill of the sward, depending apparently on the density of the fronds,

(iii) Aminotriazole formulations caused slightly less damage than glyphosate at 2.22-8.89kg/ha. The aminotriazole + NaI formulation appeared to have less effect than the others.

(iv) Asulox and Asulox + NH_4SCN had little or no effect on the sward.

Further trials are now being planned to investigate the influence of other free radical inhibitors on the effectiveness of aminotriazole and possibly asulam while depending on the results of the 1978 trials, further trials into the effect of NH_4SCN on other herbicides such as 4-CPA and 2,4-D may be set up.

Chapter Six

THE INFLUENCE OF MORPHOLOGICAL CHANGES IN BRACKEN PINNAE ON THE PENETRATION OF FOLIAR APPLIED AMINOTRIAZOLE

6.1 INTRODUCTION

Bracken is the most widely distributed of the pteridophytes, indeed, it is present throughout the world except in hot and cold desert regions and with the possible exception of a few annual weeds it is probably the most widely distributed vascular plant (Page, 1976). It is unlike many other pteridophytes which are restricted to damp, shaded environments in that it may exist readily on relatively dry heaths and hillsides (Clapham et al., 1962). In fact, it may completely dominate in such environments to the exclusion of all other plant species, thereby rendering the land quite useless for grazing. Its ability to colonise such widely differing habitats would appear to be at least in part due to its morphological plasticity and in particular its ability to assume a xerophytic frond morphology in the more exposed situations (Boodle, 1904; Woodhead, 1906; Bright, 1923). Tinklin and Bowling (1969) have suggested that the greater efficiency of stomatal control of water loss may also help to explain its success under xeric conditions while in addition to this, Bright (1923) has shown a definite decrease in the number of stomata per unit area as the degree of exposure increases. Such changes in morphology are likely to influence the rate of penetration of foliar applied chemicals and hence this could have a considerable influence on the effectiveness of a spray treatment. Obviously, such factors as frond density, surface area of pinnae, angle of pinnae etc. will all have an important role in determining the effectiveness of a treatment due to effects on spray retention. However, it was decided to choose fronds whose gross morphologies were extremes and to limit the investigation to factors influencing the penetration of their surfaces i.e. cuticle thickness, stomatal density and to rate of water loss.

For this purpose, bracken growing in three differing environments were chosen for study. These were as follows, and for the purpose of the discussion will be referred to as types (i), (ii) and (iii) :- type (i) - bracken growing under shade, type (ii) - bracken from within the main stand and type (iii) - bracken from around the edge of the stand.

6.2 SITE DESCRIPTION

Drumclog Muir in Stirlingshire (Grid reference NS 552757) was chosen as the site of study since all three types of bracken could be found within a radius of 25 metres on a shallow west facing slope.

Type (i) bracken was found growing under the moderate shade of downy birch (Betula pubescens Ehrhart) at the base of the slope and in relatively damp conditions. Numbers were sparse (1 or 2/m^2) and very variable in height (40-160cm approximately). The soil was an acid brown earth with slightly impeded drainage and with a well developed (15cm approximately) organic horizon. Other plants typical of this community included (a) two other species of fern; lady fern (Athyrium filix-femina (L.) Roth) and Dryopteris sp. and (b) the following grass species; wavy hair-grass (Deschampsia flexuosa (L.) Trinius), creeping soft-grass (Holcus mollis L.) and Agrostis sp. (Plate 6.1a).

Type (ii) bracken growing within the main stand on a freely drained acid brown earth with a fairly well developed organic horizon (7-9cm approximately) was approximately 140-160cm in height and at a density of 30-40 fronds/m^2. The other main components of the plant community were the following grasses; sheep's fescue (Festuca ovina L.), Agrostis sp., wavy hair-grass and creeping soft-grass. (Plate 6.1b).

Type (iii) bracken consisted of the small fronds ($\lesssim$ 60cm in height) growing in a blaeberry (Vaccinium myrtillus (L.) Hull) dominated community immediately adjoining the main bracken stand. The soil here was of a more podzolised brown earth type, again with a well developed organic horizon (15cm approximately). Again, the fronds were sparse in number (1 or 2/m^2) and considerably lighter in the colour of the pinnae than types (i) and (ii).

Other less dominant members of this community included heather (Calluna vulgaris L.), gorse (Ulex europaeus L.), downy birch, Agrostis sp. and sheep's fescue (Plate 6.1c).

Pinnae typical of all three types are shown in Plate 6.1d.

6.3 EXPERIMENTAL

6.3.1 Materials

Weedazol TL, aminotriazole, polysorbate 20 and glycerol were as previously described (see 2.2.1; 5.2.1)

6.3.2 Methods

6.3.2.1 Penetration studies

All penetration studies were carried out in the laboratory under diffuse tungsten lighting. Conditions in the laboratory were continually monitored and found to remain quite constant at $20\pm2^{o}C$ and 65-75% r.h.

It was decided to carry out these studies in the laboratory rather than in the field since there were likely to be differences in humidity, light intensity and possibly temperature between the sites from which the three types were selected, all of which have been shown to affect penetration (Hammerton, 1967).

Four fronds from each of the three types were selected during early morning and detached just above the first pair of pinnae. These were transferred to polythene bags, taken immediately to the laboratory and the base of the stems immersed in flasks of water.

The treatment of the plants was standardised as follows :- 10 µl was applied as ca 1 µl droplets from a 10 µl Eppendorf pipette to leaflet pairs 2-6 of each of the fourth pair of pinnules from the base of the lowermost pair of pinnae on the detached fronds. Lanolin was used to prevent spreading beyond this area. Each pinna had one pinnule treated on the upper side and one on the lower side thus making 8 pinnules treated on the upper side and 8 on the lower for each of the three types. (In one experiment, both the third and fourth pair of pinnules from the base of the pinnae were used.) After 4.5h the treated pinnules were detached and washed with 20ml deionised water and

Plate 6.1 Ecological niches containing the three types of bracken under study (a-c) and pinnae typical of each (d).

(a) Type (i)

(b) Type (ii)

(c) Type (iii)

(d) Right - left; Type (i) - type (iii)

the washings analysed for aminotriazole by the method of Storherr and Burke (1961). A preliminary study revealed that total recovery was possible by this method, provided that washing was done immediately after treatment. Two aminotriazole formulations were employed in these studies, these were as follows :-

(a) Aminotriazole (90.0 µg a.i. in 10 µl) in the presence of polysorbate 20 (19.2 g/litre) and glycerol (0.6ml/litre), a formulation based on previous results (see 2.3) and used in certain field experiments (see 5.2.2).

(b) Weedazol TL (aminotriazole application rate - 90.0 µg a.i. in 10 µl).

6.3.2.2 Cuticle membrane development

The fourth leaflet pair from the base of the fourth pinnules from the base of the second lowermost pair of pinnae were used to standardise the material from the three types. The leaflets were fixed in a 3% solution of glutaraldehyde buffered to pH 7.4. The material was then dehydrated through a series of acetone concentrations and embedded in an epoxy resin. 1 µ Transverse sections were cut, mounted and stained with toluidine blue. Ultimately, cuticle thickness was measured and the sections photographed using a photo-microscope.

6.3.2.3 Leaflet surfaces and stomatal densities

Similarly positioned leaflets to those in 6.3.2.2 were dehydrated through a series of ethanol concentrations (50-70-90-100%) followed by a further change in 100%. The material was then placed in a 1/1 mixture of 100% ethanol/amyl acetate followed by two changes in amyl acetate alone. The samples were then critical point dried and mounted on stubs, half of them adaxial surface uppermost and half abaxial surface uppermost. These were then coated with gold to a thickness of 500Å. Ultimately, they were scanned and photographed using a Cambridge Stereoscan 600 scanning electron microscope.

6.3.2.4 Rates of water loss from detached pinnae

Four fronds from each of the three types were selected during early morning and detached just above the first pair of pinnae. These were transferred to polythene bags, brought immediately to the laboratory and the bases of the stems immersed in flasks of water for a period of time (2h approximately) to bring the pinnae to maximum turgidity. The lowermost pinnae were removed and weighed at hourly intervals for 7h. The results were expressed as both percentage losses in fresh weight and percentage losses in moisture.

6.3.2.5 Statistical analysis

This was carried out as previously described (see 2.2.2.8).

6.4 RESULTS

6.4.1 Penetration studies

The aminotriazole-polysorbate 20-glycerol formulation was tested on three occasions, September 1977, July 1978 and August 1978 to determine whether the trends obtained were similar throughout the main part of the growing season, which in fact they were. For each type of bracken, penetration through the lower surface was greater than through the upper while penetration of types (ii) and (iii) was very similar but greater than type (i) for both surfaces (Table 6.1).

In the case of the Weedazol TL formulation, there was no significant difference in penetration between the upper and lower surfaces of any of the three types while type (i) still showed less penetration than types (ii) and (iii). Again, there was no significant difference between the corresponding surfaces of types (ii) and (iii) (Table 6.2).

When the Weedazol TL and aminotriazole-polysorbate 20-glycerol formulations were compared using types (i) and (iii) the following trends were noted :- (a) again in the case of the Weedazol TL formulation the lower surfaces did not show greater penetration than the upper, (b) there was no significant difference in the degree of penetration between the Weedazol TL

Table 6.1 Penetration of aminotriazole from an aminotriazole-polysorbate 20-glycerol formulation into the leaflets of types (i)-(iii). Aminotriazole application rate - 90.0 µg/pinnule (assessed after 4.5h)

Type	MQPS[a] (µg)	Mean % penetration	SD[b] (%)
(Sept. '77)			
(i) upper surface	79.9	11.2	4.0
(i) lower surface	67.0	25.6	8.0
(ii) upper surface	62.4	30.7	11.0
(ii) lower surface	40.6	54.9	4.5
(iii) upper surface	59.0	34.5	11.8
(iii) lower surface	37.0	58.9	8.9
(July '78)			
(i) upper surface	72.9	19.0	8.4
(i) lower surface	65.8	26.9	10.1
(ii) upper surface	67.3	25.2	4.3
(ii) lower surface	50.2	44.2	5.1
(iii) upper surface	65.4	27.3	15.7
(iii) lower surface	53.0	41.1	10.4
(Aug. '78)			
(i) upper surface	88.1	2.1	1.1
(i) lower surface	75.9	15.7	5.4
(ii) upper surface	77.8	13.6	6.8
(ii) lower surface	52.4	41.8	6.7
(iii) upper surface	79.1	12.1	5.3
(iii) lower surface	51.4	42.9	9.7

a Mean quantity of aminotriazole left on pinnule surface

b Standard deviation (n-1 degrees of freedom)

Table 6.2 Penetration of aminotriazole from a Weedazol TL formulation into the leaflets of types (i)-(iii). Aminotriazole application rate - 90.0 µg/pinnule (assessed after 4.5h).

Type (Sept. '78)	MQPS[a] (µg)	Mean % penetration	SD[b] (%)
(i) upper surface	77.5	13.9	8.2
(i) lower surface	73.9	17.9	6.2
(ii) upper surface	68.2	24.2	3.5
(ii) lower surface	67.7	24.8	4.7
(iii) upper surface	71.6	20.4	5.2
(iii) lower surface	69.6	22.7	6.6

a Mean quantity of aminotriazole left on pinnule surface

b Standard deviation (n-1 degrees of freedom)

and the aminotriazole-polysorbate 20-glycerol formulations for the upper surfaces of types (i) and (iii), (c) as before, there was significantly greater penetration of the upper surface of type (iii) compared with type (i) and (d) both lower surfaces treated with the aminotriazole-polysorbate 20-glycerol formulation showed greater penetration than their corresponding upper surfaces although in the case of type (iii) the difference was not significant (Table 6.3). However, the results depicted in Table 6.1 tend to reinforce the arguement for significantly more penetration through the lower surface with this formulation. Thus, it would appear that the aminotriazole-polysorbate20-glycerol formulation enhances penetration of the lower surfaces rather than reduces penetration of the upper surfaces.

6.4.2 Cuticle membrane development

The results of these studies revealed that the cuticles of types (ii) and (iii) were of similar thickness, 4.9 ± 0.7 μ and 5.1 ± 0.6 μ respectively for the upper surfaces and 2.0 ± 0.5 μ and 1.9 ± 0.4 μ respectively for the lower surfaces. The differences between types (ii) and (iii) for their respective surfaces were not significant. Type (i) showed significantly less development of both the upper and lower cuticles (2.6 ± 0.5 μ and 1.6 ± 0.4 μ respectively). These estimates of cuticle thickness were obtained from the mean $\pm$1standard deviation of 30 measurements, 10 measurements each from 3 leaflets from 3 different fronds. In addition to these measurements the following observations and measurements were made :-

(a) underlying the upper epidermis of types (ii) and (iii) there is a well differentiated and almost continuous hypoderm while such a layer is absent in type (i) except for a few cells in the region of the midrib and certain veins. Similar observations were made by Boodle (1904), (b) in types (ii) and (iii) cuticle formation is extended completely around the upper epidermal cells forming an "inner cuticle" between the epidermis and the hypoderm. (A similar observation was made by Goodman and Addy (1963) while studying apple leaves), (c) in accordance with the observations of Boodle (1904) and Bright (1928) there is greater development of the palisade tissue in types (ii) and

Table 6.3 Penetration of aminotriazole from Weedazol TL and aminotriazole - polysorbate 20 - glycerol formulations into the leaflets of types (i) and (iii). Aminotriazole application rate - 90.0 µg/pinnule (assessed after 4.5h)

Type (Sept. '78)	Form.[c]	MQPS[a] (µg)	Mean % penetration	SD[b] (%)
(i) upper surface	WTL[d]	73.8	18.0	2.0
(i) lower surface	WTL	77.0	14.5	4.1
(i) upper surface	P20[e]	73.4	18.5	2.1
(i) lower surface	P20	69.7	22.6	3.3
(iii) upper surface	WTL	66.9	25.7	11.6
(iii) lower surface	WTL	63.4	29.6	8.0
(iii) upper surface	P20	66.8	25.8	6.6
(iii) lower surface	P20	54.2	39.8	19.4

a Mean quantity of aminotriazole left on pinnule surface
b Standard deviation (n-1 degrees of freedom)
c Formulation
d Weedazol TL
e Aminotriazole + polysorbate 20 + glycerol

(iii) (the more exposed types), it occupies a greater proportion of the mesophyll while the individual cells are more elongated than those of type (i), (d) measurements of overall leaflet thickness taken from approximately midway between the midrib and the leaflet margin (mean $\pm$ 1 standard deviation of 24 measurements, 8 measurements each from 3 leaflets from 3 different fronds) revealed no significant difference in thickness between types (ii) and (iii) (222.7 ± 23.3 µ and 231.3 ± 14.0 µ respectively while type (i) was significantly thinner (138.7 ± 9.3 µ) (Plate 6.2).

6.4.3 Leaflet surfaces and stomatal densities

Again there were many similarities between types (ii) and (iii) :-

(a) both were found to bear a great number of both unicellular and multicellular hairs on their lower surfaces in the region of veins and in particular along the midrib (Plate 6.3d and f) whereas type (i) was almost totally devoid of hairs (Plate 6.3b), (b) both showed similar stomatal densities on the lower surface, greater than found in type (i) (Plate 6.3a-f), (c) the upper surfaces were very similar in appearance (Plate 6.4c-f), type (i) was quite different in appearance having a much more angular and convoluted appearance (Plate 6.4a-b).

The upper surfaces of all three types appeared to be astomatal and devoid of hairs.

6.4.4 Rates of water loss from detached pinnae

The rates of water loss expressed either as percentage losses in fresh weight or percentage losses in moisture revealed similar trends in both cases (Figure 6.1a and b). The rate of loss was slowest in type (ii) followed by type (iii) and then type (i) although there was little difference between types (i) and (iii).

Plate 6.2 Transverse sections through bracken leaflets typical of the three types under study. Magnification X46. (Top - bottom ; Type (i)-(iii))

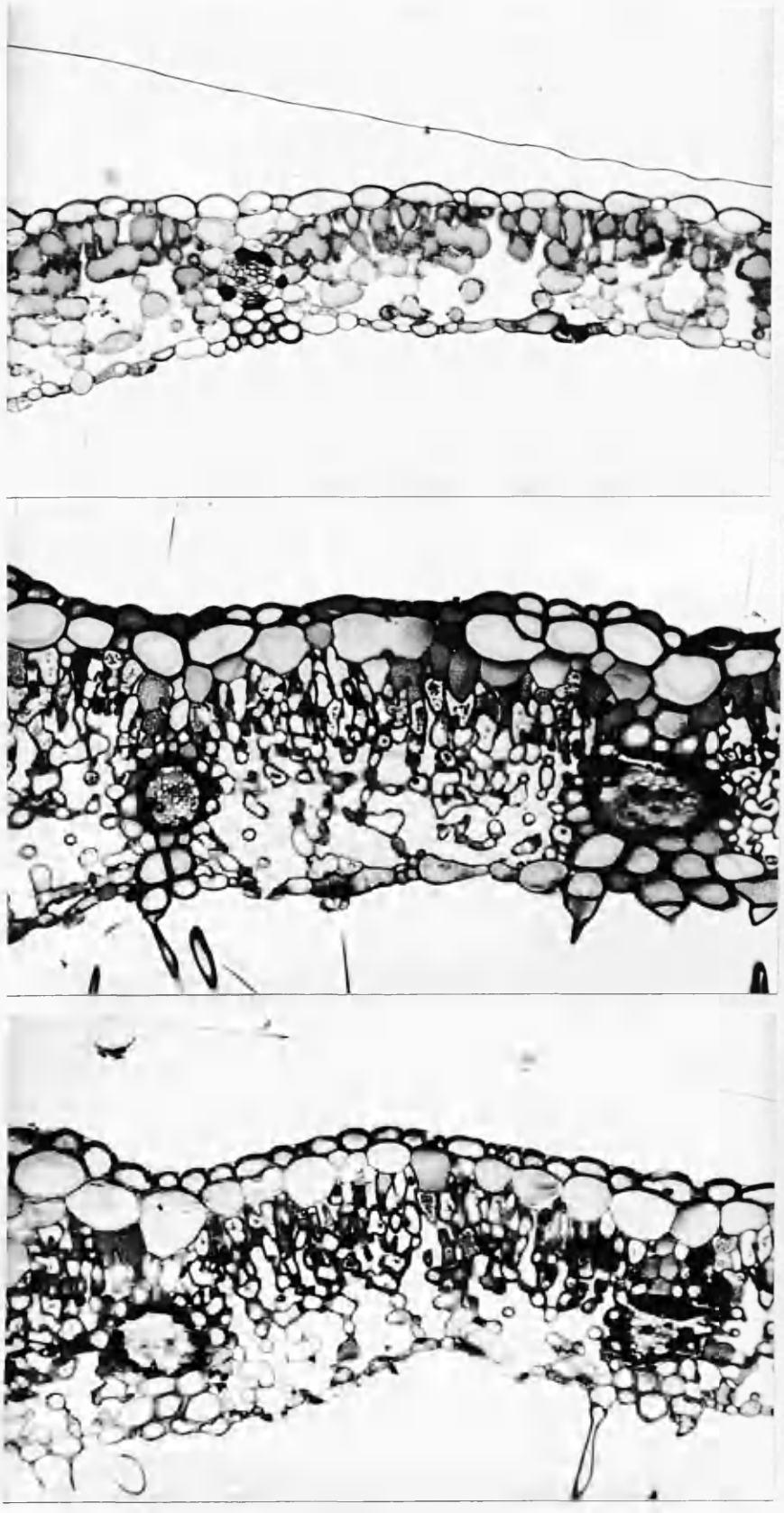
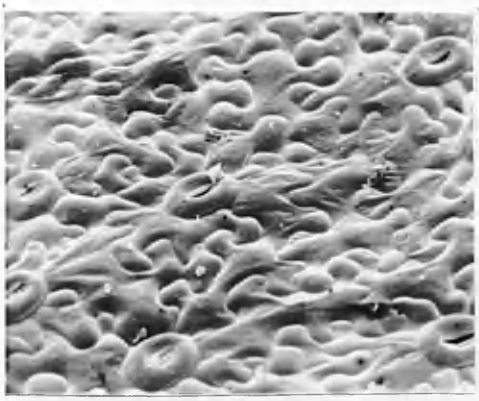
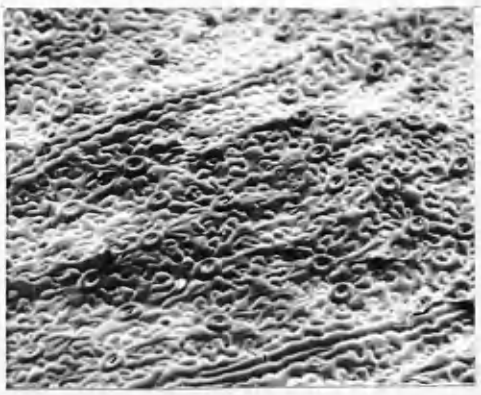


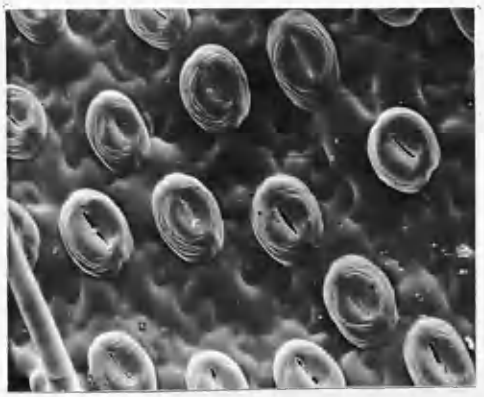
Plate 6.3 Electron micrographs of the lower surfaces of leaflets typical of the three types of bracken under study.
Magnifications X 200 and X 500.



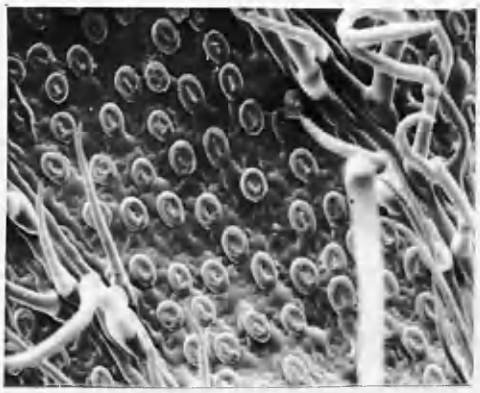
(a) Type (i) x 500



(b) Type (i) x 200

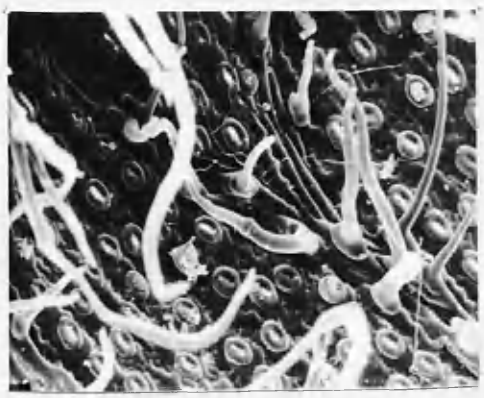


(c) Type (ii) x 500



(d) Type (ii) x 200

(e) Type (iii) x 500

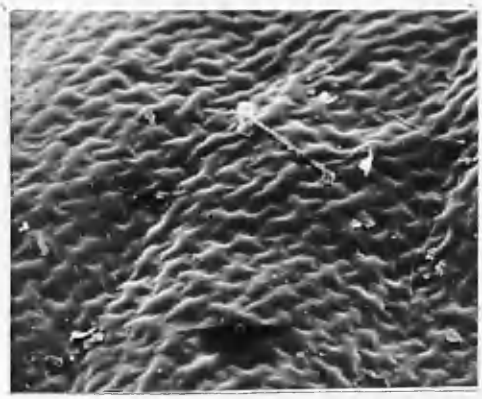


(f) Type (iii) x 200

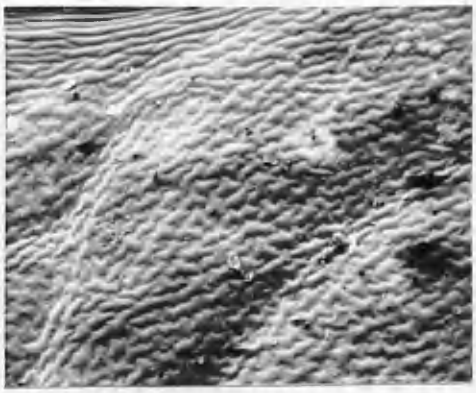
Plate 6.4 Electron micrographs of the upper surfaces of leaflets typical of the three types of bracken under study.
Magnifications X 200 and X 500.

(a) Type (i) x 500

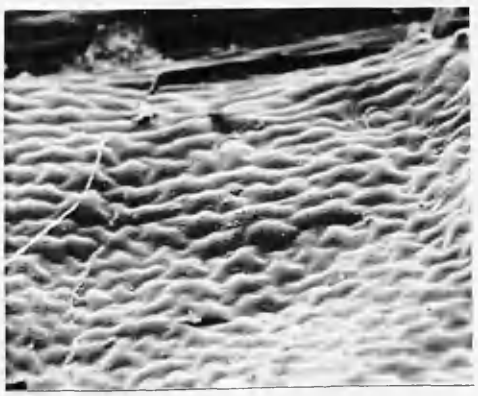
(b) Type (i) x 200



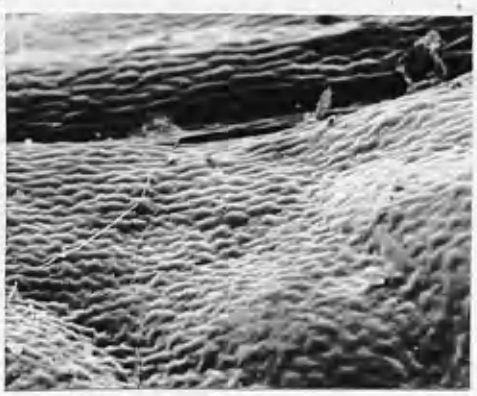
(c) Type (ii) x 500



(d) Type (ii) x 200



(e) Type (iii) x 500



(f) Type (iii) x 200

Figure 6.1a Rates of water loss from detached pinnae of types (i)-(iii) expressed as percentage losses in fresh weight. Standard deviations (n-1 degrees of freedom) were in the 1-7 % range.

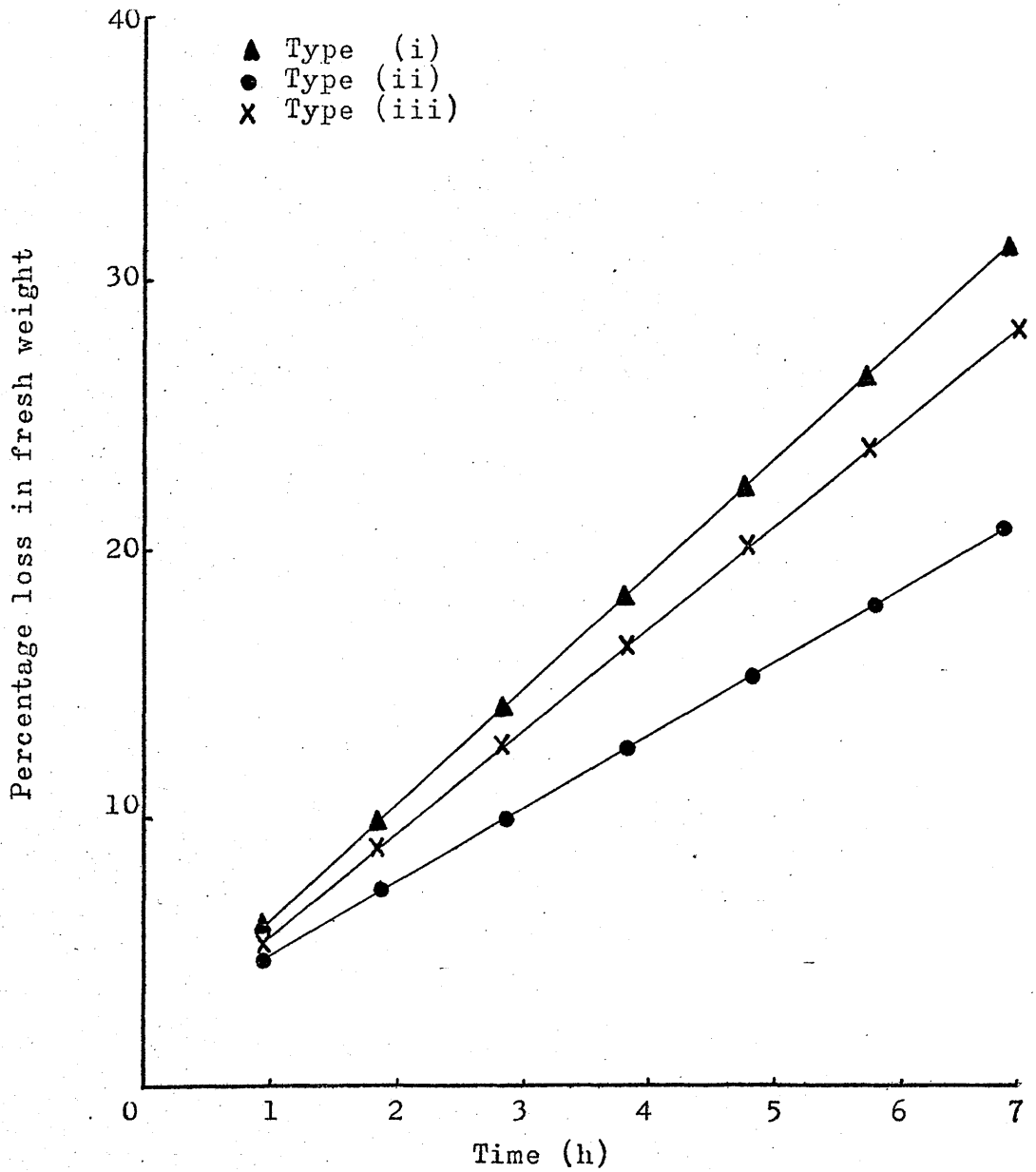
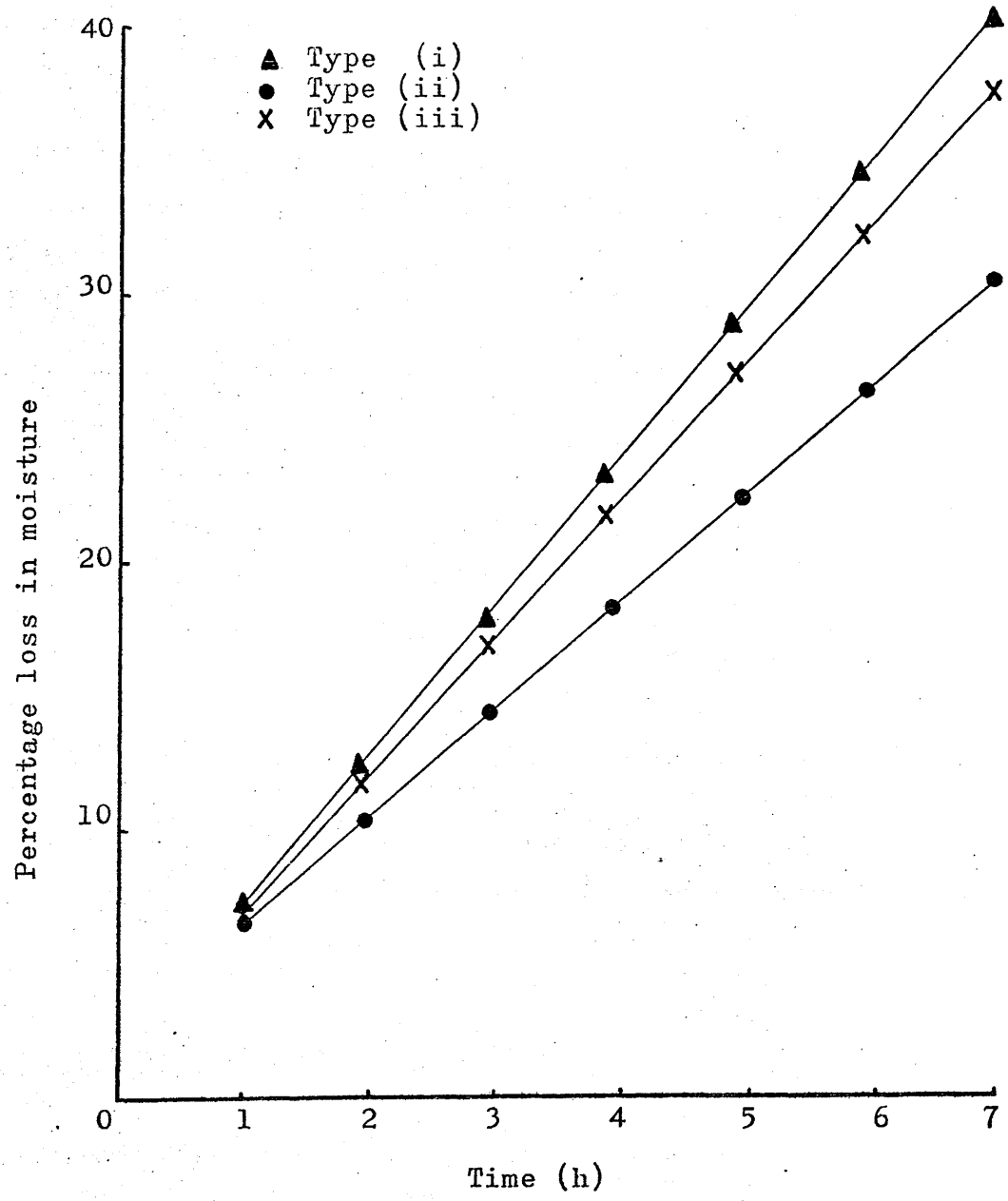


Figure 6.1b Rates of water loss from detached pinnae of types (i)-(iii) expressed as percentage losses in moisture. Standard deviations (n - 1 degrees of freedom) were in the 1-8 % range



6.5 DISCUSSION AND CONCLUSIONS

The results in Table 6.1 indicated that penetration of the upper surfaces of types (ii) and (iii) was not significantly different. Penetration of the upper surface of type (i) was however significantly less than both, while the studies on cuticle development (see 6.4.2) revealed that cuticle thickness in types (ii) and (iii) was not significantly different but that both were significantly thicker than type (i). Such trends in penetration could not be explained in terms of differential stomatal densities since all three upper surfaces appeared to be astomatal (Plate 6.4a-f). Thus, penetration must be considered to be cuticular rather than stomatal.

Considerable controversy surrounds the relationship between cuticle thickness and penetration. Pereira et al. (1971) attributed the increased resistance of one cabbage cultivar compared with a second to an increase in cuticular wax while Robertson and Kirkwood (1969) concluded that penetration must be influenced by any factor influencing cuticle thickness. Hull (1964) however, has stated that to relate the penetration of organic substances to cuticle thickness per se is rather difficult because of the profound chemical and structural differences among different cuticles. Indeed, Norris (1974) could not detect any correlation between thickness or weight of cuticle per unit area and ^{14}C-2,4-D penetration. Thus, it is possible that chemical and structural differences in the cuticle of type (i) may account for the reduced penetration. However, Edgerton and Haeseler (1959) found that exposure of apple leaves to artificial light (500 foot candles) prior to treatment reduced absorption of naphthaleneacetic acid and naphthaleneacetamide but increased cuticle thickness compared with plants grown at 50-100 foot candles. Skoss (1955) has shown that increased light intensity increases the wax content of cuticles and that the amount of wax may be the major factor limiting their permeability to water. However, the other possibility exists that the cuticles of types (ii) and (iii) may have become subject to weathering and degradation due to sunlight and the action of wind and rain (Crafts and Foy, 1962) whereas type (i)

may be less affected being at the base of the slope in a more sheltered position and under shade. Thus, since it has been proposed that aminotriazole is taken up via an aqueous route (Crafts, 1964), a comparison of the rates of water loss from the three types of pinnae was undertaken even although it is recognised that the rate of movement of water through cuticles from outside to inside may differ from the reverse process, (similar trends have also been found for inorganic ions and organic compounds (Yamada et al., 1964; 1965)). In addition, it is also recognised that this would be a measure of water loss from the pinnae as a whole rather than just the upper surface. The results of this study revealed no trends which might readily correlate with the observed increase in penetration of types (ii) and (iii) while the electron micrographs showed no indication of obvious breakdown of, or damage to the cuticle in any of the specimens examined. One conceivable explanation could be that the poorer penetration of type (i) is linked with the observation that spreading of the solution on the leaflet surface was much poorer compared with types (ii) and (iii) where considerable spreading took place. In type (i), the droplets remained almost totally intact whereas in (ii) and (iii) spreading over the leaflet midrib and a considerable portion of the rest of the leaflet took place. Enhanced uptake and activity has been shown to occur from solutions overlying veins (Crafts, 1956; van Overbeek, 1956; Leonard, 1958), possibly due to the numerous ectodesmata in the outer walls (Franke, 1960; 1961).

Such differential spreading could be due to the topography of the leaflets (Martin and Juniper, 1970). Type (i) had a much more convoluted appearance than (ii) and (iii) and this could be partly responsible although possible differences in the ultramicroscopic structure and surface wax might also be of considerable importance (Holly, 1976). The process involved in the preparation of the samples for scanning electron microscopy could have removed surface wax deposits which could influence contact and spreading (Holly, 1976). Hence, this factor would require investigation.

Similar trends to those above were also shown for penetration

of the lower surfaces (Table 6.1). Here, the differences in cuticle thickness between the three types are much less obvious. However, the more rapid penetration of types (ii) and (iii) is more readily explicable. Types (ii) and (iii) were found to bear a great number of hairs in the region of the veins and in particular along the midrib while type (i) was almost totally devoid of hairs (Plate 6.3). Hair cells themselves, their basal cells and those epidermal cells surrounding them have been shown to be particularly active sites of absorption (Franke, 1967) again possibly due to the numerous ectodesmata in their outer walls (Franke, 1960; 1961). In addition, the greater stomatal densities in types (ii) and (iii) could also have a bearing on penetration due to penetration of the cuticle overlying the guard cells which appears to be more permeable (Sargent and Blackman, 1962; Franke, 1964a) and/or penetration of the stomatal pore itself (Currier et al., 1964; Foy, 1964) followed by penetration of the cuticle lining the pore although conflicting results have been observed as to the possibility of this occurring (Sargent and Blackman, 1962; Currier et al., 1964; Foy, 1964; Middleton and Sanderson, 1965). Schönherr and Bukovac (1972) have investigated the factors governing this process in considerable detail. Numerous ectodesmata have also been demonstrated in the walls of the guard cells and cells surrounding the stomatal pores (Franke, 1964a; 1964b).

The results of Tables 6.2 and 6.3 which indicated that over the duration of the experiment the degree of penetration of the upper surfaces was unaffected by the formulation employed would seem reasonable on the basis of observations on the behaviour of the droplets which revealed no differences in wetting or spreading. In the case of the lower surfaces where reduced penetration was noted from the Weedazol TL formulation, it could be observed for types (ii) and (iii) that the droplets were held on the hairs and did not make good contact with the leaf surface proper, while for the polysorbate 20 containing formulation good wetting of the leaf and spreading was achieved quickly. Hence, for Weedazol TL, penetration associated with (a) the veins in general and in particular with the basal cells

of the hairs and those surrounding them and (b) the stomata, either associated with the guard cells and/or the stomatal pore itself might be reduced. For type (i), reduced spreading was observed with Weedazol TL, and this combined with the possible lack of penetration of the stomatal pores due to the lack of an efficient surfactant might account for the reduced penetration.

Thus, from the results of this study it would appear that the degree of spreading of the spray solution and stomatal and/or leaf hair densities are of more importance than actual cuticle thickness although the possibility of other less obvious factors being involved cannot be ruled out. Differences in the degree of penetration for pinnules treated with the same formulation, but in different exercises might be explained in terms of pinnule age and possibly variations within the temperature and humidity ranges employed.

Chapter Seven

ABSORPTION OF IRON BY BEAN LEAVES FROM FRUCTOSE - IRON CHELATES

7.1 INTRODUCTION

Although iron makes up about 5% by weight of the earth's crust and is invariably present in all soils (Mengel and Kirkby, 1978), cases of deficiency are commonplace in horticultural and agricultural crops. This deficiency normally manifests itself as a chlorosis of the leaves, and since most cases occur on calcareous soils, it is often termed lime induced chlorosis. The reason for iron chlorosis has been sought in the availability of iron in the soil solution rather than in the total iron in the soil (De Kock, 1966).

The role of chelation as a corrective measure for lime induced chlorosis must begin with an appreciation of the problems associated with the solubility of iron. In aqueous solutions ferrous ions are readily oxidised by dissolved oxygen to ferric ions, this being the preferred oxidation state under all but acidic and anaerobic conditions (Christopher et al., 1974), while the situation is further complicated by the fact that the solubility of the ferric ion at neutral pH is 10^{-17}M. Hence, at all but acid pH (< 2), ferric ions hydrolyse to form insoluble ferric hydroxide (Christopher et al., 1974). Thus, the use of soil applied inorganic iron salts generally has no effect since the iron rapidly forms insoluble oxides (Mengel and Kirkby, 1978). To overcome these solubility problems, iron is supplied to the soil in the form of chelates. The most commonly used include ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxyethylethylenediaminetriacetic acid (HEEDTA) and ethylenediamine di(o-hydroxyphenylacetic acid) (EDDHA). Due to chelate instability, the first three are of limited value in alkaline soils, EDDHA however has been used successfully (Kroll, 1957).

As pointed out by Wallace et al. (1957) and Wallace (1963) the use of foliar applied sprays confers several advantages over the use of soil applications :-

(a) the chelates are free from the many soil reactions which may lead to immobilisation and decomposition,

(b) they do not require irrigation to move them into the root zone of the soil,

(c) they are more economical,

(d) they bring about more rapid responses.

They do however have certain disadvantages :-

(a) they may bring about incomplete coverage and response since iron is generally considered to be immobile in growing plants when applied to the foliage,

(b) there is often a need for repeated applications,

(c) there is a greater chance of causing toxicity problems.

Generally, foliar applications of the polyaminopolycarboxylic acid chelating agents have not been as successful as soil applications (Wallace, 1963).

Although application to the foliage is not free from the solubility problems experienced in the soil, ferrous sulphate ($FeSO_4$) has been used with success in many cases (Bar-Akiva and Hewitt, 1959; Withee and Carlson, 1959; Neumann and Prinz, 1974; 1975; Seeliger and Moss, 1976), indeed, it has been more successful than the polyaminopolycarboxylic acid chelates (Withee and Carlson, 1959; Neumann and Prinz, 1975), probably due to more efficient penetration (Lunt and Kohl, 1956; Kannan, 1969). However, leaf scorching by $FeSO_4$ can be a problem (Boynton, 1954; Withee and Carlson, 1959; Neumann and Prinz, 1975) although, Neumann and Prinz (1975) largely overcame this problem by the incorporation of a silicone based surfactant into the spray solution. This caused the spray solution to form a film on the leaf surface rather than droplets, thereby preventing the formation of concentrated deposits as the droplets dried out. In addition to this problem, if conditions are not favourable to relatively rapid penetration then oxidation of the $FeSO_4$ with a resultant depletion in soluble iron will perhaps become important. This would be likely to further reduce penetration since penetration from solid deposits has been shown to proceed very slowly (see 2.3.1).

It would therefore appear that there is a need for a cheap iron chelate which might eliminate these problems and perhaps reduce the disadvantages associated with foliar sprays as previously described.

Various sugar acids and related compounds have been tested with some success (Lunt and Kohl, 1956; Wallace et al., 1957; Wallace, 1963), however, Wallace et al. (1957) believe that the ideal type of metal chelate for spray application may be a non-ionising molecule.

Reducing sugars and polyols have been shown to form soluble stable complexes with iron (Charley et al., 1963) and fructose chelates (normally referred to as ferric fructose) in particular have been used as iron supplements for the treatment of anaemia in man and animals (Christopher et al., 1974; Eatough et al.,1974). In addition, Larsen et al. (1960) have demonstrated that despite its excellent solubility and stability, the EDTA chelate of iron is poorly absorbed by the gastrointestinal tract of rats. This complex carries a net negative charge in the range of pH values largely encountered in this tract. On the other hand, low molecular weight complexes of iron/fructose and other simple sugars readily penetrate biological membranes such as are encountered in the mucosal wall (Charley et al., 1963).

Thus, with these many factors in mind, it was decided to investigate the possibility of using iron-sugar chelates as a relatively cheap foliar spray for the correction of iron chlorosis.

7.2 EXPERIMENTAL

7.2.1 Materials

Fructose, anhydrous ferric chloride ($FeCl_3$) and potassium acetate were purchased from Hopkin and Williams Ltd. Polysorbate 20 was as previously described (see 2.2.1).

7.2.2 Methods

7.2.2.1 Chelate preparation

It was decided to limit the study initially to fructose/iron chelates. $FeCl_3$ was used as the iron source throughout.

The nature of the ferric fructose complex is dependent on the molar ratio of fructose/ferric. At low ratios, a polymer of 65,000 can be isolated while in the presence of excess fructose a chelate of maximum molecular weight of 2000 is formed (Spiro and Saltman, 1969). These polymers are capable of existing in equilibrium with low molecular weight species and so it would appear that the rate determining step in the absorption process for these polymeric species is probably depolymerisation (Christopher et al., 1974).

Thus, four chelates differing only in the fructose/ferric ratio were formulated. These ratios were as follows 5/1, 20/1, 50/1 and 200/1. These were prepared as follows :

1.622g of reagent grade $FeCl_3$ were dissolved in distilled water and the volume made to 1 litre. This was filtered through Whatman No. 42 filter paper and 4x25ml aliquots withdrawn from the filtered solution and placed in 100ml volumetric flasks. To these were added fructose solutions sufficient to bring about the molar ratios quoted above. The pH of each was then made to 4.7 with 0.1M potassium acetate, the solutions made up to volume with distilled water and the pH re-checked.

7.2.2.2 Penetration

All plants were germinated, selected and grown as previously described in a growth room adjusted to a 16h day length and a temperature of $30\pm3^{o}C$ (see 2.2.2.1). A few hours prior to treatment, the plants were transferred to a Fisons Series 111 growth cabinet as previously described (see 4.2.2.10). The temperature here was maintained at $30\pm0.75^{o}C$ and humidity at $48\pm5\%$ r.h., $64\pm5\%$ r.h. or 95-100% r.h. Just prior to treatment, the leaves were washed with distilled water to remove any surface deposits of iron which preliminary investigations had shown to interfere with results. On drying, 200 µl of chelate was applied per leaf from a 100 µl Eppendorf pipette as ca 5 µl droplets, randomly to the upper surface. Plant age was as previously described (see 2.2.2.1). No spreading of the droplets was observed during the course of the experiments. On completion of the experiments, the leaves were washed with 25ml distilled water and the washings analysed for iron by atomic absorption

spectrophotometry. Preliminary experiments had revealed that 100% recovery was possible by this method provided it was carried out immediately after treatment. Eight leaves per treatment were used throughout.

7.2.2.3 Influence of humidity and the fructose/ferric molar ratio on iron penetration

Iron (28 µg approx. of ferric in 200 µl), in the form of four chelates of differing fructose/ferric molar ratios was applied to the leaves and the plants held at 48±5% r.h., 64±5% r.h. or 95-100% r.h. for the duration of the experiment (5h).

7.2.2.4 Influence of polysorbate 20 on iron penetration from a fructose/ferric chelate of molar ratio 5/1

Iron (28 µg approx. of ferric in 200 µl) in the form of a fructose/ferric chelate (5/1) was applied to the leaves in the presence of varying concentrations of polysorbate 20 (0.0-5.0g/litre) and the plants held at 64±5% r.h. for the duration of the experiment (4h).

7.2.2.5 Statistical analysis

Analysis of the data was carried as previously described (see 2.2.2.8).

7.2.2.6 Iron analysis

Analysis was carried out by atomic absorption spectrophotometry using an air/acetylene flame. Six 3 second integrated readings per sample were taken to cut down any error since the concentrations of iron in the final washings were relatively low for analysis by this method.

7.3 RESULTS

7.3.1 Influence of humidity and the fructose/ferric molar ratio on iron penetration

At 48±5% r.h. there were no significant differences in the degrees of iron penetration between the 5/1, 20/1 and 50/1 ratios. The 200/1 ratio showed significantly greater penetration than the other three. At 64±5% r.h. there was a gradual reduction in penetration from 65% at the 5/1 ratio to 40% at the 200/1 ratio.

A similar trend was observed at 95-100% r.h. Penetration was reduced from 95% at the 5/1 ratio to 79% at the 200/1 ratio.

From these results, the 5/1 ratio would seem to be the most promising for efficient penetration over the 64±5% r.h. and 95-100% r.h. ranges. At the lowest humidity range employed, only the 200/1 ratio showed significantly greater penetration and here, the actual percentage difference was small (30% for the 5/1 ratio and 40% for the 200/1 ratio). (Table 7.1).

7.3.2 Influence of polysorbate 20 on iron penetration from a fructose/ferric chelate of molar ratio 5/1

Penetration was significantly enhanced by polysorbate 20 at all concentrations tested. However, there was a gradual reduction in this enhancement with increasing polysorbate 20 concentration. (Table 7.2).

7.4 DISCUSSION AND CONCLUSIONS

From the data present, it is difficult to draw any firm conclusions about the mechanisms involved in bringing about the trends in penetration depicted in Table 7.1. However, they may involve a balance between the equilibrium existing between the ferric fructose polymers and low molecular weight species, the efficiency of fructose as a humectant at different humidities and the availability of the aqueous route (see 2.3.1). The equilibrium could influence the rate of penetration of iron as

Table 7.1 Influence of humidity and fructose/ferric molar ratio on iron penetration. Ferric application rate 28 µg approx. in 200 µl (assessed after 5h)

Fructose/ferric molar ratio	Mean % penetration	SD[a] (%)
48±5% r.h.		
5/1	30	4
20/1	29	2
50/1	31	7
200/1	40	8
64±5% r.h.		
5/1	65	8
20/1	55	6
50/1	43	8
200/1	40	5
95-100% r.h.		
5/1	95	2
20/1	85	4
50/1	87	5
200/1	79	4

a Standard deviation (n-1 degrees of freedom)

Table 7.2 Influence of polysorbate 20 on iron penetration from a fructose/ferric chelate of molar ratio 5/1. Ferric application rate 28 µg approx. in 200 µl. (assessed after 4h)

Polysorbate 20 (g/litre)	Mean % penetration	SD[a] (%)
0.0	55	10
0.2	78	9
1.0	72	10
5.0	64	5

a Standard deviation (n-1 degrees of freedom)

a low molecular weight chelate while the humectant effect of fructose could influence the concentration of these low molecular weight species in solution and hence influence the equilibrium distribution between this solution and the leaf surface (see 2.3.4).

The increased penetration of iron in the presence of polysorbate 20 did not appear to be due to increased spreading of the droplets. However, it is possible that the surfactant was enabling more efficient contact between the droplets and the water continuum of the plant to take place thus bringing about more efficient penetration. Other factors could however be involved (see 1.1). A similar equilibrium distribution arguement as proposed for the results depicted in Table 7.1 could account for the trends observed in Table 7.2.

Work is now being continued in this laboratory by Calum McPhail. The factors under investigation at the present time include (a) the use of other sugar/iron chelates, both radio-labelled sugars and iron are being employed to investigate translocation, (b) the use of surfactants to bring about efficient spreading. Interest is being centred around the use of silicone based surfactants as used by Neumann and Prinz (1975).

Initial results have revealed that sucrose/ferric complexes are considerably more efficient in bringing about iron penetration than EDTA, EDDHA and fructose and the use of these chelates is now being pursued.

It would therefore appear that the use of sugar/iron chelates may possibly have some future as foliar applied iron supplements. In the case of sucrose in particular it would be a cheap alternative to the polyaminopolycarboxylic acid chelates if it can match these in translocation, while it should eliminate the problems associated with $FeSO_4$ since it is stable and has not been found to scorch the leaves.

APPENDIX I

1976 - 77 Field Trials

(i) Volume application rate

Preliminary trials with the knapsack sprayer revealed that (a) the volume dispensed per minute depended upon the volume contained within the sprayer, even although the pressure was kept constant, (b) a volume rate of 1 l/plot was a good balance between foliage wetting and a volume which could be accurately dispensed.

Thus, from these points, the following procedure was developed :

2L of spray solution was added to the sprayer and the time taken for 1 l to be dispensed was recorded (78 sec). In the field, the plots were divided into three equal strips, each of which was sprayed twice, i.e. 2 x 13 sec sprayings. This enabled the foliage to be covered relatively uniformly while an accurate 1 l could be dispensed. On completion of one plot, a further 1 l was added and spraying was continued until all four plots of one treatment had been sprayed. The knapsack was then washed out and the procedure repeated with a second treatment solution.

(ii) Assessment of results (1976 trials)

(a) Frond numbers

In all cases, a 1m perimeter was discarded to allow for edge effects.

In the control plots where no aminotriazole was present in the spray solution, three $1m^2$ plots taken diagonally across the remaining 3x3m were counted. These three figures were meaned to give a figure for the number of fronds/m^2 per plot. A mean of the four replicate plots was then calculated and expressed as a mean number of fronds/m^2 per treatment ± 1 standard deviation (n-1 degrees of freedom).

In the treated plots, the number of fronds in the entire $9m^2$ were counted. This was expressed as a mean number of fronds/m^2 per plot. A mean number of fronds/m^2 per treatment was then calculated as before.

(b) Frond heights

Thirty frond heights were measured independently by three separate assessors. These 90 measurements were then expressed as a mean frond height / plot. A mean frond height per treatment was then calculated from the four replicates. When there were less than 30 fronds within the middle $9m^2$, the difference in numbers was taken from as near to it as possible. Such fronds were found not to differ significantly in height from those within the middle $9m^2$.

(iii) Assessment of results (1977 trial)

(a) Frond numbers

It was felt that in some of the plots assessed the previous year that a 1m discard around the perimeter was not sufficient to allow for edge effects. Therefore, a 1.5m discard was used in all cases and the total number of fronds in the middle $4m^2$ counted and expressed as a mean number of fronds/m^2 per plot. A figure for the mean number of fronds/m^2 per treatment was then calculated.

(b) Frond heights

A brief assessment of frond height was made by measuring 12 fronds from the middle $4m^2$ of each plot. These figures were dealt with as before and expressed as a mean frond height/ treatment. It was felt that judging from the lack of significant differences in frond densities between aminotriazole treated plots of the three formulations that detailed height measurements were not required.

1978 Field Trials

Volume application rate was doubled to 2l/plot since plot area was approximately double. Plot size was increased in the hope that a more exact assessment of results might be obtained. It is planned that a number of 1 or 2m^2 areas may be measured within each plot. This should improve the statistical analysis and enable significant differences to be revealed.

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