BEHAVIOUR AND FATE OF PESTICIDES IN PLANT-SOIL SYSTEMS

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SUMMARY

This thesis deals mainly with behavioural aspects of herbicides in plants and soils. The herbicide asulam was the main chemical under study although other chemicals including amitrole were employed in some experiments.

The work was subdivided as follows:

1. A consideration of the role of weeds in human affairs and a review of methods used in their control with emphasis on herbicide usage and factors affecting performance.

2. An examination of factors influencing the rate and efficiency of penetration of plant foliage by herbicides.

The findings can be summarised as follows:

a) An investigation by light and electron microscopy into the nature and development of the leaf surface barrier in bracken revealed that thick cuticle developed on exposed plants while a thin cuticle developed on glasshouse-grown plants.

b) Penetration of bracken fronds by asulam was doubled and spray retention was improved by incorporating Tween 20 in the spray solution.

c) Penetration of bracken fronds by amitrole was influenced by pre-spraying conditions and the incorporation of adjuvants.

With regard to surfactants, Tween 20, Triton GR-5, Triton X-100 and Tergitol NPX improved penetration by a minimum of 17% while Triton X-405 decreased penetration. Other adjuvants viz ammonium salts, glycerol etc. were also included in the study.

d) Penetration of bean leaves by asulam was shown to be much greater under high than under low humidity conditions. Different surfactants (at 0.2% $^{w}/_{v}$ behaved differently in that Tween 20, Triton GR-5, Tergitol NPX and Triton X-67 significantly increased penetration compared to the aqueous controls (46.6, 42.6, 36.3 and 25.1% respectively), while Triton X-114, Triton X-15 and Teepol produced a non-significant increase, Triton X-100 a non-

significant decrease and Triton X-405 a significant reduction (22.4%).

Glycerol enhanced penetration in the presence of Tween 20 on all occasions. Urea both with and without Tween 20 enhanced penetration. The enhancement again was more pronounced at high humidity.

Penetration in the presence of contact chemicals viz potassium ethyl xanthate and tributyl phosphate was erratic.

e) A preliminary field investigation where asulam spraying was carried out in September using two surfactants viz Tween 20 and sodium dodecyl sulphosuccinate (at 0.1% $^{w}/v$) revealed no differences between treatments with and without surfactants.

3. The behaviour of asulam in soils.

The findings can be summarised as follows:-

a) A comparative study on the adsorption of asulam, asulox (commercial formulation) and sulphanilamide onto soils collected from under bracken revealed that i) the chemicals were not adsorbed to any marked extent onto the soils and ii) charcoal resulting from the burning of bracken litter increased (considerably) the adsorption capacity of the soils.

b) An investigation into the influence of activated charcoal added to the soil revealed that large quantities of asulam could be retained by the charcoal. Activated charcoal was shown to be very effective in removing asulam from water (Freundlich constant $\underline{K}$ was of the order $2.6 \times 10^{4} \mu g/g$).

c) An investigation into the influence of soil depth, pH and composition on asulam adsorption indicated that the adsorption which did take place decreased with soil depth, was negatively correlated with pH (r for 8 soils ranged between -0.92 and -0.99) and was positively correlated with organic matter ($r = 0.94$).

d) Asulam was shown to be highly mobile in soils and the mobility was influenced by pH (increased with increase in pH). Field studies demonstrated the possibility of upward movement.

e) Asulam degradation under field and laboratory conditions revealed

i) the possibility of both biological and non-biological routes for degradation and ii) conditions conducive to increased microbial activity e.g. high organic matter, warm temperature, moist soil and the addition of yeast extract accelerated its degradation.

A bio-assay using maize plants also indicated rapid disappearance of asulam. A point to note was that in this system plant growth was stimulated at lower asulam concentrations.

4. In vitro studies on the influence of asulam and (breakdown product) sulphanilamide on the properties of horseradish peroxidase.

The findings can be summarised as follows:-

a) Both chemicals enhanced the oxidative destruction of IAA by the enzyme.

b) No change in optical density at 474nm was observed when either chemical was added to horseradish peroxidase with or without H_2O_2. However when PABA was added in the presence of H_2O_2, sulphanilamide brought about a decrease in the change in optical density while the reverse was observed with asulam.

The significance of the two reactions was discussed in the light of some of the observations noted during the course of the study.

5. An assessment of bracken eradication methods with emphasis on chemical control was made with reference to studies carried out here and allied work.

CONTENTS

A list of abbreviations for names (other than chemicals) used in this thesis.

a.i.	active ingredient
ac.	acre
Ch.	chapter
^{o}C	degrees Celsius (formerly Centigrade)
g	gramme
h	hour
ha	hectare
Kg	kilogramme
μ	micro ($\times 10^{-6}$)
mg	milligramme
ml	millilitre
min	minute
ppm	parts per million
p.	page
pp.	pages
lb	pounds
t.l.c.	thin-layer chromatography
ref.	reference
var.	varietas
>	more than
<	less than
%	percent(age)
*	pounds/acre
in.	inch
S.E.	standard error
C.L.	confidence limit
O.D.	optical density

ΔO.D.	optical density (treatment - control)
C.A.	Chemical abstract
Weeds Abst.	Weed abstract

NB Abbreviations for the names of units are the same for singular and the plural.

A list of common names and abbreviations used for herbicides and other chemicals in this thesis.

amo 1618	2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride
asulam	Methyl (4-aminobenzenesulphonyl) carbamate
bromacil	5-bromo-6-methyl-3-s-butyl uracil
CDAA	NN-diallylchloroacetamide
CDEC	2-chloroallyl NN-diethyldithiocarbamate
chloramben (amiben)	3-amino-2,5-dichlorobenzoic acid
chlorpropham (CIPC)	N-(3-chlorophenyl) carbamate
chlorthiamid	2,6-dichlorothiobenzamide
dalapon	2,2-dichloropropionic acid
4-CPA	4-chlorophenoxy acetic acid
2,4-D	2,4 dichlorophenoxy acetic acid
2,4-DB	4-(2,4-dichlorophenoxy) butyric acid
dicamba	3,6-dichloro-2-methoxy benzoic acid
2,3-DCDT (Avedex)	S-2,3-dichloroallyl NN-di-isopropylthiol carbamate
DCPA	2,3,5,6-tetrachloroterphthalic acid
dichlobenil	2,6-dichlorobenzonitrile
dichloral urea	1,3-Bis (2,2,2-trichloro-1-hydroxyethyl) urea
diquat	1,1'-ethylene-2,2'-bipyridylium ion
diuron	3-(3,4-dichlorophenyl)-1,1-dimethyl urea
dinoseb (DNBP)	2-sec-butyl-4,6-dinitrophenol
DNC	2-methyl-4,6-dinitrophenol
EPTC	S-ethyldipropylthiolcarbamate
Glyphosate	N-(phosphonomethyl)glycine
IAA	Indole-3-acetic acid
MCPA	4-chloro-2-methyl phenoxy acetic acid
MH (maleic hydrazide)	1,2,3,6-tetrahydro-3,6-dioxopyridazine

monuron (CMU)	3-(4-chlorophenyl)-1,1-dimethyl urea
neburon	1-n-butyl-3-(3,4-dichlorophenyl)-1-methyl urea
PABA	p-aminobenzoic acid
paraquat	1,1'-dimethyl-4,4'-bipyridylium ion
picloram	4-amino-3,5,6-trichloropicolinic acid
propham (IPC)	isopropyl N-phenyl carbamate
simazine	2-chloro-4,6-bis(ethylamino)-1,3,5-triazine
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,3,6-TBA	2,3,6-trichlorobenzoic acid
TCA	trichloroacetic acid
Terbacil	3-t-butyl-5-chloro-6-methyl uracil
Terbutryne	4-ethylamino-2-methylthio-6-t-butylamino-1,3,5-triazine
TIBA	2,3,5-triiodobenzoic acid
Trifluralin	2,6-dinitro-NN-dipropyl-4-trifluoromethylaniline

CHAPTER I

BEHAVIOUR AND FATE OF HERBICIDES IN PLANTS AND SOIL IN RELATION TO THEIR PERFORMANCE.

This chapter is meant to be an introduction to the subject matter of this thesis. It deals with weeds, their definition and importance in human affairs and methods of control. Some aspects of herbicide behaviour in plants and soil and the impact of the environment on herbicide performance are dealt with in a general way.

The fate of aniline-based herbicides in the soil and the bracken problem are dealt with in this introduction under the heading of specific problems.

1- Weeds in relation to man:-

1-1- Why we have weeds:-

One of the basic objectives of agricultural research is to develop more efficient plants and more productive soils and to manage them at high productivity levels.[425] The native vegetation in most geographical areas in the world is not very efficient or economical as a source of food for livestock and humans.[425] As the human population has increased, it has been necessary to replace the native vegetation with more productive, and more economical plants for feed, food and fibre.[362,425] To achieve this it is often necessary to disrupt the course of natural plant succession and substitute crop plants, which are usually grown as monocultures.[362,365] No one plant species, crop or otherwise, can fully exploit the resources of the habitat.[365] In arable land and other disturbed areas, numerous ecological niches are initially unfilled, creating enormous pressure for the invasion of aggressive, unwanted species. These unwanted plant species are termed weeds.[362,365] Left unattended cropland and other disturbed habitats return by succession to stable plant communities.[362,365]

1-2- The importance of weeds in human affairs:-

As stated by Holm,[260] the role of weeds in human affairs is quite complex and their significance in the life of people across the world is not fully appreciated. The following points show some of their roles in human affairs.

1- Weeds compete with crops for light, nutrients, water and space.[362] Weeds, like crops, vary in their competitive ability, but characteristically, they exhibit, when young, a rapidly spreading and deeply penetrating root system which gives them an early advantage in obtaining water and nutrients. Competition for space and light with concomitant reduction in photosynthesis leads to crop losses.[362]

Bhan et al.[51] found that the yield of peanuts was significantly reduced if the crop was not maintained weed free over the first 3 weeks. Hamdoun[216] reported that weeds can drastically reduce the yield of cotton if weeding is delayed beyond a critical period and when weeding was completely neglected seed cotton yield reductions of the order of 63 and 88% were noted. However different crops have different critical periods when they should be kept weed free.[85]

2- Many weeds such as quackgrass,[371] nutsedge[184] etc. exude inhibitors from their living or dead roots which further reduce crop growth. Water leachates from bracken fronds and shoots of other plants are known to exert an inhibitory effect on the germination and growth of other species.[204]

3- In dairy farming areas a serious problem is caused by weeds which give an off-flavour to milk e.g. wild onion and wild garlic.[362]

4- Serious illness or even death may result if cattle eat bracken, horse tail or horse nettle.[362,398]

5- Spiny weeds such as canada thistle interfere with harvesting, especially in hand-harvested crops.[362] In sisal and cotton fields of South Africa, Mucuna coreacea, a legume, is a serious weed.[260] The pods are covered with

hairs, which break off at maturity when disturbed. They cause a burning sensation and irritate the skin.[260]

6- Unripe seeds and stems may be harvested along with legumes or cereal crops. The decay of these moisture containing plants causes an undesirable rise in temperature in the stored crop and may lead to spoilage.[362]

7- Weed seeds may increase the cost of cleaning, and some are extremely difficult to remove from crop seeds.[171]

8- Hay containing mature thick weed stems is less attractive to livestock. Foliage of certain weeds such as bracken fern and various sedges make the hay less palatable.[362]

9- Damage to machinery or clogging of harvest equipment may occur when substantial stands of perennial weeds or brush are cut.[362]

10- Weeds may harbour pests and diseases e.g. dock plants can harbour swafly which attacks fruit in orchards. The leaf hopper, which lives on shepherd's purse carries a virus which causes curly top in sugar beet, beans and tomatoes. The common barberry is an alternative host for wheat rust.[171, 362]

11- Weeds of waterways cause enormous losses of water by evapotranspiration.[75] In irrigation areas they reduce the stream flow, cause silt deposition[260, 362] and navigation is seriously limited by aquatic weeds in many regions. Fishing, swimming and recreation may be almost eliminated by weed infestation.[260]

12- Human health is affected by poisonous plants, especially those which cause allergies e.g. johnsongrass, redroot pigweed etc.[365] A number of weeds such as corn cockle, darnel and certain species of Senecio produce seeds which are poisonous when present in flour and bread. Many people in South Africa were killed by such poisoning.[260, 294]

However, the common weeds are not unmitigated pests. In some ways they can be beneficial. They reduce soil erosion on abandoned land, add organic matter to the soil, provide food and cover for wildlife, yield

useful drugs or delicacies, beautify the landscape and constitute a potential source of domesticated plants.[365]

2- Weed control methods:-

Weed control methods are classified as:-

i) Preventive methods which include procedures aimed at limiting the spread and establishment of weeds.[365]

ii) Biological control, which employs natural enemies such as insects and plant diseases to control weeds.[365]

iii) Managerial methods which rely on a wide range of cultural, grazing, and competitive practices to reduce weed populations and their effect on land and water use.[365]

iv) Physical methods which include the full range of cultivations e.g. machinery also handpulling, burning, smothering, and flooding.[365]

v) Chemical methods which include the use of organic and inorganic chemicals as foliar sprays, soil and water treatments, fumigants, and stem applications, for selective and nonselective weed control.[365]

Of the above only the chemical methods will be treated in detail here as this is the subject of this thesis.

2-1 Chemical weed control:-

Herbicides are chemicals that kill plants or inhibit their normal growth.[362,365] Their means of doing this are diverse. In many cases the mechanisms are unknown and are theoretically as numerous as the processes essential to life.[16,181,365]

The phytotoxicity of these many chemicals varies from the destruction of living membranes as brought about by oils[136,376] to complex interactions with enzyme systems.[16] These may best be exemplified by considering the competition of dalapon with pantoate and its inhibition of pantothenic acid synthesis in microorganisms,[251,391] and the blocking of oxygen release during photosynthesis by urea, triazine and uracil herbicides[374] (see Ch.4)

2-1-1 Advantages of using herbicides:-

Using herbicides in preference to other methods of weed control may be advantageous for the following reasons:-

(1) Cost of weeding, always considered high, assumes new proportions as labour becomes scarce and expensive. The crop becomes more important to us, and new land is non-existent.[16,216]

(2) In many countries primitive, simple and inefficient means of handling weeds are still in vogue and are carried out often by women and children.[260] The application of herbicides in developing countries is essential in order to allow adequate time for the education of children and to free women so that they can provide a higher standard of living in the home.[16,260]

(3) Herbicides may be applied to weeds in row crops where cultivation would be impossible.[362,365]

(4) Pre-emergence treatments with herbicides provide early season weed control. Weed competition during the early stages of crop growth is responsible for the greatest loss in yield.[365,458]

(5) Residual herbicides applied to the soil will also provide control in cases of delayed and/or periodic weed emergence.[182]

(6) Cultivation often injures the crop root system as well as the foliage. The use of herbicides reduces the need for tillage.

(7) Herbicides reduce the destructive effects of tillage on soil structure by decreasing the need for tillage.[227,365,362]

(8) Erosion in orchards and other perennial crops can frequently be prevented by using a sod cover, kept in a state of reduced weed competitiveness by herbicides.[365]

(9) With many perennial weeds traditional methods of handweeding and cultivating have not effectively controlled them.[227,365,506] Rhizomatous or stoloniferous perennial weeds may be spread by tillage particu-

larly under moist conditions where tillage could effectively increase the population of individual plants by transplanting the cuttings.[227, 365]

Though herbicides are powerful tools it would be wrong to imply here that their use is free of problems. As pointed out by Professor Muzik[365] there is nothing magical about them. They will not substitute for good husbandry, careful management or planning. They can only reinforce them.[365] (see Ch. 5)

Everyone is cognizant of the shift from the broadleaf weed to the grass equivalent that occurred with the introduction of the chlorophenoxy compounds.[16] Similar shifts in weed species have been reported following the commercial use of DCPA and trifluralin in the Southern Western U.S.A. where annual weeds such as Echinochloa colonum which are readily controlled by these herbicides are being replaced by resistant weeds e.g. Sisymbrium irio and Euphorbia glyptasperma.[344] Faced with such a challenge scientists take it as a sign calling for the introduction of new herbicides.[344] Even with relatively new herbicides such ecological shifts have been reported e.g. asulam post-emergence treatment gave good control of johnsongrass and allowed recolonisation of the area by Cynodon dactylon.[503] The problem seems to be the logical outcome of the continuous use of one herbicide. Resistant weeds, being relieved of competition take over and become serious.[16,362] This problem was dealt with in the sugar cane plantations of Hawaii a decade ago by using herbicides in rotation.[16] In many situations mixtures of herbicides are used to broaden the spectrum of weeds that can be controlled.

In some situations the reduced soil disturbance accompanying the use of herbicides, though it helps greatly in the control of annual weeds,[227] may promote the development of herbicide-resistant perennials.[365] So herbicide treatment may need to be supplemented by tillage.[365]

2-1-2- Herbicide action:-

Herbicides exert their effects on plants by contact or systemic action.[365]

2-1-2-1- Contact herbicides:- They are most effective against annual weeds,[365] they kill the plant part(s) to which they are applied.[365] However under certain environmental conditions (high air humidity, low soil moisture and darkness) some contact herbicides may have a systemic action e.g. paraquat and diquat[71] (see Ch. 5, 2-4-1).

2-1-2-2- Systemic herbicides:- These are absorbed by plant roots and/or shoots. They are then translocated to tissues that may be away from the point of application.[365] Although systemic herbicides may be effective against all types of weeds, they are particularly advantageous against established perennial weeds or in crop situations where continuous weed control is needed in the early days of the crop.[365]

2-1-3 Timing of herbicide application:-

Timing of herbicide treatment is dependent on many factors including the chemical nature of the herbicide, its persistence, crop tolerance, characteristics of the weed species, cultural practice employed and climatic and soil conditions.[365]

Three categories of timing are recognised, pre-planting, pre-emergence and post-emergence. Pre- and post-emergence may be planned with respect to either the crop or the weed. Pre-planting treatments are before the crop is planted.[365]

3- Uptake of herbicides:-

Systemic herbicides are either applied to the plant shoots or to the soil, while contact herbicides are applied to the shoots only.[262,265] To be successful a systemic herbicide must 1) enter the plant, 2) move from point of entry by diffusion or other means through the plant,

3) escape from detoxification mechanism(s) and 4) attack at the molecular level some process vital to the plant[365] (Fig. 1). Contact herbicides must at least enter the leaf.[137]

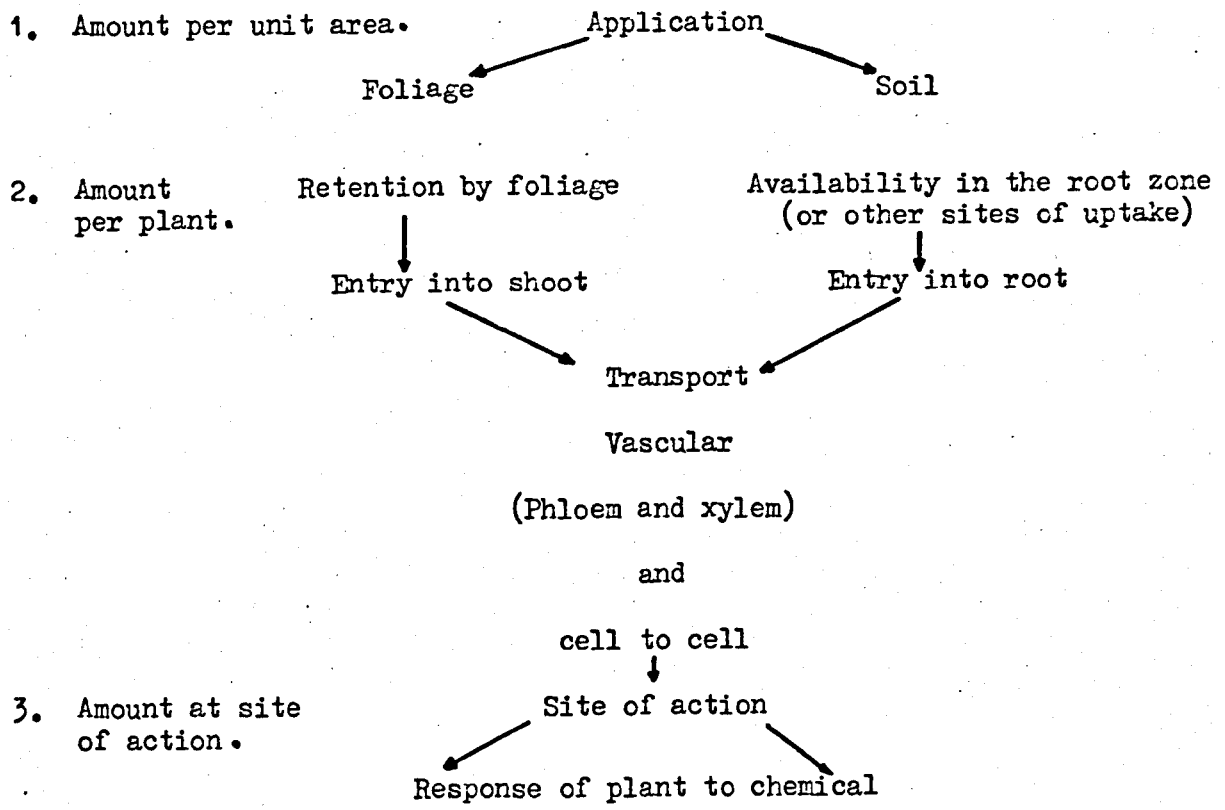


Fig. 1 A herbicide may be blocked in its action at several possible points (with modification see Ref.[361])

3-1- Foliar applied herbicides:-

3-1-1- Spray retention :- The amount of spray intercepted and retained by the foliage is of primary importance as it determines the dose of herbicide available to affect the plant whenever entry is mainly by the foliage.[219,257] Plant form, leaf shape, leaf position, leaf surface and leaf margins, greatly influence the foliar interception, distribution and retention of herbicides.[425]

The nature of the leaf surface is critically important.[425] Most leaves are covered with a hydrophobic layer of wax. Some leaves such as those found on sugar beet are smooth and without visible surface structure,

but others with a "bloom" have pronounced wax or cell protusions.[72] The amount, chemical composition and especially the physical configuration of the wax deposits on the leaf surface influence greatly the wettability of the surface (a crystalline or irregular form leads to repellency).[435] Other workers believe that the leaf surface topography (which is governed in part by the size and shape of the epidermal cells, and the underlying veins)[328] plays a role in spray retention.[190, 256, 328] All gradations occur between almost complete repulsion of the droplets e.g. peas and many grasses, to retention and spreading of all droplets on leaf surfaces of plants e.g. cotton, Brassica alba or Cyperus rotundus.[257] Retention can also be proportionally low at high volume rate if the surface is so readily wetted that the spray forms a continuous film and the excess runs off the leaf margins.[257] At commonly used low and medium volume rates this seldom occurs.[257] However, when a surface active agent is used the wettability of the leaf surface has to be taken into consideration (see Ch. 5, 2-4-2-1-3).

Wettability of leaf surfaces has been found to vary with the leaf position on the plant. Ashworth and Lloyd[18] found that with cabbage the youngest leaves at the stem apex and the oldest leaves at the base were more wettable than the middle large leaves. The upper surfaces of cabbage leaves were of greater wettability than the lower surfaces and that no significant changes in wettability were observed with some plants grown under glasshouse conditions (see Ch. 2,A). The increase in wettability of older leaves was attributed to disappearance of the wax due to weathering.[18, 257] Other workers using other plants reported increases in spray retention with age due to changes in leaf posture and that lower surfaces were more wettable than upper surfaces[257] (see Ch. 1,B and Ch. 5, 2-4-2-1-1).

Leaf wettability has also been reported to undergo diurnal fluctuations and improve after rain.[117, 173, 328] Cook[117] in this laboratory demonstrated that the leaves of Phaseolus vulgaris become more wettable when subjected

to high humidity levels.

The presence of hairs, coating the leaf surface may form a protective layer on the leaf surface by preventing contact between the spray droplets and the actual leaf surface e.g. Salvinia auriculata.[72, 257] It is however essential that the air gaps between the hairs should be small and regularly spaced.[257] If wide gaps occur the capillary pressure restricting passage of water will be small.[72, 257] The nature of hairs is also of prime importance e.g. while hairs on the leaf surfaces of Chenopodium album and Salvinia auriculata tend to repel spray droplets, the opposite[72, 257] effect can occur in that a hairy leaf is readily wetted by a water spray. The hairs themselves by forming a weak irregular mesh may be easily wetted and the droplets readily penetrate through to make contact with the leaf surface proper e.g. Salvia argentea and African violet.[72, 269]

This diversity of behaviour of spray droplets on plant surfaces may contribute very considerably towards selectivity of herbicidal sprays.[257]

In practice spray retention can be governed by:-

1) Regulating the droplet size.[328]

(a) Wettable leaves can be satisfactorily treated by using relatively large droplets.[328]

(b) Unwettable or partially hydrophobic leaves are best wetted by small droplets. Very small droplets particularly if they are blown more or less horizontally, may approach a leaf or stem but fail to make contact with it due to the boundary layer of still air over the leaf. The terminal velocities of water droplets also fall away rapidly as they decrease in size below 150 µm. The possibility of drift away from the target is so great with small droplets that further reduction in droplet size to achieve a higher degree of retention is not usually desirable.[328]

2) Using surface active agents : however the nature and concentration of the surface active agents may be critical.[189, 328]

3-1-2- Foliar Penetration :-

The penetration of the outer epidermal wall of plant foliage presents a serious problem in the case of foliar applied herbicides.[122, 368] The lipid character of the cuticular waxes and the negative charge in the cuticle make it a unique barrier for the penetration of hydrophilic compounds.[181, 368] The general belief among weed scientists is that penetration into the leaf is cuticular and sometimes stomatal.[137, 328] However many authors (see Ref.[181]) consider that the obstacle caused by the cuticle is so serious that the uptake of hydrophilic compounds could only be envisaged as stomatal.

3-1-2-1- Stomatal penetration :- The following points were considered as indicative of stomatal penetration:-

1) The observation that the uptake via the lower leaf surfaces is almost always greater than via the upper surfaces which either have no stomata or many fewer.[181]

2) Stomatal penetration of some herbicides and dyes has been reported by some workers,[138,153] but two conditions were said to be necessary.

a) The stomata should be open.[122, 138]

b) The spray fluid should contain an efficient surface active agent at a suitable concentration.[138]

Many workers question the validity of the arguments based on the above points and others discount stomatal entry for the following reasons:-

1) The ease of penetration of lower surfaces could be due both to thinner cuticles and more numerous guard cells[181] (see point 2). Moreover species differ as to which surface functions most in absorption.[137]

2) Sargent and Blackman[411] using Phaseolus vulgaris leaf discs and an aqueous solution of 2,4-D, showed that penetration was enhanced in the dark by a wetting agent (Tween 20), but the effect is not proportionally greater in the day light when the stomata are open. They showed that there is a

direct relationship between the ease of penetration of a surface and the number of stomata present on that surface, but the relationship holds even in the darkness when the stomatal pores are closed. They concluded that the pathway was through the guard cells and/or accessory cells and not via stomatal pores.

3) Furmidge[191, 192, 193] observed that injury by surfactants to apple and plum leaves bore little relation to the distribution of stomata and concluded that entry is principally other than stomatal.

4) Molecules as large as streptomycin enter the apple leaf as readily through the non-stomatous adaxial surface as through the stomatous abaxial surface.[207]

5) Middleton and Sanderson[347] found that 8-hydroxyquinoline sulphate which caused the stomata to close did not affect the uptake from solution containing a surface active agent (Lissapol).

6) The rate of uptake of 2,4-D is little affected by light below 1000f.c. even though the stomata open at about 250 f.c.[71]

It is noteworthy that some of the observations cited above are based on work employing different plants, different chemicals and in all cases different surfactants. This increases the complexity of the situation and makes it rather difficult to draw a safe conclusion regarding the possible involvement of stomata in foliar penetration of chemicals for the following reasons :-

i) The surfactants are said to promote stomatal penetration through their action on the surface tension of the spray fluid.[122, 181, 328] It is understandable that low surface tension is needed to secure entry into the stomatal chamber, but for efficient penetration factors other than the surface tension have to be taken into consideration viz the internal cuticle lining the walls of the stomatal chamber, the nature and concentration of the surfactant (see Ch. 2,D) and the possibility of herbicide-plant-surfactant interactions.[179]

ii) Some of the chemicals used may have a specific action on the plant e.g. 2,4-D may cause stomatal closure,[16] so chemicals like 2,4-D may retard their uptake through stomata as closed stomata make the cuticle barrier complete.[16,65]

On the whole one can conclude that if the right conditions are satisfied (open stomata, low surface tension spray fluid and efficient surfactant at optimum concentration) stomatal penetration would be expected to occur, due to the high humidity prevailing inside the air space of the leaf.[122] This high humidity should assure the presence of the water continuum very close to the cell surfaces and so diffusion will be encouraged.[122] However, no appreciable role for stomatal participation in foliar penetration under practical field conditions is expected for the following reasons:-

i) The opening of stomata is variable under field conditions and it may occur at an inconvenient time or for only short periods of the day. Closed stomata make the cuticle barrier complete.[16,65]

ii) Stomata are abundant on the abaxial surfaces of leaves of most species, but are often sparse, if not absent on the adaxial surfaces which usually receive by far the greatest quantities of spray materials.[328]

iii) Under many conditions, rapid drying of the spray solutions, may provide very little time for stomatal penetration. This is particularly true of application by aircraft, and under the hot dry conditions found in the regions of irrigation agriculture.[122]

3-1-2-2- Cuticular penetration :-

The consensus of opinion now is that stomatal penetration of aqueous solution is relatively unimportant and that the main route of entry of both water and lipoid-soluble material is provided by the cuticle.[347] Historadioautographic studies also indicate that dalapon, 2,4-D and monuron enter the foliage directly through the cuticle.[365]

The question arises as to whether there are pores in the cuticle serving as pathways of absorption. Pores through the cuticle exist in specialised areas e.g. the epidermal outer walls of gland cells of Drosophyllum lusitanicum,[269] trichomes and hydathodes of Cicer,[181, 328] glandular hairs of Mentha[181, 328] and salt glands of Tamarix aphylla etc. But there is no indication of a general occurrence of such openings.[181, 328]

Some investigators[137, 177, 181, 365] have reported the existence of specialized areas where penetration proceeds at least more rapidly than through the neighbouring areas e.g. hydathods, lenticels, natural fissures, insect punctures and other imperfections in the cuticle, glandular and nonglandular trichomes, directly over veins, anticlinal epidermal walls, guard cells and accessory cells.

Of interest here are the views of Robert et al.[402] concerning the cuticle of McIntosh apple leaves. They reported that these leaves are not covered with a continuous layer of cuticle since they did not detect cutin in cells covering the veins. Furthermore, they visualize the cuticle on the outer epidermal walls between veins as composed of pectinaceous substances that make up the ground matrix in which lamellae of cutin are arranged parallel to the outer walls.

Orgell[371] has stated that the cuticle may be characterized by an imbricate arrangement of lipoid platelets cemented together by hydrophilic pectinaceous materials. Thus an intercuticular penetration should be possible for aqueous solutions. The same conclusion was reached by Boynton.[64] The involvement of pectinaceous materials in foliar penetration has been demonstrated by Palmiter et al.[379] using microchemical techniques.

Crafts[123] suggested that there are two routes by which exogenous materials may traverse the distance from the cuticle surface into the living inner cells, a lipoid route and an aqueous pathway. The relative importance of either depends upon the potential of water in the plant (stressed or saturated), the nature of the molecule applied (lipoid vs. water soluble) and the formulation.[16]

4- Factors influencing herbicide penetration :-

Foliar penetration of herbicides is influenced by plant factors, environmental factors,[137,219] the composition of the spray solution[219] and the methods of application.[137] All constitute variables that should be considered in any experimental approach. However, it should be pointed out that the relationshop of penetration and absorption to susceptibility is not clear-cut.[56] Several workers have reported a lack of correlation between the herbicide absorbed or disappearing from the leaf surface and species susceptibility and between penetration and the inhibitory effect.[56,259] This is probably due to the fact that a series of barriers (any one of which may limit herbicidal action) intervene between application of the herbicide and its ultimate effect on the plant[365] (see sec. 6 and Ch. 5, 2-4-2-1-1). Under field conditions, however, any factor increasing herbicidal retention, penetration, and absorption by weeds is likely to increase lethality.[219]

4-1- Plant factors :-

These encompass the plant cuticle, leaf morphology, water balance, and stage of growth. They are dealt with in various parts of this thesis and therefore are not discussed here. For further details see sec. 3 and Ch. 2 and Ch. 5.

4-2- Envirnmental factors :-

Herbicide penetration can be influenced by environmental conditions e.g. light, humidity, rainfall etc. before, at, and after the time of application.[97,219,296]

4-2-2- Before application:- At this time the environment influences: a) the size, form and habit of the shoots which intercept the spray droplets[97,415] and b) the development and nature of the leaf surface barrier including the cuticle.[97,288,328]

4-2-3- At and after spraying:- The effects of environmental factors at

and after spraying on herbicidal penetration are discussed together with particular reference to light, temperature rainfall and humidity.

4-2-3-1- Temperature:- Temperature, if not excessive,[137, 219] promotes penetration through its effects on: a) physico-chemical processes - diffusion and viscosity.[137, 423] Prasad et al[392] observed increased penetration of dalapon at 43°C compared with 26°C. The difference was attributed to increased diffusional movement. In this connection Franke[181] mentioned that the temperature may exert its effect through an increase in the ultraporosity of the cuticle.

b) physiological processes - through metabolic control of certain steps in penetration processes and/or through bringing about a steeper concentration gradient in the tissue.[137, 423] Sargent and Blackman[410] observed that at low temperatures entry of 2,4-D is reduced in darkness and more markedly in light. They concluded that rates of penetration in darkness are governed largely by physical parameters but those in light are limited by metabolic factors (see -a- above)

However the rate of drying of the spray droplets could nullify the enhancing effect of temperature on penetration.[306] This is claimed to be one of the causes of the contrasting recommendations in the rates of application of DNC between the Netherlands and England. When the temperature exceeds ca. 18°C lower rates are used in England and higher rates in the Netherlands.[306]

4-2-3-2- Light:- Light promotes penetration a) directly by stimulating the opening of the stomata[137] (see 3-1-2-1) and/or indirectly through its effects on photosynthesis,[137] metabolic factors and/or through its effect on permeability of the cytoplasm and active transport processes.[76] However light reduces the activity of paraquat and diquat if it follows immediately after treatment.[71] Diquat uptake by tomatoes is less under light conditions than in the dark.[71] Photodecomposition of the herbicide by light may decrease

the quantity of the herbicide which is finally absorbed.[317,461]

4-2-3-3- Rainfall :- Rainfall could either enhance the penetration of herbicides or wash them off depending on 1) the quantity of rain involved[219,296] 2) the time interval between spray application and when it occurs[219,296] 3) the solubility characteristics of the herbicide[296] and 4) the physical nature of the leaf surface.[296]

Thus it is conceivable that the natural wetting of a leaf by dew or rain could either increase or decrease the quantity of a herbicide which is finally absorbed (see Ch. 2, D-3-3). Linscot and Hagin[317] found that simulated rainfall of 0.1, 0.5 and 2.0 in. applied to six crops in the field right after treatment with 1.5lb/acre of the dimethylamine salt of 2,4-DB resulted in an average herbicidal loss from the foliage of 21, 60 and 93% respectively.

Removal of herbicides from leaf surfaces by rainwash is claimed to be the reason for using much higher concentration of 2,4-D sodium salt in Europe than in the U.S.A. (European rainfall, on average, is much higher than the U.S.A. rainfall in the corresponding growing season.)[22]

4-2-3-4- Humidity :- Air humidity after spraying is most critical.[71] Many if not all foliar applied herbicides penetrate more readily under high humidity conditions e.g. 2,4-D,[16,71] dalapon[122,392] picloram,[423] MH[71,122] etc. Humidity may exert its effect via a) persistence of liquid deposits on leaves. Penetration appears to cease with droplet desiccation.[71,219,296] However, Middleton and Sanderson[347] and Cook[117] in this laboratory demonstrated that at high relative humidity the presence of large quantities of water is not obligatory. b) affecting the plant water stress, stomatal opening, and cuticular permeability.[71,137]

4-3- Formulation:-

4-3-1- Structure of toxicant:- Various 2,4-D formulations gave increased effectiveness in the order Na salt, NH_4 salt, amines and esters.[137] Penetration is said to be a controlling factor in those changes.[137,238]

Recently Hee Que and Sutherland[243] have found that absorption of long chain amine formulations (alkyl moieties larger than butyl are fairly insoluble in water) sprayed in diesel oil as a carrier correlates well with the quick absorption of esters of increasing chain length. The amine formulations of 2,4-D have several advantages over the ester formulations a) they are more stable on storage[243] b) they are less volatile and so present less environmental contamination [42, 243] and c) the ester formulations may exhibit limited translocation[369] (see 6-2- i)).

4-3-2- Spray additives:- The activity of a foliar applied herbicide can often be changed without changing its molecular structure.[137] This can be achieved by using suitable additives.[137] Enhancement of penetration by these additives may enable reductions to be made in the recommended rates. The resulting use of smaller amounts of herbicide[461] 1) reduces the cost,[461] 2) may improve selectivity 3) reduces long term soil effects 4) minimizes environmental contaminations[120, 461] and 5) adjuvant-herbicide mixtures are frequently more active than herbicides alone when applied in adverse environmental conditions.[333]

4-3-2-1- Surfactants:- The term surfactant is a general one and denotes molecules with two opposing characteristics (lipophilic and hydrophilic).[47] In the agricultural field the chemical categories of importance are nonionic, cationic and anionic depending on the nature of the active species.[47]

Numerous studies have demonstrated that surfactants increase the activity of herbicides on plants and in many instances they make the differences between no toxicity and effective weed control.[442] For example the addition of polyoxyethylene thioether surfactants to dalapon increased the control of johnsongrass.[341] Ethoxylated nonionic surfactants increased the activity of diuron on large crabgrass.[340] Biodegradable alkylpolyoxy ethylene glycol surfactants enhanced phytotoxicity of paraquat, dalapon and amitrole to maize[443] etc. The way(s) the surfactants exert their role

is obscure.[130] Several authors proposed various explanations. Some of these proposals will be discussed where relevant (see Ch. 2 and Ch. 5). For further details see Behrens,[47] Hull,[296] Crafts and Foy[130] and Smith and Foy.[179]

4-3-2-2- Oils :- Several workers have shown that oil or oil-in-water emulsions are superior to water as a carrier for herbicides.[35,37,131] Aya and Ries[23] reported that paraffinic and naphthenic oils as additives to water enhance the activity of amitrole on quackgrass. Barrentine and Warren[37] reported that chlorpropham and terbacil showed enhanced activity on giant foxtail and ivyleaf morning glory when applied in isoparaffinic oils rather than in water. Similar results were reported with other herbicides.[35,131] The increased activity was attributed to enhanced penetration brought about by oils in all cases.[23,35,37]

The enhanced penetration of herbicides by oils may be due to 1) the lower surface tension and higher wettability brought about by oils leading to spreading of the spray droplets and hence a greater surface area of contact between the spray and the leaf surface;[23,131] 2) oils may soften or solubilize the cutin layer;[37] 3) oils may modify the cuticle and may form a lipophilic pathway as proposed by Jansen.[281]

Water-in-oil (W/O) emulsions significantly reduce the extent of spray drift, and droplet shattering on impact with the plant surface (viscous, less volatile and low surface tension spray fluid) during aerial spraying when compared with the behaviour of conventional water-based sprays applied in a similar manner.[107] However, when using oils as solvents, additives, or for spray drift control, it should be remembered that the active ingredient has to penetrate into the plant tissue and not to stay in the formulating solvent. If the solvent cannot penetrate the plant cuticle; as is the case with some oils, its continued presence will tend to hold back the active ingredient.[223]

4-3-2-3- Miscellaneous additives:- Various other additives e.g. humectants, salts,[43, 322, 461] phosphate esters,[461] sugars,[137] urea,[137] acids[43] etc., have been shown to enhance foliar uptake of herbicides and will be discussed in some detail in Ch. 2.

5- Translocation of foliar-applied herbicides:-

There are two tissue systems by which herbicide molecules may move rapidly in plants; the phloem and the xylem.[122] Much evidence has been accumulated to show that most of the export of herbicides from leaves takes place via the phloem.[122] However, there are exceptions. Some herbicides e.g. substituted urea, uracils and triazines may penetrate the cuticle and move in the apoplast with the water of transpiration but they do not commonly enter the phloem and move with the food.[16, 122, 219, 368] Under certain conditions movement of foliar applied herbicides may occur in the xylem.[352]

1. By means of an induced backward water stream in the xylem.[158]
2. Under special conditions of high humidity.[158]
3) Herbicides with an extremely rapid toxic action may under certain prerequisites move from the leaves in the xylem e.g. diquat.[32, 158]

5-1- Phloem transport :- Herbicides that enter and move freely in the symplast migrate to the phloem, and translocate in the lumina of the sieve tubes in the assimilate stream along a source-to-sink route. In other words, the nature of movement of the assimilate stream governs the rate of herbicide transport (rapid or sluggish).[16, 122] Evidence for this is the common observation that compounds such as 2,4-D may move from a given leaf wholly in a basipetal direction into the roots or wholly in an acropetal direction into the upper leaves or shoot tips or in both directions to root and shoot tips.[122] The direction of movement under these conditions seems wholly determined by the pattern of food distribution.[16, 122, 67]

5-2- General pattern of assimilate distribution in plants:- The general pattern of assimilate distribution in plants is that the lower leaves act as a main source of assimilate for roots whereas the upper leaves perform this function for the shoot apex, and leaves in an intermediate position supply assimilate in either or both directions.[486] However when considering the direction of movement of the assimilates from the leaf it should be remembered that 1) the plant is not a static organism and that the position of the leaf relative to the shoot apex is continually changing throughout development.[486] 2) The pattern of assimilate movement from any one leaf is dependent not only on the proximity of the leaf to the growing regions, but also on the supply of assimilate from other leaves on the shoot.[486] 3) the stage of development of the leaf is also of prime importance.[352,486] Very young leaves obtain carbohydrates required for their growth from older leaves.[352] Once such a leaf has become photosynthetically active, and at least partially self-sufficient, photosynthate is translocated from the leaf.[352] Leaves have been shown to be most active in exporting assimilate after reaching their maximum size. The rate of export subsequently declines with leaf age.[352,486]

5-3- Carbohydrate content as a guide to timing for weed control methods:- From the foregoing discussion (5-1 and 5-2) it is clear that the outflow of assimilates from a leaf is dependent on factors within the leaf itself, as well as on growth activity and assimilation in other parts of the plant. The changes from vegetative growth to production of flowers and fruits or of a rapidly developing storage organ will markedly alter the pattern of assimilate distribution.[486] The occurrence of such alterations in assimilates distribution should always be taken into consideration in weed control for the following reasons:-

1) Some herbicides e.g. chlorophenoxy compounds, dalapon and amo-1618 may accumulate in edible plant parts e.g seeds, particularly if the herbicides

are applied during flowering or seed setting.[352,486]

2) Most perennial weeds possess the ability to regenerate from underground organs.[362] New shoots produced after a dormant period draw their food supply from the carbohydrate reserves in the storage organs.[362] Later in the growing season replenishment of the food reserve occurs. So invariably in perennial weeds the carbohydrate reserve passes through a low point.[120, 209, 236, 337, 342, 405] The period of low carbohydrate content is generally considered to be the time when plants are most susceptible to injury.[95] Researchers have found that :-

a) The least amount of regrowth occurs when the top growth is removed when the carbohydrate reserves in the plants are low.[120, 209, 362, 405]

b) Translocation of foliar applied herbicides to the underground organs is at a maximum during the period of rapid build up of the carbohydrate reserves after the initial drop in the early growth stages.[95, 104, 169] Chemical control of established perennial weeds during the early growth stages generally result in failure, due to the lack of adequate translocation of the herbicide to the underground organs. Upwards translocation towards the shoot tips may occur.[335]

6- Factors influencing herbicide translocation and movement:-

6-1- Plant factors:-

Unfortunately no such clear cut behaviour (as mentioned above) for assimilates or herbicides occurs in practice for the following reasons:-

i) The changes in the annual cycle of carbohydrate reserves in[28, 95, 362] perennial weeds is closely associated with the start of the growing seasen. The beginning of the growing period is dependent upon climatic conditions[28, 95] e.g. temperature, rainfall, and physiological processes within the plant.[28, 95] The rapidity with which the food reserves are utilized depends on the growth of the plant (stunted or vigorous) which in turn depends on climatic and

edaphic conditions.[85] Thus variations in the time when low carbohydrate reserves occur should be expected between sites.[337] Such fluctuations in carbohydrate content is claimed to be one of the reasons for the erratic results obtained with foliar applications of dalapon and other herbicides used in johnsongrass control and also for the non-effectiveness of cultivations in controlling many perennial weeds.[337,362] So the use of herbicides or cultivation operations should be timed according to the development of the weed and not by the calender.

ii) If the underground organs of the perennial weed (roots, rhizomes etc.) are not actively growing but simply storing starch with all buds in a dormant state the herbicide may have little effect on the storage tissue and the treatment may result in failure.[121] Hull[272] observed that inactive rhizome buds in established johnsongrass fail to accumulate assimilate or dalapon. Similar observations have been reported with other perennial weeds[275,358] (see Ch. 5, 2-4-2-1-3).

The efficiency of control measures might be increased if the vegetative buds could be chemically stimulated into active growth, or at least into a physiologically receptive state, prior to application of a herbicide.[53,272,352] This requirement for effective systemic herbicidal control was emphasized by Oyer.[377] Chemical stimulation of dormant buds, increased basipetal translocation of phloem-mobile herbicides and improved perennial weed control have been reported by some workers.[53,352,358] (see Ch. 5, 2-4-2-1-3).

6-2- Herbicide factors :-

1) The retention of the herbicide along the translocation path results in deficient basipetal translocation.[122,158] Differences in translocatability have been reported for various phenoxyacetic acid formulations (2,4-D and 2,4,5-T).[369] The ester formulations have been reported to be less translocatable than the acid or amine salts.[369] The reduced translocatability

of the ester probably results from a concentration effect in the treated leaves due to greater absorption (see point ii).

Disregarding concentration effects due to high rates of absorption, little is known concerning the extent to which ester forumlations influence the translocatability of herbicides. A few investigators have demonstrated that ester formulations may be hydrolysed on the leaf surface or in the plant following absorption.[124,203,359] If ester hydrolysis is complete or nearly so, a point still in doubt,[453] then translocation of the herbicide will be in the acid form as proposed by Crafts.[124] Long-chain alkyl esters do not partition readily into the water phase, and it is doubtful whether extensive hydrolysis proceeds in the lipid phase.[369] It would seem that only the amount of ester which partitions into the aqueous phase will be available for hydrolysis and transport.[369]

ii) High toxicity may interfere with translocation and thus prevent the herbicide from reaching the site of action. For example extremely toxic foliar herbicides kill the phloem so quickly that translocation to active metabolic sites is prevented.[365] Effective use of phenoxy herbicides is dependent upon maintaining live phloem cells.[365] Excessive rates of application of such herbicides can result in killing these cells and halting translocation to underground parts.[240,322,365] Although above ground parts may die, the plant swiftly resprouts.[365] Use of such translocated herbicides, therefore, requires low dosage of chemicals. Such behaviour has been encountered in canada thistle control with 2,4-D. Loomis[322] reported that treatments of canada thistle with 2,4-D at 1lb per acre, were ineffective. The recommended dosage was stepped up to as much as 8lb per acre with no improvement in control. However lower rates of applications 1/4 to 1/2 lb per acre were considerably more effective than the massive doses formerly recommended.

iii) Norris and Freed[369] reported that translocatability of herbicides

may be influenced by a specific plant-herbicide interaction. Some workers have reported that movement of 2,4-D from treated leaves is greater than that of 2,4,5-T in bean, cotton, corn and potato plants.[369] Others have observed 2,4,5-T to be more mobile than 2,4-D in both wild and cultivated cucumber plants.[369,438]

iv) Some herbicides 2,3,6-TBA, MH, dalapon, and possibly others have an additional property that is important to their systemic effects on plants. These compounds are freely mobile in the mesophyll and phloem and thus are commonly carried into the underground organs of weeds.[122] However these herbicides may leak from the phloem into the xylem and move acropetally in the transpiration stream (the same sort of behaviour has been reported with 2,4-D, 2,4,5-T and picloram).[44,158,424] Having reached the leaves the herbicides may 1) re-enter the symplast and repeat this performance; that is, like phosphorus they may circulate in the plant,[122] 2) get trapped into very young leaves or older leaves e.g. picloram and 2,4-D.[158,424] The removal of 2,4-D from the lower plant parts and its acropetal movement in the transpiration stream is considered by some authors to be an important obstacle to the basipetal translocation of this herbicide and hence a contributing factor to its efficacy.[158] Crafts and Yamaguchi [132] proposed that the ability of a herbicide to leak from the phloem to the xylem is related to its ability to be exuded by roots. This property imposes different problems with respect to 1) Weed control. Fits et al.[170] found that jimsonweed (Datura stramonium) a 2,4-D resistant plant transports 2,4-D to the basal tissues and roots and most of the 2,4-D reaching the roots is lost into the surrounding media. 2) Pesticide residues. Sharma et al.[424] and Reid and Hurrtt.[369] reported that picloram was released into the soil following leaf application to canada thistle (Cirsium arverse) and red maple (Acer rubrum) and 3) Untreated plants growing alongside, may be adversely affected particularly if selectivity is based solely

on depth protection.[122, 273]

6-3- Environmental factors:-

The very nature of translocation of phloem-mobile herbicides (dependence on the pattern of food distribution) makes it liable to be influenced by environmental variables e.g. light, temperature etc.[127] These variables exert their influence by:-

1) affecting the production of the photosynthate. Photosynthesis is the primary process that provides green plants with the solute responsible for the driving force of pressure flow (the mechanism by which assimilates move).[71, 127, 181]

2) affecting source-sink relationships. Normal translocation requires an active source and an active sink.[127]

3) affecting the ratio of basipetal/acropetal translocation in the case of herbicides which leak from the phloem into the xylem.[44, 158]

The effects of environmental factors on translocation is considered here with particular reference to water potential, temperature and light.

i) Water potential :- The water availability affects a) photosynthesis, and hence the provision of osmotically active solute;[127] b) velocity and concentration of the assimilate in the sieve-tube system and c) growth of stems and roots and hence the activity of sinks.[127, 362]

Conditions will be optimum for translocation in the presence of ample water. Crafts[126] reported that plants in dry soil do not translocate chemicals as readily as those in moist soil. However, under conditions of soil moisture stress and high atmospheric humidity, it is possible that complete reversal of the transpiration streams might take place, resulting in the movement of the herbicide deep into the root system.[102]

ii) Temperature :- Temperature, if not excessive, will increase the metabolic activity and concomitantly translocation to the metabolic sinks.[336] Cool air around the tops of Saccharum officinarum plants reduced transport appreciably. Cooling the roots reduced transport by 50% in 24h, and by

82% in 80h.[127] Pallas[378] reported an increase in basipetal translocation of 2,4-D in Phaséolus vulgaris with increase in temperature between 20° and 30°C. However in the case of herbicides which leak from the phloem into the xylem high temperature may increase acropetal translocation through its effect on transpiration.[44,158] Other factors which increase transpiration rates e.g. low humidity and air movement will also increase acropetal translocation unless the closing of stomata intervenes.[44,365]

iii) Light :- Since light is required for the production of photosynthate, adequate illumination of leaves, especially young ones, is critical as far as translocation of exogenous materials is concerned.[351] Translocation of 2,4,5-T is significantly reduced in plants treated in the dark compared to plants placed in the light at the time of treatment.[28] Similar findings have been reported by Rohrbaugh and Rice[407] with 2,4-D. Moreover, they found that translocation from destarched leaves in the dark could be restored by the addition of sugars to the foliage.

7- Soil-applied herbicides :-

Herbicides may reach the soil directly from pre-emergence spraying[34,78,458] or indirectly from post-emergence sprays[98,128,144,430] (see Ch. 3, 1). In both cases the herbicide may be absorbed by plants and may exert a toxic action depending on the herbicide itself, the plant in question and other factors (discussed below). This discussion is confined mainly to pre-emergence herbicides (see 2-1-3 for definition)

7-1- Pre-emergence herbicides :-

With the discovery of Anderson and Wolf[10] and Anderson and Ahlgren[11] of the pre-emergence action of 2,4-D, the era of soil-borne organic herbicides was ushered in. Studies since that time have shown that a great number of water soluble compounds are readily absorbed by plant roots.[16]

Like foliar applied herbicides, the effectiveness of soil-applied

herbicides is governed by a chain of events, set in motion after the application of the herbicide to the soil[257,362] (see Figs.1 and 2).

7-1-1 Root uptake and upward transport :-

Comparative studies show that plant roots absorb and accumulate some herbicides very rapidly (2,4-D, picloram, monuron, simazine) and others more slowly (MH, dalapon, amitrole).[16] Movement of these chemicals into roots and upward into the shoot occurs to varying degrees. Substituted urea and triazine compounds move into the xylem and upwards into the transpiration stream very rapidly,[16] amitrole, dalapon and MH move more slowly and 2,4-D and trifluralin may be retained in root cells.[16] However there are certain exceptions, e.g. diuron (a substituted urea) accumulates in the lysigenous glands and trichomes in cotton (resistant).[448] The susceptibility of sorghum and wheat to terbutryn (a triazine) correlates well with its translocation. Terbutryn accumulates in the root and stem of sorghum (resistant) and little reaches the leaves (site of action), while the reverse is true in the case of wheat (susceptible).[159]

Present evidence suggests that herbicides enter the roots via the same pathways and by similar mechanisms as inorganic ions.[122,365] Herbicides are taken up by both passive and active (requiring energy derived from respiration and hence a carbohydrate substrate and oxygen) mechanisms.[122,141,354,356]

7-1-1-1- Passive uptake:- Passive entrance is primarily along with absorbed water, and herbicides may continue to move with water throughout the plant in the apoplast system.[356] Shone and Wood[432] reported that the movement of simazine in barley is largely a passive process. Similar behaviour has been reported for linuron uptake in maize, soybean and crabgrass.[363] The recent microradioautographic studies of Strange and Rogers[448] using ^{14}C-diuron in cotton seem to support this.

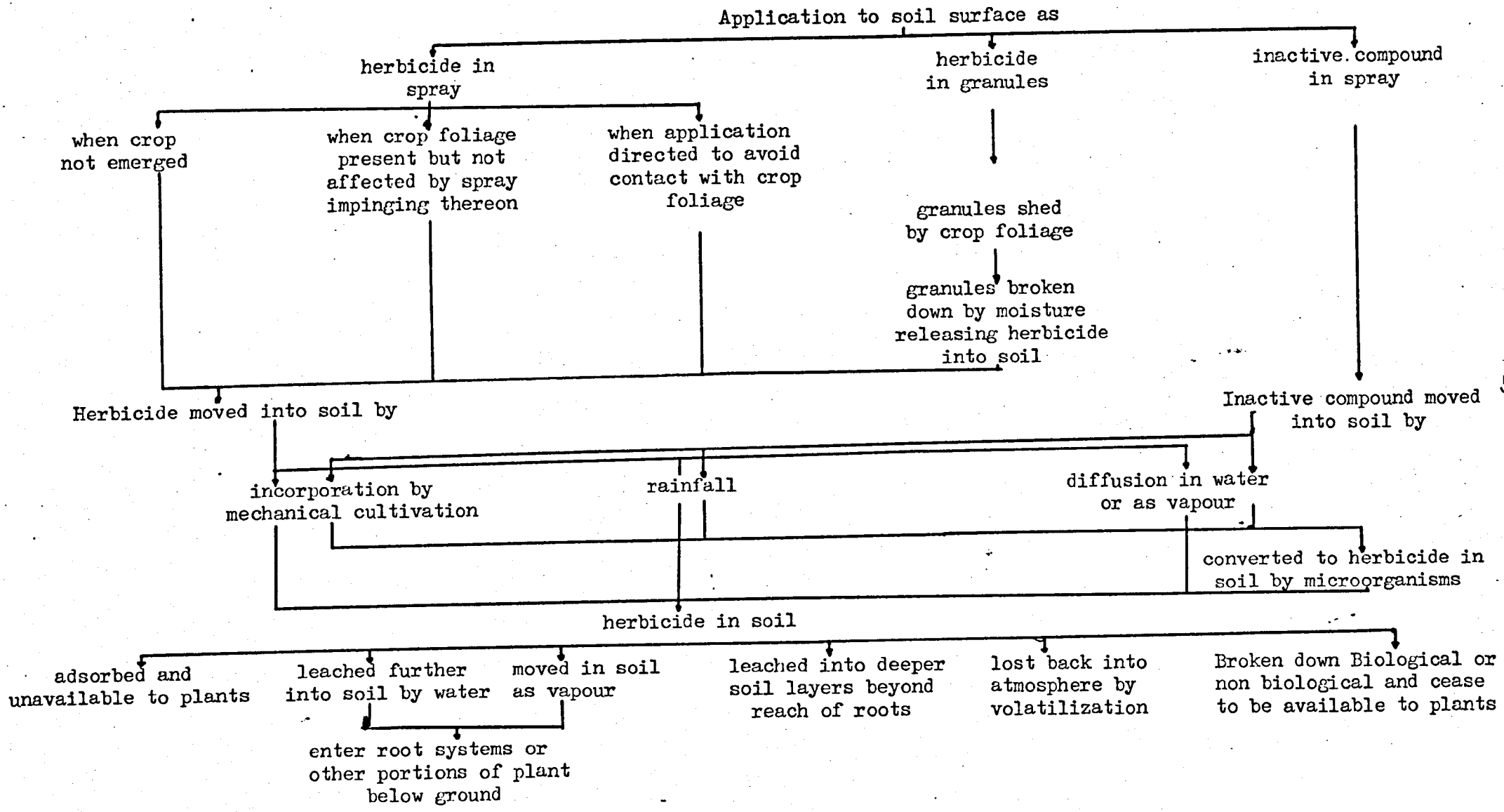


Fig.2 Scheme showing the sequence of events following application of herbicides acting through the soil (with modifications after Holly; see Ref.257)

7-1-1-2- Active uptake :- Active uptake involves the entrance into the protoplasm and movement via the symplast system.[122,365] Accumulation of herbicides in plant roots or shoots against a concentration gradient (indicative of active uptake) has been reported by many workers and it has been reduced by the use of metabolic inhibitors[365] e.g. uptake of monuron by tomato root,[237] TIBA by duckweed[363] and diquat by elodea.[141]

7-1-2- Shoot uptake :-

Work within the last decade has proved that some compounds may be absorbed from the soil by coleoptiles and young shoots as they push upward through the soil following the germination of seeds.[16] Dawson[142] studying the response of barnyardgrass (Echincchloa crusgalli) to soil-applied EPTC found that exposure of the roots did not lead to injury, whereas exposing the coleoptile did. Dawson[142] concluded that the leaf tissue of barnyardgrass is the main site of EPTC uptake and also the prime site of injury. However, Appeleby et al.[14] and Yamaguchi[511] obtained evidence of root uptake of EPTC by several plants including barnyardgrass and they demonstrated that there is no retention of EPTC by roots. Movement takes place from the roots to the shoot, but it is apparently inadequate or occurs too late to cause damage. It seems probable as stated by Parker[380] that this low rate of movement could be associated with the lack of any transpiration stream in the seedling prior to emergence. This assumption (inadequate and/or late translocation from the root to the shoot) seems to be supported by the finding of Friesen et al.[185] who demonstrated that the herbicidal activity of 2,3-DCDT (Avadex) applied to the soil is primarily via the coleoptile rather than the root system of wild oat (Avena fatua) and wheat. They demonstrated also that the degree of activity was distinctly influenced by 1) the age of the coleoptile when it came in contact with the treated soil, 2) the concentration of the herbicide in the treated soil, 3) the depth of the treated soil layer and 4) the length of time that

the coleoptile was in contact with the treated soil. However, the situation is not always so clear-cut as differences between plants in shoot vs. root response to a given herbicide occur. Colby[106] using soybean found that 10.1 Kg/ha chloramben on the seed inhibited root and shoot growth by 21% whereas the same rate applied 0.64 cm below the seed caused a 54% inhibition. Kanke et al.[289] on the other hand found that chloramben applied in the root zone of green foxtail (Setaria virdis) was only half as effective in reducing dry weight as applying chloramben to the shoot zone. The difference in response between these two plants may be due to the difference in contact time between the apical meristem and the young developing tissues with the treated soil. The apical meristems and young developing tissues will have a brief contact period with the treated soil in the case of soybean (dicotyledenous) and a longer contact period in the case of Seteria virdis (monocotyledenous). However other possibilities cannot be excluded (see Ref.[380]). Differences in susceptibility on the basis of shoot vs. root exposure is not limited to dicotyledenous and monocotyledenous plants. Ivany and Sweet[279] in their studies on the response of cucurbits to certain analogs of chloramben found that response differs between species and is partially dependent on which plant part is exposed to the herbicide (shoot or root).

7-3- Factors influencing field performance of herbicides :-

From the above review it seems that the shoots of seedlings may be a major site of uptake of some herbicides. The roots may predominate in the case of others and some may enter plants by either tissue. Guidelines may be developed from such work for herbicidal usage under field conditions. However it should be remembered that the field situation is by far the most complicated.

Weed control by soil-applied herbicides by its very nature - no

direct contact between the toxicant and the target organism ; purely physical processes (diffusion and water flow) are relied upon to bring about this contact[235] - is subject to variables of diverse origin and complex nature. The relationship of dosage applied to the soil, dosage received by the plant and biological response is more complex than the corresponding relationship in most branches of toxicology.[235] Not only are weather dependent physical processes important, but the relative position, eveness of distribution[325,468] and availability of the herbicide at the site(s) of uptake and the factors governing uptake are of paramount significance in determining the success or failure of a herbicidal treatment.[468]

7-3-1- Loss of herbicide from sphere of activity :-

Apart from some irreversible adsorption processes - not clearly established[235] - the herbicide can be lost from the sphere of possible activity in four ways :-

1) photodecomposition and evaporation from the soil surface.

2) deep leaching[21,314,468] (see Ch. 3,C).

3) biological and non-biological decomposition.[235,258,468]

4) uptake by resistant plants.[235,258]

The latter point is not going to be discussed further.

7-3-1-1- Loss from the soil surface:- When a herbicide is sprayed onto a dry soil surface without receiving subsequent incorporation into the soil by, cultivation, rainfall or overhead irrigation it may then be liable to loss by :-

1) Photochemical decomposition : It has been reported that some herbicides e.g. triazines, substituted urea, phenoxyacetic acids, amiben, trifluralin etc. undergo molecular changes when exposed to sunlight, or to specific artificial light sources.[468] Many researchers (based on laboratory work) agree that photodecomposition could be an important mechanism of herbicide detoxication on soil under field conditions.[468] However, Messersmith et al.[346]

Wright and Warren[509] have demonstrated that photodecomposition of trifluralin is a minor detoxication route under field conditions. This was attributed to the possibility that the soil may present an uneven surface which may alter the degree of direct radiation that an applied material may receive and/or the degraded end products may possess herbicidal activity.

2) Volatilization : Loss by volatilization is probably more significant than is generally realized for many surface-applied herbicides. This is known to occur with many herbicides e.g. DNBP, 2,4-D esters, EPTC, CIPC, CDEC, CDAA and trifluralin.[258]

The loss of herbicide vapour from the soil may have the double disadvantage of undesired plant injury and loss of herbicide from the regions of its intended activity.[468] Volatilization is affected by 1) soil temperature (soil temperature may be considerably higher than air temperature[258]); 2) soil moisture content[468] (water moving upward as a liquid to replace evaporated water may concentrate the herbicide at the soil-air interface - wick evaporation,[238] soil water also may compete with the herbicide for adsorption sites in the soil).[258] 3) soil pH through its effects on adsorption of herbicide onto the soil or in cases where ion selection plays a role in herbicide volatilization e.g. DNBP.[468] 4) the nature of the herbicide formulation e.g. 2,4-D acid, alkali metal salts or amine formulations are less volatile, while the 2,4-D ester formulations (low alkyl esters) are more volatile[3,42] and 5) the herbicide carrier e.g. technical grade EPTC applied to soil in water is more persistent than when applied in kerosene.[468] Volatilization may also be less from granular formulations.[468]

Herbicides subjected to photochemical or volatility losses need to be incorporated into the soil.[258,468] Sometimes incorporation is desirable within an hour or so of spraying. The maximum time interval permissible

between application and incorporation depends on the chemical, its formulation, the temperature and level of soil moisture.[258] Generally when herbicides are incorporated into the soil, they are in soil containing more moisture, less oxygen and more carbon dioxide.[429, 468] The vapours which form are subjected to no air movement and the temperature of the soil below the surface varies less than that of the surface layer.[429]

In places of low or erratic rainfall or in places where furrow irrigation is adopted (see 7-3-1-2) soil incorporation may also be required to obtain an optimum placement for the herbicide.[17, 468]

In all cases the depth of incorporation should be great enough to obtain the maximum activity-selectivity possible from a plant morphological-physiological standpoint and obtain enhanced activity due to control over volatility, photodecomposition and high temperature effects[258] (see 7-3-1-2), yet it should be shallow enough to minimize the soil dilution effect (EPTC, CDEC and CDAA become less effective as they are incorporated deeper into the soil).[17] However in view of the variability of equipment from farm to farm and the attendant uneven distribution obtained with some instruments as demonstrated by McWhorter and Wooten[343] for disk machines taken in conjunction with 1) the necessity for even distribution of herbicides particularly those which have got localized soil action e.g. MCPA[258] and 2) the possiblity of unfavourable effects obtained with mobile herbicides upon receipt of appreciable additional water (crop injury and/or loss of weed control);[468] mechanical incorporation of herbicides is likely to be fraught with an element of risk.

7-3-1-2- Leaching :- The use of pre-emergence herbicides when natural rainfall is low and/or sporadic has not been uniformly successful.[17] In general the results have been erratic.[17, 343] Excellent weed control has been obtained sometimes and complete lack of weed control at other times.[17] It is likely that the desirability of rainfall after application is

attributed at least in part to the movement by leaching of the herbicide to the zone of herbicidal uptake by the germinating weed seedlings.[468] However, the importance of post-application rain varies with each herbicide and herbicide formulation due to differences in such factors as water solubility and ionic charge of the herbicide[468] (and hence adsorption and mobility see Kirkham classification of pesticides refs. 159,296)

Excess leaching may take the herbicide down to the roots of a crop relying partly on depth protection for safety.[468] In some instances leaching may occur so readily that the herbicide disappears beyond reach of most roots and may be lost as far as biological effects are concerned,[258] (see Ch. 3,C). However herbicides vary in their leachability and they may leach differently in different soils e.g. TCA, dalapon and chlorate are very readily leached in all soils;[258] 2,3,6 TBA and CDAA are readily leached in all but organic soils;[258] dinoseb and dichloral urea are leached in sandy soils but not in loams or organic soils;[258] simazine and monuron are not readily leached in any soil;[258] neburon and chlorpropham are almost completely resistant to leaching.[258]

The impact of these factors (leachability, rainfall and herbicide physico-chemical properties) on actual field performance is best illustrated by the contrasting results reported by Linscott et al.[318] with amiben derivatives. In 1966 (low rainfall) the ammonium salt, the acid, the amine salt (all water soluble) and the high rates of esters (methyl and butoxyethyl) gave excellent weed control, while the low rates of esters and the amide salt (esters and amide being fairly water insoluble tend to stay on the soil surface) gave poor weed control. In 1967 (excessive rainfall) the amide and the esters gave excellent weed control, while the acid, ammonium and amine salts gave poor weed control due to excessive leaching. For further details see Ch. 3,C.).

7-3-1-3 Adsorption :- The extent of adsorption onto soils regulates the

availability of herbicides in the soil.[258,468] Experience with pre-emergence herbicides showed that the minimum dose required for effective weed control differs from soil to soil.[230] Higher rates of soil-applied herbicides are needed on fine textured or high organic soils than on coarse textured or low organic matter soils.[235] When the same dose is used regardless of soil type, there is a risk of crop injury in some soils and insufficient herbicide for weed control in other soils.[230] This difference is attributed at least in part to adsorption onto soil[230,258,468] (see Ch. 3, A and B). However, adsorption may be affected by a specific interaction between a soil constituent (organic matter, clay content) and individual herbicides. Harris and Sheets[230] in their study of the influence of soil properties on adsorption and phytotoxicity of CIPC, diuron and simazine found that CIPC was adsorbed the most, diuron intermediate and simazine the least in some soils. In other soils (high in clay content) they found that simazine was adsorbed the most followed by CIPC and diuron in this order. Similar behaviour was reported by Harris and Warren[231] and Hilton and Yuen[250] for simazine and monuron on different soils. Holly[258] working with different herbicides and different soils showed that :-

1) With herbicides like TCA neither adsorption nor phytotoxicity is affected by soil type;

2) With herbicides like diuron and chlorpropham great variation occurs in both adsorption and phytotoxicity with soil type.

7-3-1-4 Decomposition :- A given pre-emergence herbicide should be available to weeds over a specified period determined mainly by the crop and/or the weed in question (see 1-2), yet it must not resist decay and must not persist in the soil longer than one growing season to avoid problems due to delayed action on subsequent susceptible crops.[230,235] Unstable compounds will not be suitable in view of the expected lack of weed control and compounds having long persistence are undesirable in view of the expected injury to subsequent crops.[235]

Herbicides in the soil could be inactivated by biological or non biological decomposition[258] (see Ch. 3,D). Biological decomposition is by far the most important.[258] The dependence of most herbicides on biological decomposition for inactivation makes it rather difficult to expect most herbicides to show similar persistance and performance under different climatic and edaphic conditions. This is illustrated by:-

1) Herbicides like amitrole and dalapon are known to be quite susceptible to microbial breakdown.[468] Work carried out by Day et al[144,145] showed that their decomposition in the soil is highly variable (their decomposition being very rapid under conditions conducive to increased microbial activity - see Ch. 3,D) and that their soil residues caused tree damage in various localities in California. Tree injury resulting from dalapon (sodium salt) has been a limiting factor in its use for controlling perennial grasses in citrus and avocado orchards.[145]

2) Crops sown more than 2 years after trifluralin, prometryne, atrazine or diuron application suffered severe losses when sown according to instructions on the labelled containers without paying attention to climatic or edaphic conditions.[218]

3) As pointed out by Holly[258] sometimes repeated application of a herbicide to a soil may result in a build up of a microbial population capable of rapid detoxication of that particular herbicide. Therefore there is a danger that a situation may arise whereby the same soil acting herbicide applied to the same area at too frequent intervals may not give weed control for the expected period because of quicker breakdown (see 2-1-1).

7-4- The influence of environmental factors on the herbicide-plant system:-

In several instances, extensive herbicidal damage to crops (which are otherwise tolerant) from soil-applied herbicides has been reported.[90,151,239,318A] Usually the degree of injury seemed to be augmented by environmental condtions favouring vigorous seedling growth, such as high temperature and plentiful soil water supply[421] (conditions which could lead to increased

microbial activity).[468] On the other hand conditions which retard seedling growth e.g. low soil temperature and low soil moisture may lessen the effectiveness of a pre-emergence treatment.[481] This suggested that herbicide effectiveness should be evaluated not only in terms of the effect of the environment on the herbicide-soil system but also in terms of the effects of the environment on the herbicide-plant system.

Most investigations have been directed at isolating each environmental factor and studying its individual effect on phytotoxicity of herbcides. It is important that this should be done, but it is often forgotten that the environment under practical field conditions is a complex of many different factors and interactions. Of all environmental factors light, temperature and moisture appear to be the most important.[267] Though the inter-relationship between these factors is so intimate (they play a collective role in affecting the phytotoxic action of herbicides[267]) the division between them is necessary for the convenience of presentation. However it must be stressed that this division is rather arbitrary and some overlap is inevitable.

7-4-1 Light :- Light is essential for the effectiveness of herbicides which act via photosynthesis e.g. substituted urea, triazines and the bipyridylium herbicides (paraquat and diquat).[347] Apart from this, light through its effect on the carbohydrate balance of plants may provide the energy necessary for active uptake (see 7-1-1-2).[267]

7-4-2- Soil water content :- The effect of a change in soil water content on herbicide concentration is clearly dependent on the magnitude of adsorption. Soil drying results in an increased concentration in soils with low herbicide adsorption[212,235] (depending on the water solubility of the herbicide).[512] However, this increase in concentration does not mean that a herbicide will be more phytotoxic under dry conditions. Other factors in plant-soil-water-herbicide systems may interact in such a way that the

opposite effect occurs.[212] Upchurch[469] under greenhouse conditions and Sedgley and Boersma[421] under field conditions observed that diuron is more phytotoxic at high soil moisture contents. Geissbühler et al.[199] observed a lower plant uptake from soil than from nutrient solution at the same calculated solution concentration, thus indicating restricted herbicide transport in the soil. Herbicide transport in the soil takes place by mass flow and/or molecular diffusion. (Both mass flow and molecular diffusion are expected to diminish with decreasing soil water content).[212]

Additional effects of soil water stress are :-

1) Passive uptake of herbicides may decrease (uptake of water decreases at high soil water tension),[196,428] also active uptake may be affected through an indirect effect of water stress on some metabolic processes in the plant[413] (see 7-1-1-2).

2) Translocation of the herbicide may be reduced by restricted transpiration.[380,413,481]

3) Soil water also affects some plant physiological functions e.g. photosynthesis (see 7-1-1-2 and 7-4-1-) and root permeability.[413] Root permeability as influenced by soil water potential is claimed to be one of the possible reasons for the encountered reduction of bromacil uptake by plant roots at low soil water contents.[413]

7-4-3- Temperature :- It has been demonstrated that high temperature results in increased phytotoxicity of certain herbicides.[267] Burnside and Behrens[88] reported that increased soil temperature results in an increase in the toxicity of simazine to maize. Schneider[444] cited observations where plants growing under ideal conditions were killed more rapidly by simazine than plants growing at low temperature. Vostral et al[481] observed that low soil temperature reduces the pre-emergence activity of soil-applied atrazine. They concluded that low absorption under low temperature conditions is likely to reduce the effectiveness of pre-emergence application of herbicides made early in the spring, particularly at high relative

humidity. Similar observations were reported by Sheets,[428] he found that uptake and translocation of simazine by oat (susceptible) and cotton (Gossypium hirsutum) (resistant) were greater at higher temperature and low humidity conditions favouring increased transpiration. Increase in uptake and activity of soil-applied herbicides with increase in temperature may be due to 1) increased transpiration[428] 2) increased metabolic activity[88] 3) increased desorption of the herbicide (adsorption processes are exothermic)[88] and 4) increased diffusion rate (both in the soil and plant roots.)[421]

If one considers that the susceptibility of plants to a herbicide is governed by concentration of the unchanged herbicide which reaches the active site (such a point seems to be supported by many observations, see Rogers and Funderburk,[407] Moreland and Hill,[356] Strang and Rogers,[447,448,449] Sedgley and Boersma,[421] and Good[206]), then the role of soil temperature and soil moisture could be visualised as thought by Sedgley and Boersma[421] to arise from their influence on the rate of arrival of the herbicide at the active site. However, it must be borne in mind that the rate of accumulation of the toxic moiety relative to the threshold level of the plant in question rather than the rate of arrival per se is the important factor in determining the susceptibility of a plant to a given herbicide. (The threshold level of a plant is not a constant property, it varies with the physiological status of the plant and the intrinsic phytotoxicity of the herbicide).[225]

Accumulation of a herbicide at the active site(s) depends on :-

1) the availability of the herbicide in the zone of uptake (shoot or root). This is affected by several soil and environmental factors (see 7-3).

2) several plants processes e.g. absorption and translocation which are affected by environmental factors.

3) the rate of increase in plant size (growth dilution) and rate and pathway of metabolic change of the herbicide. These are also affected by the environment.

So it could be concluded from this review (see 3 to 7) that susceptibility to a herbicide is not a constant property of a species but varies with variations in both environmental and intrinsic factors. This dependence of susceptibility on the environment is undoubtedly responsible for the encountered variations in herbicide performance in different seasons [145, 225, 235] and in different localities. [122, 144, 158, 306]

8- Specific problems :-

The fate of aniline-based herbicides in the soil and the bracken problem are treated briefly under this heading. Asulam (which could be considered as an aniline-based herbicide) and bracken are subject matters dealt with in this thesis.

8-1- The fate of aniline-based herbicides :-

A substantial proportion of the currently used herbicides contain unsubstituted or variously substituted anilines, e.g. acylanilide, phenylcarbamate and phenylurea. Among the attractive features of some of these herbicides are their effectiveness, selectivity and biodegradability (see ref. 38). The proved or postulated degradation pathway of these compounds in soil involves the release of the corresponding aniline. [38] Plant tissues (through degradation of the herbicide) may be an additional source of aniline that may enter the soil. [38]

It was believed that the released anilines are then readily metabolised by established mechanisms, including ring hydroxylation and cleavage to an aliphatic product that would be further oxidized (see ref 40). The work of Bartha and others [40, 59, 60, 291] proved this notion to be erroneous. Instead they showed that some of the anilines released are

oxidized in soils via enzymic (soil peroxidases) reactions, that are polymerising rather than degradative[40] (see Ch. 4, A-1). These reactions lead to the formation of azobenzenes and/or to polymeric products of higher complexity which have extended residual activity and largely unknown biological activity.[62] However, some azobenzene compounds are known to be carcinogenic.[38,40] Evidence now exists that some of these products are formed in the soil under practical field conditions.[291] Previous reports denying their formation,[200] or admitting their formation but under unrealistic rates of application[222] could be due to 1) the complex nature of the transformation products and their adsorption onto soil.[60] Such conditions could prevent the detection of some transformations, and prevent any quantitation of the results[60] and 2) the occurrence of competition reactions.[291] Plimmer et al.[389] reported triazene formation in soil treated with 3,4-dichloroaniline. Tweedy et al.[466] reported quantitative conversion of p-bromoaniline to acetanilide after an incubation period of 7 days. Both acetylation and triazene formation are regarded as competition reactions lessening the possibility of azobenzene formation.[291,466]

Moreover it should be borne in mind that transformations in soils are the result of the interactions of a number of processes:

1) Soil characteristics have been recognised as important factors in determining the quantitative relationships between metabolites of a pesticide.[389]

2) The microbial flora may determine the qualitative relationships between metabolites of a pesticide.[389]

3) Susceptibility to transformation is dependent primarily on the electron distribution in the molecule. Anilines become increasingly susceptible to enzymic transformations with increasing electron density at the amino group.[60]

8-2 The bracken problem :-

Species of the genus Pteridium and their varieties, hereinafter

refered to by the common name of bracken, are unpalatable, poisonous perennial ferns, with world wide distribution in humid and sub-humid regions.[404] They are highly competitive with other vegetation because of their tall growth habit under favourable environment, shade tolerance and deep massive rhizome system.[285, 404] Recently it has been demonstrated that leachate from bracken fronds is inhibitory to the growth of many grass seedlings.[204] This suggests that suppression of other plants by bracken is not due wholly to mere competition for water, mineral, food or light, but at least in part to allelopathic substance(s) which could be leached from bracken fronds by rain.[204]

Bracken dominates considerable areas of unimproved or degenerate hill land throughout Britain, being particularly a problem on land inaccessable to the plough.[226] In Scotland (where about 450,000 acres of pasture are infested with bracken[285]) the spreading of bracken has been encouraged by 1) the curtailment of cattle-grazing on the hill land in favour of sheep-rearing[70] 2) the former use of bracken for thatching, litter, alkali manufacture and other purposes is now virtually extinct[70, 285] and 3) the decrease in the intensity of agriculture in the upland areas since the middle of the last century encouraged bracken to take over the better drained land[285] (impeded drainage discourages bracken growth[390]).

Before the advent of modern herbicides, cultural methods were the only feasible means of control.[404] Since cultural control is often impractical (see Ch.5), chemical means have received increasingly greater investigation. By the end of the 1950s the phenoxy and phenoxybutric compounds had been screened in Britain by Conway[111] and Conway and Forrest[115] with varying results. In the 1960 s other herbicides were tested on bracken. Most effective among newer compounds were picloram and dicamba.[404] Research is still going on, on these chemicals.[167] Many other chemicals appeared in the 1970s e.g. asulam and later glyphosate.[285] However inconsistency

in performance, at different sites, different years and the ability of bracken to regenerate after treatment are common features assocciated with some of these chemicals if not all of them.[285] The possible reasons for such behaviour are discussed in Ch. 2, and Ch. 5.

CHAPTER II

This chapter deals with studies on A) The bracken frond surface B) Penetration of bracken fronds by asulam C) Penetration of bracken fronds by amitrole D) Penetration of bean leaves by asulam and E) A preliminary field experiment on bracken control.

A- The bracken frond surface:-

A-1- Introduction :-

It is commonly assumed by botanists that plants originated in water as simple unicellular organisms, and that by specialization of many sorts they have attained their present complexity of form and organisation.[127] However only the acquirement of a cutinised surface enables them to grow permanently in a terrestrial environment with foliar organs exposed to the air.[127] This cutinised surface (the cuticle) provides a distinct barrier to optimum herbicide penetration[328,433](see Ch.1, 3-1-2).

The thickness and composition of the cuticle vary with the plant species and with the environmental conditions under which the plant develops.[353] It is most prominent on leaves and stems of xerophytic plants.[353]

Unlike many Pteridophytes which are restricted to damp, shady habitats, Pteridium is also found in grassy places and open hill side where conditions are usually relatively dry.[459]

Opinions as to whether the ability of bracken to occupy such diverse ecological niches is accompanied and/or made possible by changes in the plant cuticle, as is the case in many plants with similar powers,[328] are contradictory.[57,328] Such modifications (if they occur) will be of paramount importance in the control of bracken by foliar applied herbicides and could be responsible in part for variations noticed in practice between different sites and different years (see Ch.5).

This preliminary investigation set out to (a) compare cuticle

development on a glasshouse-grown plant and a field plant using light microscopy b) compare stomatal structure in exposed field plants after and before a chloroform wash using electron microscopy and c) compare variations in amount of cuticular waxes with 1) plant habitat 2) pinnae age (on the same frond) and 3) pinnules age (on the same pinna).

A-2- Experimental:-

a) Cuticle development:- Bracken pinnae were collected from the field from exposed isolated plants. Glasshouse-grown bracken samples were supplied by Dr. C. Page of The Royal Botanic Gardens, Edinburgh. Small pinnae segments were fixed with 3% phosphate buffered glutaraldehyde and postfixed with 1% S-collidine buffered osmium tetraoxide at pH 7.4. The tissues were dehydrated in 70, 90 and 100 per cent acetone series, followed by acetone change (of 100% acetone for ½h) and 2h in two changes of propylene oxide. The material was embedded for 2h in 50/50 propylene oxide/Spurr's resin, followed by overnight infiltration in Spurr's resin, The samples were then embedded flat in Spurr's resin polymerised overnight at 60°C, sectioned and viewed with a light microscope.

b) Stomatal structure :- Small pinnae segments (one sample was given 10 sec immersion in chloroform) were dehydrated in an acetone series (50% and 100%) followed by two changes of acetone (100%), air dried, coated on the lower surface with gold (500 Å) and viewed with a Cambridge S600 scanning electron microscope.

c) Cuticular waxes :- The samples were collected from the field in July. Fronds with four fully expanded pinnae were selected for uniformity. The method used for extraction of cuticular waxes was adopted after Radler and Horn.[393] Two successive emersions in chloroform at room temperature (30 sec each) with agitation were decided upon after a preliminary trial (Table 1).

A-3- Results and Discussion :-

a) In the exposed frond (Fig.1) the cuticle on the upper epidermis is

Table 1 Extraction of cuticular waxes with chloroform, preliminary experiment.

Number of immersion (30 sec immersion time)	Yield as per cent (w/w) of the total extracted in 6 successive immersions
1	67.4
2	20.7
3	4.7
4	2.7
5	2.5
6	2.0

considerably thicker and there is a well differentiated hypoderm. A notable feature of the epidermal and hypodermal cells is the variation in cell size and shape. A feature responsible perhaps for the observed roughness of exposed field bracken frond surfaces (the topography of the leaf surface is governed in part by the size and shape of the epidermal cells).[328] The cuticle on the lower epidermis is comparatively thinner.

In the case of the glasshouse-grown plant the cuticle on the upper epidermis is very thin and only a trace of cuticle is visible on the lower epidermis (Fig. 2 a and b).

The differences in cuticular thickness on glasshouse-grown plant and exposed field bracken show that bracken grown under glasshouse or controlled environment conditions may differ in cuticle characteristics from others of the same variety grown in the open. This finding confirms the findings of Boodle[57] about 73 years ago and provides an explanation of surface roughness observed by him.

Because of the inhibited cuticle formation in glasshouse-grown bracken it may well be asked whether such plants will give representative responses to herbicides formulated for field use (see Ch.5).

Fig. 1 A transverse section of a bracken pinna segment. (x 90). Specimen from an exposed field plant.

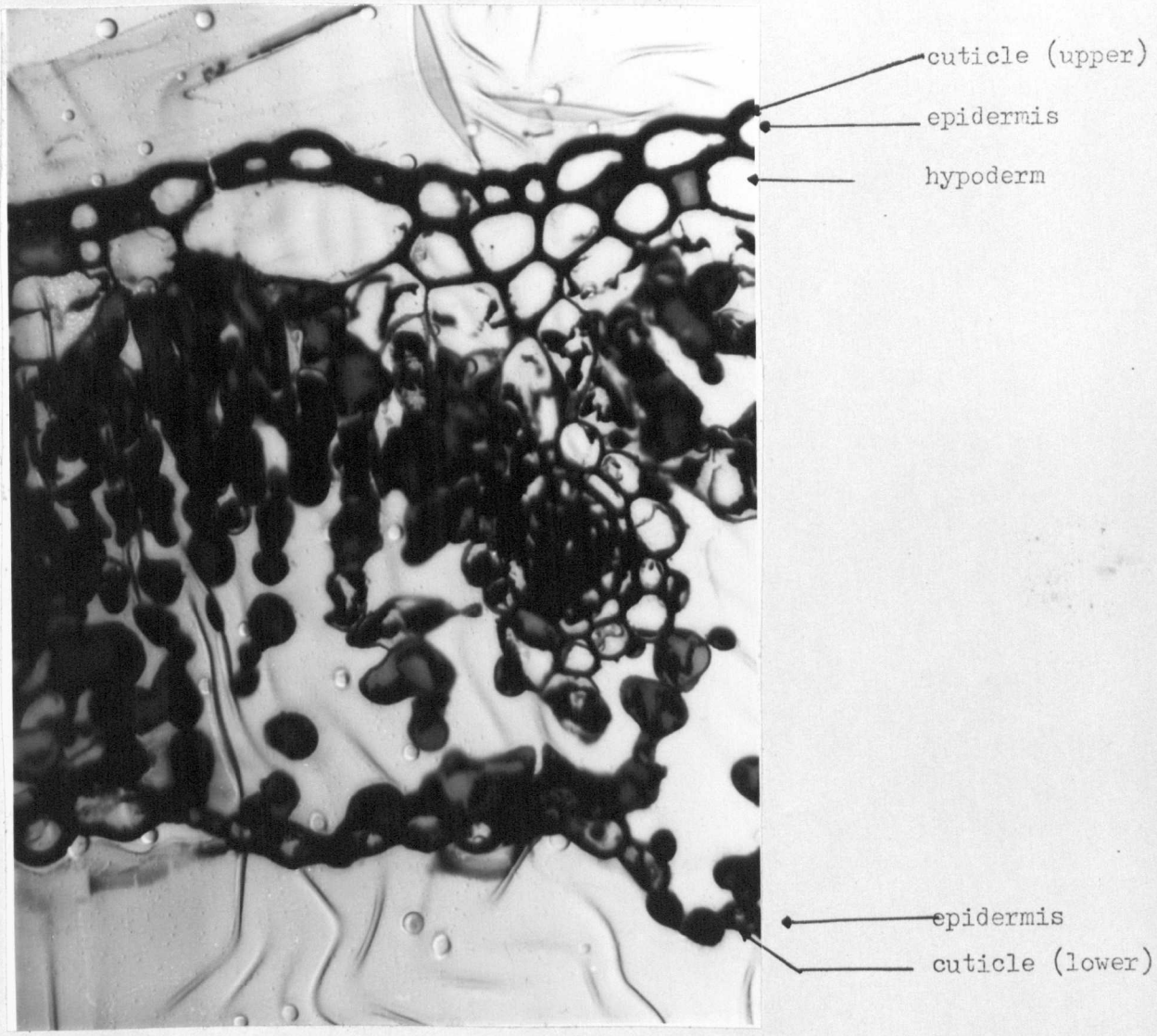
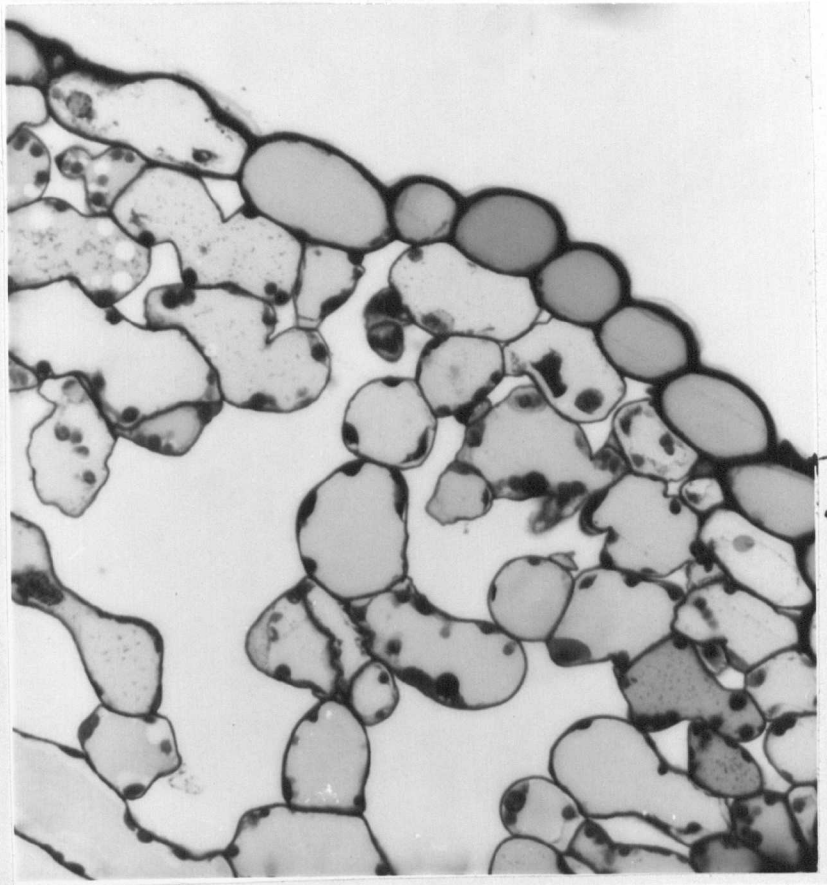
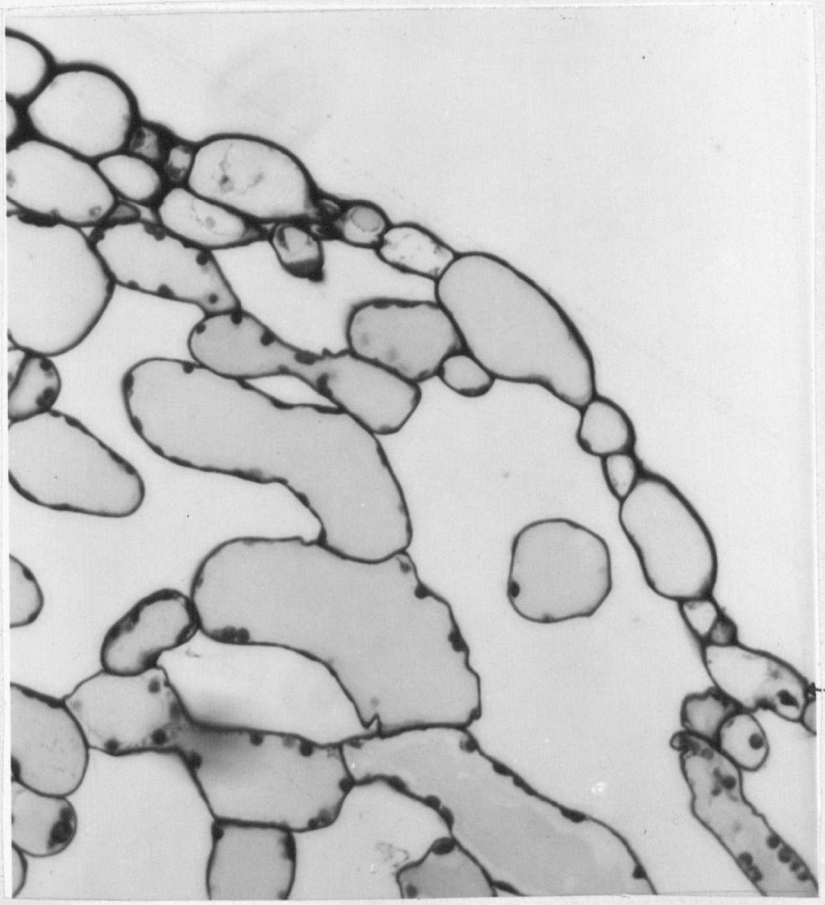


Fig. 2 A transverse section of a bracken pinna segment (x90). Specimens from a glasshouse-grown plant.





b) Stomatal structure: The scanning electron microscope studies (Fig. 3 a and b) demonstrated clearly the presence of a resinous or a fatty structure (could be removed by chloroform) within the substomatal chambers. The presence of such a structure has been reported in various plants.[269,416,464] It is conceivable as pointed out by Hull[269] that such structures might not influence gaseous diffusion greatly but that they might inhibit seriously the penetration of pesticide solutions or other liquids, particularly those not formulated with an oil or surfactant (see Ch.1, 3-1-2-1). Moreover the presence of such a structure in bracken substomatal chambers may explain the observation made by Tinklin and Bowling[459] who suggested the existence of a mechanism of control over stomatal transpiration in bracken.

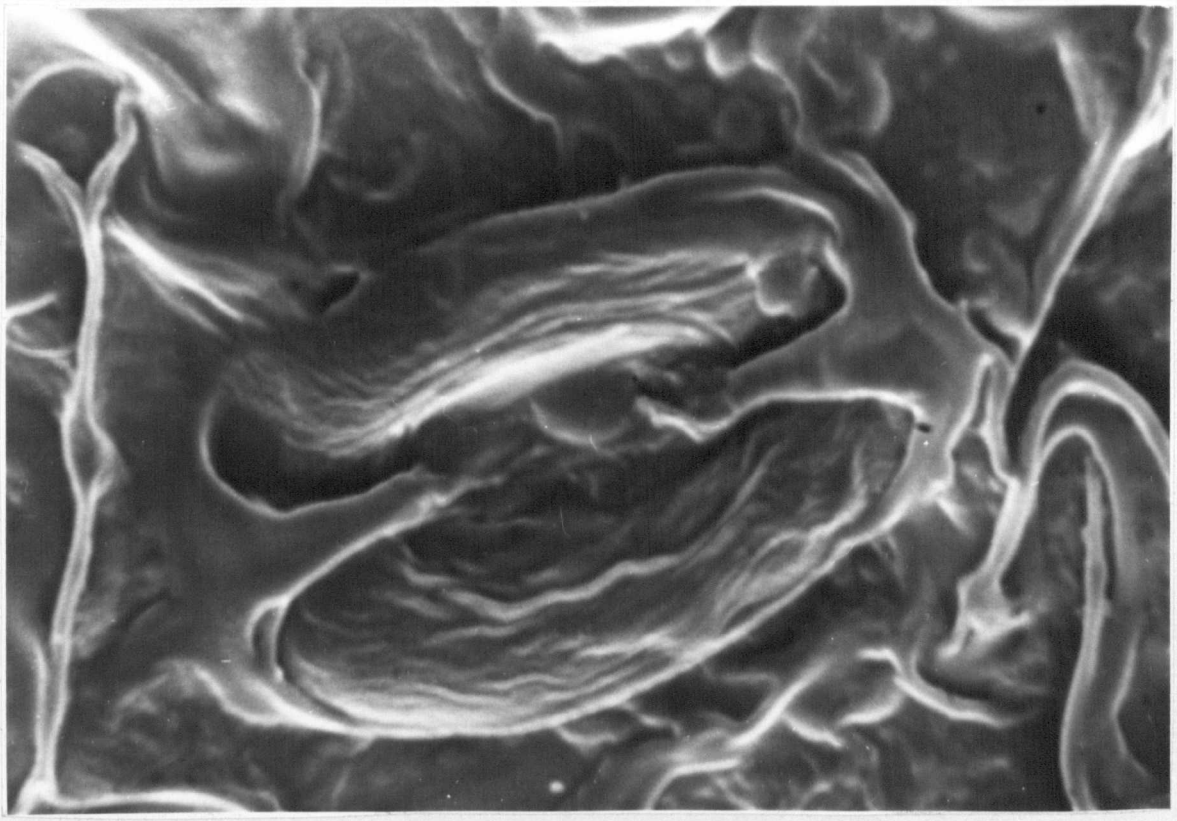
Table 2 Variation in amount of cuticular waxes expressed on dry weight per cent basis.

Treatment	Wax yield %			Standard deviation ±	Mean wax yield %
a) Variation with plant habitat.					
Exposed bracken	0.6	0.6	0.6	0.0	0.6
Bracken from under trees	1.0	1.0	1.0	0.0	1.0
Bracken from inside the canopy	1.1	1.1	1.1	0.0	1.1
b) Variation with pinnae age (pinnae numbered from top to bottom).					
1	0.5	0.9	0.8	0.2	0.7
2	0.6	0.7	0.7	0.0	0.6
3	0.6	0.7	0.6	0.0	0.6
4	0.6	0.6	0.7	0.0	0.6
c) Variation with pinnule age.					
Pinnae top	0.8	0.8	0.8	0.0	0.8
Pinnae base	0.5	0.6	0.6	0.0	0.6

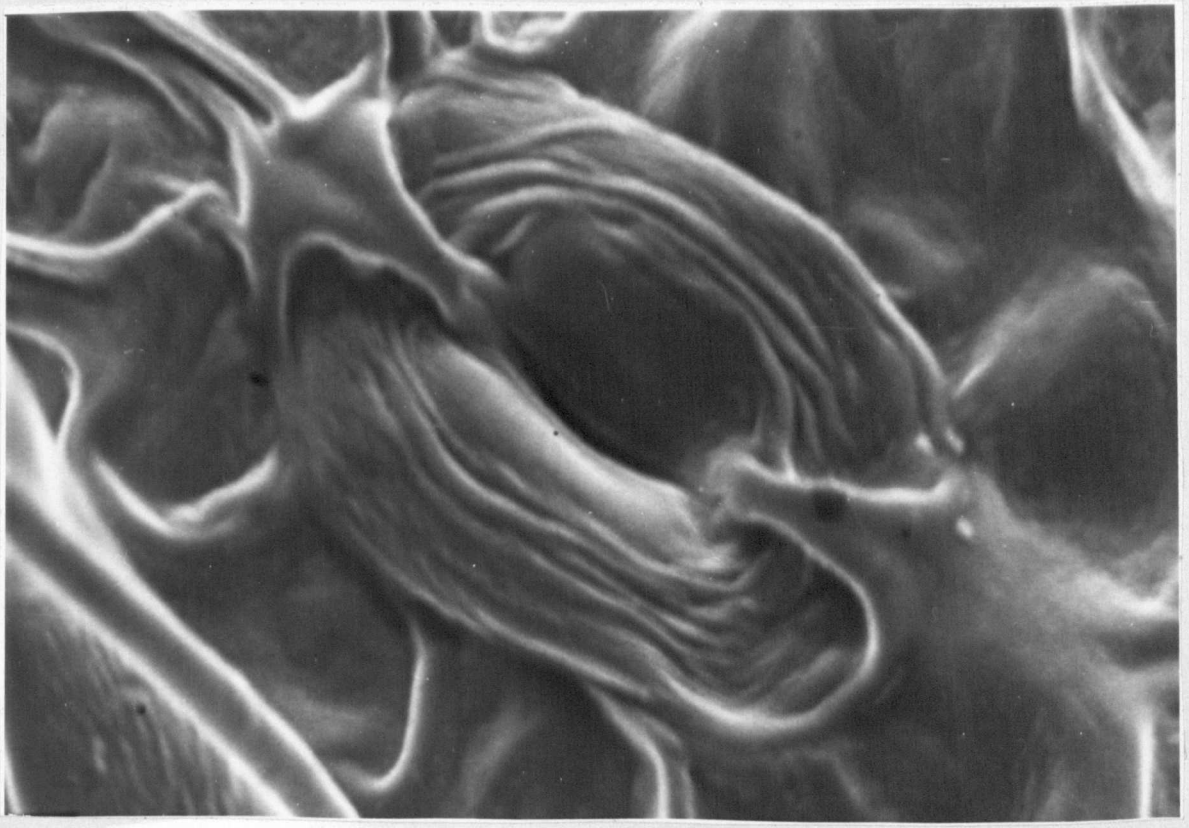
c) Cuticular waxes :- The cuticular waxes expressed on dry weight per cent basis shows that 1) exposed fronds contain less wax than their

Fig. 3 Electron micrographs of bracken frond (lower surface x 200)

a) Specimen receiving no chloroform pretreatment.



b) Specimen receiving chloroform pretreatment.



equivalents collected from under trees or from inside the bracken canopy (Table 2a); 2) the wax content of young pinnae is slightly but not significantly higher than from their old equivalents (Table 2b) and 3) the wax content of pinnules from pinnae tops is greater than from their base equivalents (Table 2c).

It is probable that cuticular waxes (cuticle is not all wax)[270] in exposed bracken and old pinnae or pinnules are subjected to loss by abrasive action of rain, wind and dust on the cuticle[328] (see Ch.5, 2-4-2-1-1 ii), or by flaking or sloughing of the cuticle as the plant matures.[305] However, it is also possible as pointed out by Kurtz[305] that an increase in plant dry weight with age may be responsible for the observed decrease in wax yield.

B- Penetration of bracken fronds by asulam as influenced by the addition of surfactants to the spray solution and by pH:-

B-1- Introduction:-

Asulam has shown promise as a herbicide for the control of bracken when applied to the foliage.[261] However, in common with many foliar-applied herbicides the erratic behaviour of asulam noted in the West of Scotland and elsewhere and attributed to climatic factors, could be caused by slow penetration[137,261] (see Ch.1, 4-2-3-3). The West of Scotland being a high rainfall area makes this aspect particularly relevant (see Ch.1, 4-2-3-3)

This investigation set out to assess (a) the effect of Tween 20 on spray retention by bracken fronds and (b) the influence of Tween 20 and pH of spray solution on penetration. As the morphology of the bracken fronds will also have a bearing on spray retention (see Ch.1, 3-1-1), it was decided to take this into consideration. Pinnae were therefore divided into three distinct regions : 1) the less-developed, curled top; 2) the flat well-developed middle section and 3) the slightly

curled base. Tween 20 was selected as it had previously been shown to enhance sulphonamides uptake by plants (see D-3-1).[94,134,266,353] A phosphate buffer as the sodium salt was selected to cover the required pH range (2 to 9).

B-2- Experimental:-

Asulam (methyl (4-aminobenzenesulphonyl) carbamate, technical ingredient 99.5% pure) was purchased from the National Physical Laboratory. Asulox, the commercial formulation containing 40% $^{w}/_{v}$ asulam as the sodium salt, was supplied by May and Baker Ltd. Tween 20 (polyoxyethylene sorbitan monolaurate) was obtained from Koch-Light Laboratories Ltd.

For spray retention measurements a modification of the method of Day and Jordan,[143] as used with bermudagrass, was employed. Pinnules from the top, middle and base of the pinnae were weighed, and water or alternatively Tween 20 solution (1.6% $^{w}/_{v}$) was applied to the upper surface with a syringe as an evenly distributed film. Then the pinnules were suspended from a balance hook and reweighed when dripping ceased.

All penetration experiments were carried out in the field under a metal framework (1.5 x 1.5 x 1.0M m) covered with polythene sheeting to standardize the experimental conditions. However, some variability had to be tolerated in the field due to a lack of control of such factors as temperature and humidity.

Penetration was determined, as for 2,4-D, by a method described by Szabo and Buchholtz.[454] Asulam (40µg) or Asulox, containing the same amount of active ingredient in a total volume of 0.2 ml was applied to adjacent pinnules at the bottom of the third pinna from the base of the frond (selected solely for uniformity) with a 1ml graduated cylindrical pipette. These conditions were selected to give maximum coverage with no run-off. Similar additions were carried out with different concentrations of Tween 20 and or phosphate buffer solution. After 3h the treated pinnules

were washed with 20 ml of deionized water. Preliminary exercises had confirmed that 100% recovery of chemical was obtained by this washing treatment provided the pinnule was washed immediately after application.

Asulam concentrations were determined by the Bratton-Marshall reaction as described for asulam by Brocklesby and Muggleton.[78]

B-3- Results and Discussion:-

B-3-1- Spray retention:- Although some variation is noted between individual samples the retention of water by bracken pinnules is shown to be very poor in all cases examined (Table 1). This could be due at least in part to the leaf surface topography of field bracken (see A). However, the inclusion of surfactant (1.6% $^{w}/_{v}$) brings about a substantial increase of the order of 2-5 fold that of the water controls.

It is noteworthy that the mean water retention of the middle section is greater than that of either the top or base equivalents. This could be accounted for in terms of a flatter exposed surface and possibly less cuticle (see A). However, all three surfactant-treated pinnules retain much more solution than the controls, a situation which could be interpreted in terms of influence on spray solution as well as on leaf surface. Bearing in mind the posture of the pinnae in the field one would expect the middle pinnules to play an even greater part in the overall interception and retention of spray than is suggested by the figures (Table 1) (see Ch.1, 3-1-1).

B-3-2- Asulam penetration:- With regard to the penetration of the chemical a considerable improvement, of the order of 100%, results on all occasions when surfactant is added and compared with control samples (Table 2).

For the commercial formulation a penetration figure of 25.8% results with deionized water compared with 53.1% when 0.08% surfactant is incorporated. The comparable figures for the pure asulam are 34.9% and 67.2% respectively.

Table 1 Influence of surfactant on water retention (8 pinnules).

Pinna section	Surfactant % (w/v)	Water retention g/g (fresh weight)								Mean	Variation
Base	0.0	0.09	0.00	0.05	0.02	0.01	0.08	0.10	0.09	0.05	0.00 - 0.10
Middle	0.0	0.06	0.04	0.10	0.03	0.05	0.03	0.10	0.03	0.06	0.03 - 0.10
Top	0.0	0.04	0.00	0.00	0.06	0.03	0.01	0.00	0.08	0.03	0.00 - 0.08
Base	1.6	0.17	0.08	0.08	0.10	0.09	0.17	0.09	0.16	0.13	0.08 - 0.17
Middle	1.6	0.21	0.18	0.24	0.22	0.26	0.33	0.14	0.19	0.22	0.14 - 0.33
Top	1.6	0.15	0.22	0.10	0.15	0.10	0.21	0.15	0.19	0.16	0.10 - 0.22

Table 2 Influence of surfactant on penetration of asulam. Application of 40 µg active ingredient to each of 5 pairs of pinnule as (a) Asulox or (b) Asulam.

Surfactant % (w/v)	Amount (µg) left on pinnule surface					Mean	Standard deviation ±	Penetration %
(a) Asulox								
0.00	30.8	29.4	29.4	29.4	29.4	29.6	0.6	25.8
0.08	23.8	18.2	22.4	15.4	14.0	18.7	3.8	53.1
0.18	15.4	19.6	14.0	19.6	13.7	16.5	2.6	58.8
0.26	19.6	14.0	19.6	19.6	14.0	17.4	2.7	56.6
(b) Asulam								
0.00	25.4	26.1	28.1	24.7	26.1	26.1	1.1	34.9
0.09	13.4	10.7	13.4	14.0	14.0	13.1	1.3	67.2
0.17	11.4	14.0	14.0	10.7	13.6	12.7	1.4	68.2
0.25	12.0	11.4	12.0	12.7	11.4	11.9	0.5	70.3

A point of some practical significance derived from these results (Table 2) is the observation that an increase in the amount of surfactant above a concentration of around 0.1% (w/v) only enhances the penetration of asulam to a negligible extent. These figures suggest that concentrations of Tween 20 of the order of 0.1% will considerably enhance the performance of asulam in the field.

Table 3 indicates that the presence of phosphate buffer, selected to cover the pH range 2 to 9, depresses the penetration of the chemical. With few exceptions the percentage penetration with phosphate is more than 10% less than the aqueous control without surfactant as given in Table 2. However, the addition of Tween 20 to the phosphate solution enhances penetration of asulam compared to penetration from the phosphate solution without Tween 20.

Unlike the behaviour predicted for weak acids,[451] the pH plays no effective role in penetration of asulam in these experiments thereby implying

Table 3 Influence of pH on penetration of asulam. Application of 40 µg active ingredient to each of 5 pairs of pinnules as Asulox.

pH	Amount (µg) left on pinnule surface					Mean	Standard deviation ±	Penetration %
(a) Phosphate buffer (0.5M[a]) without surfactant								
2.0	39.2	39.2	35.0	37.8	37.2	37.7	1.6	5.8
3.0	35.0	35.0	35.0	32.2	34.3	34.3	1.1	14.2
4.7	35.0	39.2	35.0	37.1	39.2	37.1	1.9	7.3
7.0	25.2	28.0	25.2	28.0	26.2	26.5	1.3	33.8
9.0	30.8	32.2	30.8	30.8	31.2	31.2	0.5	22.0
(b) Phosphate buffer (0.5M[a]) with 0.1% (W/v) surfactant								
2.0	12.6	14.0	14.0	12.6	12.6	13.2	0.7	67.0
3.0	21.0	19.6	19.6	16.8	12.6	17.9	3.0	55.3
4.5	12.6	15.4	22.4	19.6	21.0	18.2	3.6	54.5
7.0	21.0	14.0	16.8	14.0	14.0	16.0	2.7	60.0
9.0	22.4	26.6	21.0	21.0	23.6	22.9	2.1	42.8
(c) Phosphate buffer (0.125M[a]) without surfactant								
2.0	36.4	35.0	35.0	35.0	35.0	35.3	0.6	11.8
3.0	35.0	36.4	35.0	33.6	30.4	34.1	2.0	14.8
4.5	30.8	35.0	35.0	35.0	36.4	34.4	1.9	13.9
7.0	35.0	35.0	35.0	35.0	35.0	35.0	0.0	12.5
9.0	35.0	35.0	28.0	35.0	30.8	32.8	2.9	18.1
(d) Phosphate buffer (0.125M[a]) with 0.1% (W/v) surfactant								
2.0	11.2	14.0	14.0	11.2	14.0	12.9	1.4	67.8
3.0	19.6	11.2	19.6	16.8	11.2	15.7	3.8	60.8
4.7	14.0	15.4	21.0	19.6	19.6	17.9	2.7	55.0
7.0	21.0	15.4	16.8	15.4	14.0	16.5	2.4	68.7
9.0	19.4	22.4	19.0	19.6	22.4	20.7	1.4	48.3

[a] With respect to sodium in Na_2HPO_4 adjusted to pH with 50%(W/v) phosphoric acid.

uptake by an aqueous route[123] (see Ch.1, 3-1-2-2). However, changes in pH of the spray solution on the pinnule surface due to the buffering capacity of the latter cannot be ruled out.[322,507]

The influence of phosphate, and presumably other salts, in reducing penetration could help to explain the lower uptake of the commercial formulation in comparison to that of the active ingredient (Table 2). Although of low concentration the presence of salts or other substances within the commercial formulation (preliminary analysis of asulox revealed the presence of traces of an anionic surface active agent see Ch.3, A-1 and D-3-1 this Ch.) may retard penetration. Salts may compete with the herbicide for adsorption and/or ion exchange sites. Such reactions are known to take place when spray droplets are drying out on a leaf surface.[373] However deductions made by comparison between the data in Table 2 and Table 3 needs further investigation as the experiments were carried out on different days and variables e.g. temperature and humidity were not controlled (see D this Ch.). The lower penetration at pH 9 with surfactant is noteworthy (Table 3).

In field trials Holroyd et al.[261] observed that heavy drizzle following asulam application led to poor bracken control, but asulam treatments containing 0.1% (W/v) Agral 9C were not adversely affected. Soper[444] who made no reference to climatic conditions, found little difference between bracken control with and without the addition of Shellestol (0.025% W/v). Possibly, the penetration of asulam without the addition of a surfactant, though slow, will proceed satisfactorily provided no rain immediately follows the application. However the possibility that there is a specific herbicide-plant-surfactant interaction[178] including the hydrophilic-lipophilic-balance (HLB) of the system[19,55] cannot be dismissed at this stage without further investigation (see D-3-3 and Sec. E this Ch.).

C- Penetration of bracken fronds by amitrole as influenced by pre-spraying conditions, surfactants and other additives :-

C-1- Introduction :-

Conflicting reports have appeared in the literature on the use of amitrole as a foliar applied herbicide for the control of bracken. Work in Scandinavia with amitrole yielded promising results.[94] On the other hand, findings in the U.K. are contradictory in that experiments carried out in England and in the East of Scotland confirm the Scandinavian findings[162,255] while poor results were recorded in the West of Scotland[109,299] (see Ch.1, 4-2-3-1 and 4-2-3-3). The work of Holroyd et al.[261] revealed that the toxicity of activated amitrole could be eliminated by rain. It is therefore feasible that, as for asulam,[27] the erratic behaviour noted with amitrole in the West of Scotland could be due to slow penetration.

This investigation set out to assess the effects of different surfactants and other additives on the penetration under field conditions of bracken pinnules by amitrole, where such variables as humidity, temperature etc. were not controlled but were taken into account by incorporating suitable blanks.

The extreme sensitivity of the bracken plant to environmental conditions makes a field study rather than a glasshouse exercise much more relevant.[57,270]

C-2- Experimental:-

Amitrole (3-amino-1,2,4-triazole) was obtained from Koch-Light Laboratories. Tween 20 was as described before (see B-2). Triton X-405 [4-(1,1,3,3-tetramethylbutyl) phenoxy polyethoxyethanol], 70% w/v solution in water was obtained from Sigma London Chemical Co. Ltd., and Triton GR-5 [di-(2-ethylhexyl) sodiosulphosuccinate] 60% a.i. in propan-2-ol-water (1/1, v/v), Triton X-100 [iso-octylphenoxy (polyethoxy) ethanol] 100% a.i., Tergitol NPX (alkylphenyl ether of polyethylene glycol) 100% a.i. from B.H.D. Ltd.

All penetration experiments were performed in the field as previously described (see B-2). Amitrole (40 µg a.i.) in a total volume of 0.2 ml or alternatively 0.02 ml when surfactants were used, was applied with an Eppendorf pipette as discrete droplets to the upper surface of the pinnule. Continuous films resulted when surfactants were included with the exception of Triton X-405.

The treated pinnules were washed as before (see B-2). Preliminary experiments had confirmed that 100% recovery of chemical was obtained by this washing treatment provided the pinnule was washed immediately after application.

Amitrole concentrations were determined by the method of Shorherr and Burke.[433]

C-3- Results and Discussion :-

C-3-1- Influence of surfactants on penetration :-

The enhancement of penetration of amitrole in the presence of surfactants is quite obvious when compared with aqueous controls run simultaneously (Table 1). With a surfactant concentration of approximately 0.1% (w/v) increases greater than 17% result with all surfactants tested with the exception of Triton X-405. This latter surfactant does not encourage spreading of the droplets on the pinnule surface at the concentrations used and resulted in a slight but significant reduction of 3.3% in the amount of chemical absorbed. It is noteworthy that in this particular case the penetration of amitrole from the aqueous control is 47.5% compared with the other examples tested which range between 0.0 and 23.8%. Although some variation in penetration takes place there is no significant difference in the values obtained at different concentrations of individual surfactants.

A point of interest revealed by this investigation is the wide variation which takes place in penetration of amitrole from aqueous solution (0.0 - 47.5%). The values obtained underline the influence of the environmental conditions at the time of spraying (see Ch.1, A-2-3). In general the

most efficient penetration occurs when heavy rain precedes the application of the chemical while poor results are recorded under dry conditions. An 8% difference in moisture content is noted between fronds taken at the time of treatment of the above two samples.

Table 1 Influence of surfactants on penetration of amitrole. Application 40 µg active ingredient to each of four pairs of pinnules.

Surfactant % (w/v)	Amount µg left on pinnule surface				Mean	Standard deviation ±	Penetration %
(a) Tween 20							
0.00	31.5	31.5	31.5	31.5	31.5	0.0	23.8
0.10	21.0	23.6	26.2	23.6	23.6	2.6	40.9
0.20	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.30	26.3	21.0	26.3	26.3	25.0	2.3	37.5
0.10 + 0.5% NH_4SCN	26.3	21.0	21.0	26.3	23.7	2.7	40.8
0.20 + 0.5% NH_4SCN	21.0	26.3	26.3	21.0	23.7	2.7	40.8
0.30 + 0.5% NH_4SCN	21.0	26.3	26.3	26.3	25.0	2.3	37.5
(b) Triton X-114							
0.00	31.5	36.8	36.8	36.8	35.5	2.3	11.3
0.03	26.3	26.3	26.3	26.3	26.3	0.0	34.3
0.06	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.10	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.03 + 0.5% NH_4SCN	26.3	26.3	26.3	26.3	26.3	0.0	34.4
0.06 + 0.5% NH_4SCN	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.10 + 0.5% NH_4SCN	26.3	21.0	21.0	26.3	23.7	2.7	40.8
0.00 (leaf surface scratched)	10.5	10.5	10.5	10.5	10.5	0.0	73.8
(c) Triton X-100							
0.00	40.0	40.0	40.0	40.0	40.0	0.0	0.0
0.011	31.3	31.3	31.3	31.3	31.3	0.0	21.9

Table 1 continued

Surfactant % (W/v)	Amount µg left on pinnule surface				Mean	Standard deviation ±	Penetration %
0.11	26.3	26.3	26.3	26.3	26.3	0.0	34.4
0.22	26.3	26.3	26.3	26.3	26.3	0.0	34.4
0.11 + 25% glycerol	36.8	36.8	36.8	36.8	36.8	0.0	8.0
25% glycerol	40.0	40.0	40.0	40.0	40.0	0.0	0.0
(d) Triton GR-5							
0.00	31.5	31.5	36.8	36.8	34.2	2.7	14.5
0.02	21.0	26.3	26.3	26.3	25.0	2.3	37.5
0.06	26.3	26.3	21.0	26.3	25.0	2.3	37.5
0.08	26.3	26.3	26.3	26.3	26.3	0.0	34.3
0.10	21.0	26.3	26.3	26.3	25.0	2.3	37.5
(e) Tergitol NPX							
0.00	31.5	31.5	36.8	36.8	34.2	2.6	14.5
0.02	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.06	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.08	26.3	26.3	26.3	26.3	26.3	0.0	34.3
0.10	21.0	26.3	26.3	26.3	25.0	2.3	37.5
(f) Triton X-405							
0.00	21.0	21.0	21.0	21.0	21.0	0.0	47.5
0.02	26.3	26.3	26.3	21.0	25.0	2.3	37.5
0.04	21.0	21.0	26.5	26.5	23.8	2.8	40.9
0.08	26.3	26.3	26.3	21.0	25.0	2.3	37.5
0.10	26.3	21.0	21.0	21.0	22.3	2.3	44.2
0.10 + 25% glycerol	40.0	40.0	40.0	40.0	40.0	0.0	0.0
25% glycerol	36.8	36.8	36.8	36.8	36.8	0.0	8.1

The rate determining role of the pinnule surface for the penetration of amitrole can best be illustrated by noting the six-fold increase which results (Table 1) when the upper surface of the pinnule is slightly abraded compared with control samples. Similar results have been recorded

for propham.[33]

These findings, coupled with the fact that amitrole is taken up by the leaf via an aqueous route,[123] indicate that a water continuum is essential for efficient penetration of the leaf surface barrier. Presumably surfactants help to increase penetration by establishing or maintaining this water continuum, and bring about increased contact between chemical and leaf surface and thereby encourage penetration by diffusion[328] and/or through preferential absorption sites or fissures on the leaf surface[177] (see Ch.1, 3-1-2-2 and A this Ch.).

C-3-2- Effect of amitrole concentration and rewetting on penetration:-

The percentage penetration is unaffected by amitrole concentration (Table 2) (similar findings were observed by Cook[117] in this laboratory). This would suggest that more chemical should be absorbed by the foliage as the aqueous droplets dry out on the pinnule surface. However, it would appear that the droplets dry out too quickly for this effect to be noted in practice.

Table 2 Influence of amitrole concentration on penetration.

Concentration added (µg)	Amount (µg) left on pinnule surface				Mean	Standard deviation ±	Penetration %
80.0	63.0	68.3	67.8	68.3	66.9	2.2	16.4
160.0	147.0	126.0	126.0	141.8	135.2	9.4	15.5
240.0	199.5	204.8	204.8	189.0	199.5	6.5	16.9
288.0	236.23	241.5	241.5	241.5	240.0	2.3	16.7
80.0+0.5% NH_4SCN	47.3	47.3	52.3	52.3	49.8	2.5	37.8
160.0+0.5% NH_4SCN	105.0	110.3	110.3	110.3	109.0	2.3	31.9

Rewetting of the dried droplets on a dry day brings about an increase in penetration but only the first rewetting is significant (Table 3). This behaviour can be accounted for in terms of lack of contact with the

water continuum of the plant.[324] Similar observations have been reported by Sharma et al.[423]

Table 3 Effect of repeated rewetting on penetration.

Treatment	Amount (µg) left on pinnule surface						Mean	Standard deviation ±	Penetration %
No re-wetting	39.4	39.4	36.8	39.4	36.7	39.4	38.5	1.2	3.8
One re-wetting	36.8	36.8	34.1	31.5	31.5	34.1	34.1	2.2	14.7
Two re-wettings	29.4	34.7	29.9	29.9	34.7	34.7	32.2	2.5	19.5
Three re-wettings	31.5	27.3	31.5	31.5	29.9	29.9	30.3	1.5	24.3

C-3-3- Influence of humectants on penetration:-

The incorporation of humectants into the amitrole solution in an attempt to slow down the rate of drying reveals that both glycerol[259] and sorbitol[439] either alone or in the presence of other additives cause significant retardation compared to aqueous samples (Table 1 and 4). Carbowax[399] on the other hand gives similar results to the aqueous control (Table 4).

The retarding or the negligible effects noted with these humectants and the poor response may indicate that the limiting factor to penetration concerns the leaf surface and the plant water continuum. However, the possibility that the effects of humectants may be influenced by the environment[24] (see D-3-4) and/or the humectants may have a tendency to hold back the herbicide and so limit its diffusion into the plant tissue,[233,234] cannot be ruled out (see Ch.1, 4-3-2-2 and D-3-4- this Ch.).

C-3-4- Influence of ammonium thiocyanate on penetration:-

Ammonium thiocyanate (NH_4SCN) has been used in amitrole formulations to increase its effectiveness and has been reported to influence absorption

Table 4 Influence of additives other than surfactants on amitrole penetration.

Treatment	Amount (µg) left on pinnule surface				Mean	Standard deviation ±	Penetration %
(a)							
water	26.3	31.5	31.5	31.5	30.2	2.3	24.5
1% sorbitol	36.8	36.8	36.8	36.8	36.8	0.0	8.1
1% sorbitol + 0.5% NH_4SCN	36.8	29.9	29.9	36.8	33.4	3.5	16.5
1% sucrose	26.3	21.0	26.3	29.9	25.9	3.2	35.4
0.1% NH_4NO_3	26.3	26.3	26.3	26.3	26.3	0.0	34.4
0.1% phosphoric acid	29.9	31.5	36.8	36.8	33.8	3.1	15.5
0.1% phosphoric acid + 0.1% NH_4NO_3	26.6	31.5	36.8	36.8	32.9	4.2	17.8
(b)							
water 1.5h after application	36.8	39.4	36.8	40.0	38.3	1.5	4.3
water 3.0h after application	34.1	34.1	34.1	34.1	34.1	0.0	14.8
1% $(NH_4)_2SO_4$ 1.5h after application	36.8	40.0	39.4	34.1	37.8	2.3	5.5
" $(NH_4)_2SO_4$ 3.0h after application	34.1	31.5	36.8	34.1	34.1	1.8	14.8
2% glycerol 1.5h after application	40.0	40.0	39.4	40.0	39.9	0.3	0.3
2% glycerol 3h after application	40.0	40.0	40.0	40.0	40.0	0.0	0.0

continued

Table 4 continued.

Treatment		Amount (μg) left on pinnule surface				Mean	Standard deviation ±	Penetration %
(c)								
water alone		34.1	34.1	31.5	31.5	33.2	1.2	17.0
0.15% NH_4SCN		29.9	29.9	26.3	26.3	28.1	1.8	29.8
0.75% NH_4SCN		23.6	23.6	23.6	26.3	24.3	1.2	39.3
(d)								
water	30 min after application	40.0	40.0	40.0	-	40.0	0.0	0.0
water	1.5h after application	34.1	34.1	39.4	-	35.9	2.5	10.3
water	3.75h after application	34.1	34.1	39.4	-	35.9	2.5	10.3
water	6.0h after application	31.5	31.5	34.1	-	32.4	1.2	19.0
0.45% NH_4SCN	30 min after application	39.4	39.4	39.4	-	39.4	0.0	1.5
" NH_4SCN	1.5h after application	34.1	34.1	28.9	-	32.4	2.5	19.0
" NH_4SCN	3.75h after application	31.5	31.5	28.9	-	30.6	1.2	23.5
" NH_4SCN	6.0h after application	28.9	26.3	26.3	-	27.2	1.2	32.0
(e)								
water		34.0	30.0	34.0	34.0	33.0	1.7	17.5
0.5% carbowax		34.0	34.0	31.0	37.0	34.0	2.1	15.0

and/or translocation as well as the metabolism of amitrole.[16] However, Ford[174] has noted an adverse effect of NH_4SCN on the penetration of amitrole and van der Zweep[516] found that it reduced the necrotic foliar toxicity of amitrole. Whenever NH_4SCN is included in the spray solution an enhancement of penetration results compared with aqueous controls. With sorbitol the amount of penetration (16.5%) is greater than from sorbitol-water solution (8.1%) but less than from the water control (24.5%) (Table 4). This latter effect could be due to the retarding action of sorbitol. With surfactants, penetration is similar to the results obtained with surfactants alone (Table 1) possibly due to a levelling effect of the surfactants and/or the spreading brought about by the surfactants might result in lowering the concentration of NH_4SCN on the pinnule surface per unit area and so reduces its effect (see D-3-6).

The enhancement of penetration of amitrole by NH_4SCN is significant and is not due to a saving action as suggested by Crafts[123] but to an effect on the pinnule surface. NH_4SCN has defoliant properties[21] and the use of some defoliants to increase herbicidal penetration through their effects on the leaf surface is not uncommon.[462] However, the synergistic effect of NH_4SCN could be due to physiological interaction with amitrole within the plant. NH_4SCN itself has herbicidal properties.[21] A point of interest is that NH_4SCN will not, other conditions being favourable, slow down penetration and increase leaching from the pinnules by rain. However, the effect of NH_4SCN on amitrole translocation in bracken needs to be studied (see Ch.1, 6-2, Ch.5 and C-3-5 this Ch.).

C-3-5- Influence of ammonium sulphate, ammonium phosphate and phosphoric acid on penetration:-

Ammonium sulphate $(NH_4)_2SO_4$ is a common activator,[123] the activity being due to its buffering capacity.[123] However ammonium salts in general have been shown to exhibit a synergistic interaction with herbicides due to other factors beside buffering capacity.[463,507]

$(NH_4)_2SO_4$ when tested here, exhibits no effect (Table 4) while NH_4NO_3 enhances penetration. On the other hand phosphoric acid and phosphoric acid-NH_4NO_3 both have detrimental effects. These latter effects could be attributed to rapid leaf injury[66] (see D-3-6).

C-3-6- Influence of sucrose on penetration :-

Although the differences recorded are variable and not significant the addition of sucrose does enhance the penetration of amitrole (Table 4). This finding is of some practical significance as the translocation of herbicides can be encouraged by sugars particularly when the receiving sites are not themselves actively assimilating[66,403] (see Ch.1, 6-3 ii).

From this study with bracken, it can be deduced that the pinnule surface is the main barrier to penetration and that the properties of this barrier are very much influenced by the environmental conditions (see Ch.1, 4 and A this Ch.). For adequate performance high humidity and the maintenance of an efficient water continuum in the frond would appear to be essential requirements.

D- Penetration of bean leaves by asulam as influenced by adjuvants and humidity:-

D-1- Introduction :-

Previous investigations (see B and C this Ch.) deal with penetration of bracken fronds by asulam and amitrole under field conditions. This investigation set out to assess the effect of different surfactants and other additives and their interaction with humidity on penetration by asulam of bean (Vicia faba var. Maris Bead) foliage under growth room conditions. Variation in temperature (15 - 25°C) was taken into account by incorporating suitable blanks. Bracken was not used in this study due to its sensitivity to the environment[57,270] (see A and C this Ch.). The bean plant was selected for quick growth and ease of handling.

D-2- Experimental :-

Asulam, Tween 20, Triton X-405, Triton GR-5, Triton X-100 and Tergitol NPX were as described before (see B and C this Ch.). Triton X-67 (polyoxyethylene ether of fatty alcohol), Triton X-114 [octylphenoxy (polyethoxy) ethanol], Triton X-15 [octylphenoxy (polyethoxy) ethanol] were obtained from Sigma London Chemical Co. Ltd. Teepol 610 (sodium salt of a sec-alkyl sulphate) 34% a.i. was obtained from B.D.H. Ltd.

All penetration experiments were performed in a growth room adjusted to a 16h day length. The plants were germinated in trays on vermiculite and seedlings were selected on the basis of size and transplanted into pots and grown on the same growth medium. The plants were treated at the three-leaf stage (2-3 weeks old). The chemical was placed on the second leaf pair in a total volume of 0.02 ml/leaf using an Eppendorf pipette. The solution was applied as discrete droplets randomly on the upper surface of the leaf. The treated plants were either left exposed in the growth chamber 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). The enclosure of plants in polythene bags would 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.[424] The relative humidity under bags was expected to approach 100%.[103]

For each treatment, a minimum of six and a maximum of ten leaves were treated. The treated leaves were then washed with 10 ml deionized water. Preliminary experiments confirmed that 100% recovery of asulam was possible by this treatment provided it was performed immediately after application. Asulam concentration was determined as previously described (see B-2).

D-3- Results and Discussion :-

D-3-1- Influence of surfactants on penetration:-

On the basis of the observed behaviour of water droplets transferred

by pipette to a plant leaf surface[436] it is noted that the leaf surface of the bean plant, although not water repellent, does not show a high affinity for water as judged by the non-spreading of the water droplets. Moreover, none of the surfactants used brings about spreading which is in marked contrast to the results obtained with bracken fronds where spreading is encouraged even at low (< 0.02%) surfactant concentrations[26] (see C-3-1). Such differences can be explained in terms of differing affinities of leaf surfaces for surfactants.[189]

The inability of surfactants to enhance spreading in the case of bean could help to account for the slow rate of penetration obtained compared with bracken[27,64,177] (see A).

In spite of the non-spreading action of surfactants used here with beans a differential response occurs (Table 1). Some surfactants viz Tween 20, Triton GR-5, Tergitol NPX and Triton X-67 bring about a significant increase compared with the aqueous controls (46.6, 42.6, 36.3 and 25.1% respectively) others, viz. Triton X-114, Triton X-15 and Teepol, produce a non-significant increase. A non-significant reduction with Triton X-100 and a significant reduction (22.4%) in the case of Triton X-405 are also noted.

Of the surfactants which bring about a significant increase in penetration, leaf injury occurs with Triton GR-5 and Tergitol NPX. Triton X-67 and Tween 20 on the other hand cause no visible injury to the leaf.

Such variations when observed with other herbicides,[183] have been attributed to a specific interaction between the surfactant, the herbicide and the plant leaf surface.[183,283]

The enhancement of asulam uptake noted here with Tween 20 can be partly attributed to the possibility of Tween 20 acting as a cosolvent for asulam, thereby preventing molecular asscciation and aggregation. These effects are known to occur with both carbamates and sulphonamides.[36,99] A

Table 1 Influence of surfactants (0.2% $^{w}/_{v}$) on asulam penetration with plants held at high humidity level. Application rate 22.3 µg asulam; assessed 20h.

Surfactant	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
Without surfactant	9.3	12.8	8.5	13.5	13.5	11.9	11.6	2.0	48.0
Tween 20	1.4	0.7	1.4	1.4	0.7	1.4	1.2	0.3	94.6
Triton X-405	15.7	16.4	17.1	19.2	15.0	16.4	16.6	1.3	25.6
Triton X-100	15.0	12.1	14.2	16.4	15.7	11.9	14.2	1.7	36.3
Triton GR-5	1.4	1.4	4.3	1.4	1.4	2.9	2.1	1.1	90.6
Triton X-114	10.0	7.1	8.5	7.1	11.4	8.8	8.8	1.5	60.5
Without surfactant[a]	8.5	10.7	7.1	7.8	10.7	12.8	9.6	2.0	57.0
Triton X-100[a]	11.4	13.5	11.4	11.4	11.4	10.7	11.6	0.9	48.0
Triton X-67	2.9	5.0	3.7	1.4	5.0	5.7	4.0	1.5	82.1
Triton X-15	11.4	8.5	8.5	7.1	5.7	7.8	8.2	1.7	63.2
Tergitol NPX	0.7	2.9	1.4	0.7	1.4	2.1	1.5	0.8	93.3
Teepol	3.7	6.4	7.1	5.7	9.3	6.4	6.4	1.7	71.3

[a] Treatment repeated

finer dispersion of product will thus be possible[353] and, as Tween 20 is non-volatile, recrystallisation of asulam on the leaf surface will be prevented.[266] Such behaviour is said to account for the increased potency of sulfamerazine sprays[266,353] (see B-1).

D-3-2- Influence of humidity on penetration:-

When the penetration of asulam is assessed with and without the addition of Tween 20 at both high and low humidity levels uptake is always greater at higher humidity. With no surfactant, differences between the two humidity levels at 29 and 48h after treatment of 33.7 and 48.7% respectively are recorded (Table 2). In the presence of Tween 20 the comparable figures are 72.5 and 69.4%. These figures demonstrate that humidity has a profound effect upon the absorption of asulam.

Table 2 Influence of humidity and Tween 20 (0.2% $^{w}/_{v}$) on asulam penetration Application rate, 22.2 µg asulam.

Time (h)	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
(a) Without Tween 20 plants held at low humidity level (LHL).									
29	18.7	20.4	20.4	20.4	20.4	22.2	20.4	1.0	8.1
48	19.5	20.4	19.5	18.7	17.8	20.4	19.4	0.9	12.6
(b) Without Tween 20 plants held at high humidity level (HHL).									
29	18.8	11.5	13.3	8.9	10.7	14.2	12.9	3.1	41.8
48	12.4	8.9	5.3	6.2	9.8	8.9	8.6	2.3	61.3
(c) With Tween 20 plants held at low humidity level (LHL).									
29	15.1	20.4	17.6	18.7	17.8	17.8	17.9	1.6	19.4
48	17.8	17.8	17.8	11.5	15.1	12.4	15.4	2.6	30.6
(d) With Tween 20 plants held at high humidity level (HHL).									
29	5.3	5.3	1.8	1.8	1.8	1.8	3.0	1.6	91.9
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

The enhancement by high humidity may, as reported for other herbicides,[73,375,392] be due to the hydration of the cuticle, which will

modify the permeability of this barrier to penetration and/or to modification of internal factors related to the water continuum of the plant[26,63,392] (see Ch.1, 3-1-2-2).[74] Plants are known to absorb water from the atmosphere by negative transpiration which may modify the overall process of penetration.[392]

The augmenting by Tween 20 of the humidity effect could, in addition to its possible cosolvent role, be due to its hygroscopic nature, which will encourage condensation to take place on the leaf surface at the site of deposition of the spray droplets, thereby maintaining a continuous supply of asulam[103,127] and establishing contact with the water continuum.[26,324] This will facilitate uptake by encouraging diffusion[324] and/or by reducing surface energy due to the presence of the surfactant.[283] Equally relevant is the possibility particularly under high humidity conditions, of penetration of the surfactant itself.[353] Two obvious advantages could result : a) the bulky surfactant is claimed to encourage the swelling of the cuticle and thereby increase the area of the hydrophilic channels,[283,284] and b) as Tween 20 is a cosolvent its distribution may influence the uptake of asulam in that penetration is a function of the equilibrium distribution ratio of the toxicant between the carrier and the leaf surface.[16,233] This latter point could help to explain the observations that penetration of certain herbicides decreases or remains unaffected with rising surfactant concentration after an initial increase.[283,284,411] Work with Phaseolus vulgaris (Table 3) shows that asulam uptake increases with increasing Tween 20 concentration (0.0 - 0.3%) and then falls again.

D-3-3- Influence of asulam concentration on penetration:-

In the absence of surfactant the concentration of asulam has no effect on penetration under high humidity conditions (see C-3-2). With Tween 20 present (penetration was always greater than with the corresponding treatment without surfactant) 100% penetration occurs 48h after application

Table 3[a] Influence of Tween 20 concentration on asulam penetration with plants held at high humidity level. Application rate 22.0 µg asulam, assessed after 21h.

Tween 20 concentration (% w/v)	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
0.00	18.0	16.2	18.0	16.5	18.7	18.7	17.7	1.0	19.6
0.10	16.5	16.2	14.1	13.4	12.7	15.1	14.6	1.4	33.6
0.20	11.3	7.4	8.5	6.7	10.6	10.6	8.9	1.8	59.6
0.30	2.8	8.1	6.7	7.7	7.7	3.9	6.2	2.0	71.8
0.50	3.5	3.5	8.5	8.5	7.7	10.2	7.0	2.6	68.2
1.00	9.5	9.4	11.3	12.7	13.4	13.4	11.6	1.7	47.3
3.00	18.0	14.8	16.2	16.2	7.4	7.4	13.3	4.3	39.6
5.00	14.8	14.8	10.2	10.2	10.2	14.1	12.4	2.2	43.6

[a] Phaseolus vulgaris has been used in this experiment.

for the lower concentration of asulam with a slight decrease at higher concentrations (Table 4). These observations suggest that a) the effect of Tween 20 on penetration will persist for some time b) diurnal changes in humidity will affect asulam penetration c) the incidence of high humidity which would be expected to predominate during the night and early morning[92] will be more pronounced in the presence of Tween 20. Improvement in performance of certain herbicides e.g. picloram and 2,4,5-T on Acacia farnesiana and Rosa bracteata following evening application has been reported,[63] and is attributed to favourable changes in humidity and the water status of the plant.[63]

D-3-4- Influence of glycerol on penetration:-

The use of glycerol with the spray solution containing Tween 20 results in a significant enhancement of asulam at high humidity but only a slight increase at low humidity compared to the control samples containing Tween 20 (Table 5). The differences in penetration between the two humidity levels in the presence of 0.5 and 1.0% glycerol are 55.2 and 64.7% respectively. The corresponding increases over the surfactant treatment are 9.2 and 13.1% at low humidity and 35.2 and 48.6% at high humidity respectively.

These figures suggest that the action of glycerol and perhaps humectants in general is dependent on humidity and good contact with the plant surface made possible by the incorporated surfactant.

It would thus appear that the conflicting behaviour reported for sprays containing glycerol in the literature[26,210,211,505] could feasibly be accounted for in terms of variations in humidity and that glycerol action cannot be considered to be solely restricted to the slowing down of spray drying. Other roles including an effect on leaf permeability[128,245] have been suggested. Some of the adverse effects of glycerol at low humidity have been attributed to dehydration of the cuticle[128] and to its remaining on the leaf surface, thus retarding herbicidal penetration (see D-3-2). The

Table 4 Influence of asulam concentration on penetration plants held at the high humidity level.

Asulam concentration (µg a.i.)	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
(a) Without Tween 20 assessed after 40h.									
44.5	21.4	19.9	19.2	17.1	21.4	21.4	20.1	1.6	54.8
26.7	12.8	10.0	13.5	16.4	14.2	19.2	14.4	2.9	46.1
8.9	4.3	2.9	2.1	1.4	5.7	7.1	3.9	2.0	56.2
(b) With Tween 20 assessed after 40h.									
44.5	3.6	4.3	2.9	5.0	5.0	7.1	4.7	1.3	88.4
26.7	2.9	4.3	2.1	1.4	5.7	7.1	3.9	2.0	85.4
8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

Table 5 Influence of glycerol on asulam penetration in the presence of Tween 20 (0.2% $^{w}/_{v}$) and at different humidity levels. Application rate, 23.0 µg assessed after 15h.

Treatment	Amount (µg) left on leaf surface								Mean	Standard deviation ±	Penetration %
Glycerol (0.5% $^{w}/_{v}$) (LHL)	16.9	16.9	16.9	16.9	17.3	17.3	18.4	18.8	17.4	0.7	24.4
Glycerol (0.5% $^{w}/_{v}$) (HHL)	5.9	5.5	5.9	4.1	7.7	4.8	2.2	1.8	4.7	1.9	79.6
Glycerol (1.0% $^{w}/_{v}$) (LHL)	15.1	15.1	15.8	17.7	18.8	14.0	15.8	19.9	16.5	1.9	28.3
Glycerol (1.0% $^{w}/_{v}$) (HHL)	2.2	2.2	2.2	1.1	1.1	1.1	1.1	1.8	1.6	0.5	93.0
Without glycerol (LHL)	18.8	19.9	19.9	18.8	18.8	19.1	21.0	19.9	19.5	0.7	15.2
Without glycerol (HHL)	11.0	14.0	11.0	14.0	14.0	13.3	13.3	11.8	12.8	1.2	44.4

fact that visual inspection of the leaf surface reveals that glycerol remains only at low humidity and disappears at high humidity tends to support this belief.

D-3-5- Influence of urea on penetration:-

The addition of urea increases the penetration of asulam. At high humidity level increases of 45.4 and 5.6 are brought about by 1.0% additions of urea compared with aqueous and surfactant treated samples respectively. The difference in the latter case is not significant [Table 6 (a)].

The incorporation of urea into the spray solution containing Tween 20 brings about very efficient penetration under high humidity conditions, viz. 82.9% compared with 56.2 for aqueous urea and 50.8% for Tween 20 at a concentration of 0.2% [Table 6 (a)].

The same overall pattern prevails at low humidity. Differences in uptake between the two humidity levels, from urea solutions, with and without Tween 20, of 67.0 and 26.0% respectively are recorded [Table 6 (b)]. Urea concentrations ranging from 0.05 to 0.3% in 0.2% Tween 20 are similar to surfactant controls at high humidity. However higher concentrations of urea (0.60 - 2.00%) in presence of Tween 20 bring about significant increases compared with surfactant controls (Table 7).

Of particular note is the influence of time on the penetration of asulam from urea solution including Tween 20 (Fig. 1). There is a rapid initial uptake followed by a levelling off after 12h. Uptake after 6h is comparable in amount to that of the surfactant blank run for 30h. In the presence of urea, penetration has increased by a further 25.7% 12h after treatment. This pattern of uptake for asulam in the presence of urea parallels that reported for urea itself.

It would thus appear that conditions which favour urea uptake, viz. incorporation of Tween 20[64] and high humidity,[479] will also favour asulam uptake from urea solution. Urea is said to accelerate the uptake of other

Table 6 Influence of urea (1% $^{w}/_{v}$), Tween (0.2% $^{w}/_{v}$) and humidity on asulam penetration.

Treatment	Amount (µg) left on leaf surface										Mean	Standard deviation ±	Penetration %
a) Urea (1% $^{w}/_{v}$) and Tween 20 (0.2% $^{w}/_{v}$) with plants held at high humidity level. Application rate 44.5 µg asulam assessed after 16h.													
Aqueous control	42.0	37.2	37.7	38.5	42.6	39.9	-	-	-	-	39.7	2.1	10.8
With Tween 20	20.7	26.4	22.1	22.8	19.2	19.9	-	-	-	-	21.9	2.4	50.8
With urea	19.9	22.8	19.9	17.1	18.5	18.5	-	-	-	-	19.5	1.8	56.2
With urea and Tween 20	10.0	12.8	7.1	8.5	2.9	4.3	-	-	-	-	7.6	3.3	82.9
b) Humidity and Tween 20 (0.2% $^{w}/_{v}$) in the presence of urea (1% $^{w}/_{v}$). Application rate 22.7 µg asulam; assessed after 16h.													
With Tween 20 (LHL)	20.3	20.3	20.3	22.5	22.5	22.5	21.8	21.8	13.1	21.4	20.7	2.7	8.8
With Tween 20 (HHL)	5.1	5.1	5.8	4.0	4.0	4.0	6.5	6.5	8.0	5.8	5.5	1.2	75.8
Without Tween 20 (LHL)	23.2	23.2	20.3	19.6	22.5	21.1	21.1	22.7	22.7	22.7	21.9	1.2	3.5
Without Tween 20 (HHL)	16.0	18.2	18.2	13.1	11.6	18.2	16.0	16.7	16.0	16.0	16.0	2.1	29.5

Table 7 Influence of urea concentration on asulam penetration in the presence of Tween 20 (0.2% $^{w}/_{v}$) with plants held at high humidity level.

Urea concentration (% $^{w}/_{v}$)	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
a) Application rate 22.3 µg asulam, assessed after 18h.									
0.00 without Tween 20	15.7	14.3	15.0	16.4	14.3	15.7	15.2	0.8	31.8
0.00 with Tween 20	12.1	11.4	10.7	12.1	10.0	10.7	11.2	0.8	49.8
0.05 with Tween 20	12.8	13.6	9.3	12.1	10.7	10.0	11.4	1.5	48.9
0.08 with Tween 20	7.1	13.2	8.7	13.2	12.8	9.3	10.7	2.4	52.0
0.15 with Tween 20	10.0	7.9	7.1	7.9	10.0	11.4	9.1	1.5	59.2
0.30 with Tween 20	12.8	13.6	10.7	10.0	10.0	7.9	10.8	1.9	51.6
0.60 with Tween 20	2.1	2.1	6.1	6.1	3.6	3.6	4.0	1.7	82.1
0.75 with Tween 20	2.1	2.1	3.6	1.4	6.1	3.6	3.2	1.5	85.7
b) Application rate 44.6 µg asulam, assessed after 18h.									
0.00 without Tween 20[a]	35.4	36.0	34.1	35.4	35.4	41.2	36.3	2.3	18.6
0.00 with Tween 20[a]	28.3	24.5	24.5	22.5	28.3	28.3	26.1	2.3	41.5
0.80 with Tween 20	3.9	6.4	3.2	5.2	3.9	3.2	4.3	1.2	90.8
1.20 with Tween 20	3.2	1.3	1.3	0.6	5.2	3.2	2.5	1.6	94.4
1.60 with Tween 20	1.3	1.4	1.3	0.6	1.3	4.5	1.7	1.3	96.2
2.00 with Tween 20	1.9	4.5	0.6	1.3	1.3	1.9	1.9	1.2	95.7

[a] Treatment repeated.

chemicals by facilitated diffusion resulting from changes in the cuticular membranes.[510] For urea to exert such an effect it will have to penetrate, therefore conditions favouring urea penetration would be expected to enhance uptake. This could help to explain some of the controversial results which have been reported when urea additions have been made to herbicidal sprays.[319,510]

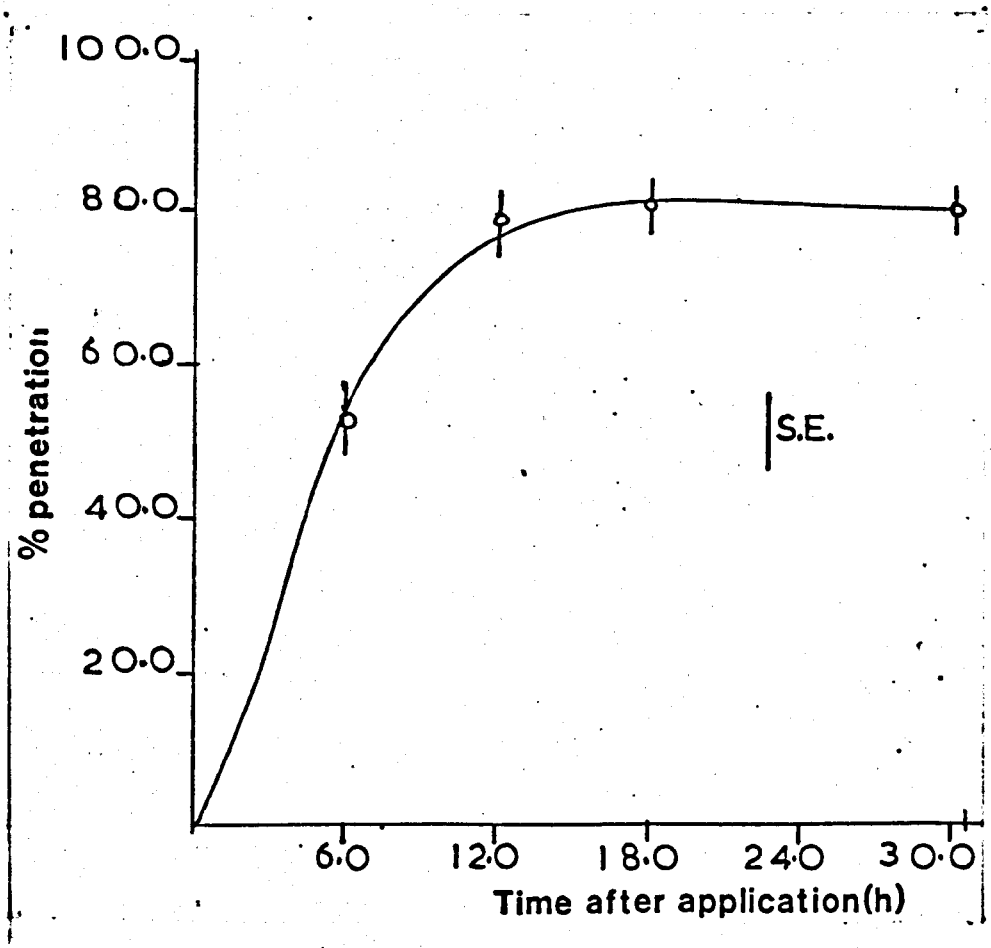


Fig. 1 Influence of time on asulam penetration in the presence of urea 1% (W/v) and Tween 20 (0.2% W/v) with plants held at high humidity level. Application rate, 44.0 µg asulam.

D-3-6- Influence of contact chemicals on asulam penetration:-

The incorporation of potassium ethyl xanthate in spray solutions with Tween 20 causes an initial increase in uptake of asulam under high humidity conditions followed by a decrease at higher xanthate concentrations. Penetration at the 1% level of potassium ethyl xanthate, is comparable to that

Table 8 Influence of potassium ethyl xanthate and tributyl phosphate on asulam penetration in the presence of Tween 20 (0.2% w/v) with plants held at high humidity level.

Concentration of reagent	Amount (µg) left on leaf surface						Mean	Standard deviation ±	Penetration %
a) Potassium ethyl xanthate; application rate 22.6 µg asulam, assessed after 20h.									
0.00	11.6	11.6	12.3	11.6	11.6	11.6	11.7	0.3	48.2
0.04	11.6	11.6	9.4	7.2	9.4	7.2	9.4	1.8	58.4
0.08	6.5	5.1	8.0	4.3	9.4	8.7	7.0	1.9	69.0
0.20[a]	1.5	3.6	4.3	5.1	1.5	1.5	2.9	1.5	87.2
0.40[a]	9.4	7.2	7.2	10.9	10.9	6.5	8.7	1.8	60.6
1.00[a]	10.1	10.9	12.3	10.9	6.5	5.8	9.4	2.4	58.4
b) Tributyl phosphate; Application rate, 22.0 µg asulam, assessed after 20h.									
0.00	10.6	10.6	9.2	8.4	8.4	7.0	9.0	1.3	59.1
0.10	7.0	7.0	9.2	5.6	9.9	10.6	8.2	1.8	62.7
0.15	12.7	7.0	11.3	7.0	5.6	4.9	8.1	2.9	63.2
0.20	7.0	7.0	11.3	9.2	11.3	11.3	9.5	1.9	56.8
0.30	9.9	10.6	7.0	7.7	6.3	7.7	8.2	1.5	62.7
0.50[a]	9.9	8.5	6.3	7.0	9.9	7.0	8.1	1.4	63.2
0.75[a]	2.8	4.2	0.7	0.4	4.2	2.1	2.4	1.5	89.1
0.80[a]	0.4	0.4	4.9	0.7	4.2	2.8	2.2	1.8	90.0
1.00[a]	0.7	2.1	0.7	0.7	0.7	2.8	1.3	0.8	94.1

[a] Visible leaf injury.

occurring with Tween 20 control (Table 8a). This reduced uptake could be attributed to rapid injury to the leaf brought about by potassium ethyl xanthate under high humidity conditions (leaf damage is readily observed within 30 min of application of the chemical at the 1% level in the presence of Tween 20 at HHL).

The damage depends on humidity and the presence of Tween 20. Under low humidity conditions a 4% solution of potassium ethyl xanthate without Tween 20 causes no damage and the salt crystallises on the leaf surface. On the other hand in the presence of Tween 20 serious damage to the leaf occurs under similar conditions. A potassium ethyl xanthate concentration of 1% in aqueous solution and 0.2% in the presence of Tween 20 cause comparable damage under high humidity conditions.

With tributyl phosphate in the presence of 0.2% Tween 20 at the high humidity level the effect on penetration is not consistent, less pronounced and not significant at lower concentrations (0.00 - 0.50%). However, at higher concentrations (0.75 - 1.00%) the effect on penetration is significant and leaf injury is visible. The leaf injury which occurs at higher concentrations (0.5 - 1.0%) is slow to develop and less severe than in the case of the potassium ethyl xanthate treatment (Table 8b).

The fact that the leaf surface is the main barrier hampering the penetration of many foliar applied chemicals is well recognised.[16,137,319] Slight damage by abraiding the leaf surface has been shown to increase penetration of many herbicides.[1,26,33] From a commercial point of view the use of contact chemicals would appear to offer more promise.[1] However, when this approach is adopted, difficulties are experienced in controlling the extent of leaf injury[462] particularly when the effect of the treatment is readily influenced by environmental conditions. The effect of the injury is particularly pertinent with systemic herbicides[462] as their activity depends on the physiological status of the leaf particularly if translocation follows the assimilates[462] (see Ch.1, 5).

Therefore chemicals which cause contact injury to the leaves may

not be practical (see C-3-4). The inclusion of other additives such as urea and glycerol may prove to be a more constructive approach when their roles in herbicide uptake are better understood.

To facilitate the selection of optimum conditions for the use of an additive with a herbicide on a field scale logarithmic screening is appropriate. This enables the extent of leaf damage and herbicidal behaviour to be observed prior to the selection of herbicide-adjuvant levels.

Finally variability between different groups of experiments was detected. Generally such variation is attributed to plant age.[210] However, it is felt in this investigation that temperature differences may have contributed to this variability.

E- Bracken control : a preliminary field trial.

E-1 Introduction:-

During this investigation it was found that Tween 20 enhanced asulam uptake by bracken (see B). Other workers[261] reported that Agral 90 improved bracken control when rain followed application.

A small non-replicated experiment was carried out with the objectives of assessing the effect of two surfactants, Tween 20 and sodium dodecyl sulphosuccinate (SDS) at 0.1% ($^{w}/_{v}$) on bracken control with asulam.

E-2- Experimental:-

Asulox and Tween 20 were as described before (see B-2). Sodium dodecyl sulphosuccinate was obtained from B.D.H. Ltd.

The plots (4.5m x 4.5m) were laid out at Carbeth, Stirlingshire on September 15th, 1973 and sprayed on the same day. Asulox (4.5 Kg a.i./ha) was applied at a volume rate of 115L/ha using a knapsack sprayer.

E-3- Results and Discussion:-

The short term (1 year) control achieved is more or less the same in all treatments (ranges between 81 and 87%) (Table 1). This supports the

surmise that penetration of asulam without the addition of surfactants will proceed satisfactorily provided no rain follows application (see B-3-2). Such was the case in this experiment where no rain was observed for at least 3 days after application.

Table 1 Results of field experiment : 4.5 Kg/ha asulam applied September 1973. Assessment August 1974 and September 1975.

Treatment	August 1974 % Reduction in fronds number	September 1975 % Reduction in fronds number
No surfactant	81	54
" "	80	35
0.1% ($^{w}/_{v}$) Tween 20	81	50
" "	87	69
0.1% SDS	80	50

However, the degree of control (short-term) is less than reported in the literature [336,444] and regeneration is considerably greater than reported [336,444] (Table 1). This can be due to late application and/or to some influence that is specific to this particular site. This matter is discussed in detail in Ch.5.

CHAPTER III

ASULAM-SOIL INTERACTIONS.

1- Introduction:-

There are two main avenues by which asulam can reach the soil:-

(a) Directly from pre-emergence sprays designed to control weed growth in crops.[8,34,77,78]

(b) Indirectly, as spray drift, rainwash and the decomposition of plant materials, from post-emergence treatments. Such problems are encountered with all agricultural chemicals applied to plants.[40,98,129,224] The situation will be aggravated when aerial application methods are adopted as is the case with bracken, particularly with aqueous sprays where spray drift and spray droplet shattering may come into play.[98,107,332,426] The slow foliar penetration which has been noted for asulam, makes the possibility of rainwash of asulam deposits from the leaf surface and hence soil contamination a distinct possibility, particularly in high rainfall areas (see Ch.2,B).

These two aspects (a and b above) make a study of asulam-soil interactions pertinent to a clearer understanding of the behaviour and fate of this chemical in the environment. An understanding of the behaviour and fate of pesticides (of which herbicides form a major segment) in the environment is a prerequisite to their intelligent and economic use in controlling target pests and minimising possible hazards and environmental pollution which could arise through their misuse.[130]

The studies which are described in this chapter deal mainly with asulam adsorption, movement and persistence in the soil. Though these aspects are interrelated they are treated separately mainly for convenience of presentation. However it should be borne in mind that the overall behaviour and fate of a herbicide in the soil is determined by the interactions of these factors with the total environment rather than with a single aspect or factor[160,161,370] (Ch.1).

1-2- Collection and description of soil samples:-

The use of asulam as a pre-emergence as well as a post-emergence herbicide[8,34,77,78] implies that in practice asulam will come in contact with an infinite number of soils of different composition and will be used under different climatic conditions and agricultural practices. Its success as a pre-emergence herbicide will be determined by its ability to function over a fairly wide range of conditions with adequate reliability. Its impact on the environment (from pre-emergence as well as post-emergence applications) will depend on the balance between the factors involved in its dissipation and their interactions with the environment.[26] Some of these factors e.g. soil-type, temperature, moisture and pH were taken into account in the studies described below.

The soils used in these studies were divided into two groups according to the nature of the vegetation on them.

1-2 a) Soils from under bracken:- The major use of asulam in the United Kingdom is for bracken control.[330,331] Soils under bracken may have a deep litter layer, and have a high organic matter content.[351] If the litter is burned they can contain carbon. The practice of burning is quite common in this area and in certain countries e.g. New Zealand.[330] The effect of organic matter on adsorption and phytotoxicity of herbicides is well documented in the literature.[144,145,213,231,247,250,276] Charcoal accumulating in soils as a result of burning of woody plants is reported to increase the adsorption capacity of soils for many herbicides,[241,249] and it has been used to protect sensitive crops.[7,121,241,312]

The soil samples employed here were collected from under bracken at three sites in West-Central Scotland. Emphasis was placed on the surface layer (A horizon) which is generally high in organic matter. However, one sample from a B horizon was also included for comparison. Only humified material was included, all undecomposed plant material being discarded. Brief descriptions of the soils used are as follows:

(1) A deep organic rich horizon of mull humus developed on a bank.

(2) A clearly defined B horizon from a freely drained acid brown earth developed on basalt till.

(3) A fairly deep organic rich mull. The A horizon from the same site as 2 above.

(4) A disturbed H horizon of a peaty podzol developed on coarse sand. The mor humus contained a high proportion of sand.

1-2 b) Soils from under grass:-The soil samples chosen represent major soil types prevailing in hill farming areas in West-Central Scotland (Darleith Association).[350] Though the soils in question are not covered by bracken at the time of collection, infestation by bracken of such soils is not uncommon.[285] Soil samples were taken from different depths down the selected profiles to facilitate the collection of samples of varying composition. Adsorption and disappearance of asulam in such soils (organic rich topsoils to mineral rich subsoils) should cast some light on asulam performance in different soils. The variation of asulam adsorption with soil depth should also be revealed. This is of practical interest, since the adsorption, availability, persistence and movement (upward or downward) of herbicides are known to be affected by their location with respect to soil depth[26, 160, 314] (see C-3). The subsequent interactions that a herbicide undergoes in subsoils are to some extent dictated by the surrounding environment which in itself is affected to varying degrees by the subsoil composition.[87, 318, 370, 419, 512] Soil descriptions are as follows:-

i) Darleith series :- soil developed on till derived from calcareous sandstone lavas. They are shallow, freely drained, brown forest soils. Soil samples were collected at regular intervals, (ca. 2 in. segments) down the profile to a depth of ca. 10 in., which is well below the B layer (soils 5 to 9).

ii) Dunlop series :- soil developed on base rich material, inherently fertile,

fine textured (well aggregated) imperfectly drained brown forest soil (soils 10 to 12).

Other adsorbents viz, humic acid, clays, cation exchange resin, charcoal, cellulose and ignited soils were used in attempts to elucidate the factors involved in asulam adsorption.

A: Adsorption of asulam, asulox, and sulphanilamide onto soils collected from under bracken and onto other adsorbents:-

A-1- Introduction:-

The nature of the formulation, the type of salts formed by the herbicide in the soil and the presence of breakdown products are known to affect herbicide adsorption onto soils.[29,45,194,226,303,430] Asulox, the commercial formulation of asulam (used for bracken control) contains 40% (w/v) asulam as the sodium salt. Preliminary analysis of asulox revealed the presence of traces of an anionic surface active agent tested for by the method of Longwell and Maniece[321] and sulphanilamide a breakdown product of asulam or a commercial impurity, which was demonstrated by thin layer chromatography.[168] The adsorption of asulox, asulam and sulphanilamide onto bracken soils and other adsorbents was investigated to assess the effect of these factors on adsorption.

A-2- Experimental:-

Asulam and asulox were as previously described (Ch.2, B). Sulphanilamide was purchased from May and Baker Ltd. and was recrystallized from hot water.[384] Zeo-carb (SRC 16) H^+ form was purchased from Permutit Co. Ltd. and was pretreated with M-HCl, washed with water till chloride free and dried at 30°C over P_2O_5 under reduced pressure. Norit A charcoal was dried similarly after several washes with hot water.

The soil samples were wet sieved through a 2mm BS. sieve and finally air-dried at 30°C in a force draught oven. Different methods are used by different workers for drying soils.[210,397] The method adopted here is in

common use[221] and was adopted as a standard treatment. C.E.C. pH, sand, silt and clay were estimated by standard soil analytical procedures.[280] The organic matter was estimated by the Walkley-Black method.[484] Soil analytical data is given in Table 1.

Table 1 Soils from under bracken, Analytical data.

Soil	pH	C.E.C.	% Sand	% Silt	% Clay	% Organic matter	% Loss on ignition
1	4.6	39.9	31.7	41.4	10.1	17.8	19.2
2	4.2	40.0	36.7	36.5	12.5	14.3	17.1
3	4.1	33.0	38.7	23.1	10.3	27.9	34.1
4	4.3	19.6	71.7	8.3	9.9	10.1	19.6

Adsorption, unless otherwise stated, was determined by shaking the following amounts of adsorbents - 2g soil, 0.1g charcoal and cation exchange resin with 10ml solutions of the appropriate chemical for 16h at 18°C on an end-over-end shaker and then filtered through Whatman No. 42 filter paper. In the case of pre-treated soil, periodic hand shaking was employed and the soil was treated with 5ml of buffer alone followed after 40 min with 5ml of 5.6µg/ml asulam solution or alternatively with 5ml of the asulam solution followed after 40 min with 5ml of buffer (see Ref.212)

The concentrations of chemical used were selected after preliminary analysis of each soil in turn (concentration range covered was 0.5 - 15.0µg/ml). Higher concentrations were employed with carbon and cation exchange resin due to their high adsorption capacities (range 147.0 - 300.0µg/ml for carbon and 30.0-60.0µg/ml for cation exchange resin).

A minimum of 5 concentrations were employed for each adsorbent. The treatments were carried out in triplicate with the inclusion of appropriate blanks to cater for the possibility of interference from aromatic compounds including aromatic amines. The filtrates were further checked by t.l.c.[168]

Asulam and sulphanilamide concentrations were determined by the Bratton-Marshall reaction (see Ch.2,B).

A-3- Results and Discussion:-

The adsorption behaviour of sulphanilamide, asulam and asulox onto soils and other adsorbents unless otherwise mentioned was compared by means of the empirical Freundlich adsorption isotherm which may be written as :-

$$\log \frac{x}{m} = \log K + \frac{1}{n} \log c \qquad \text{(see Ref.}^{224}\text{)}$$

where $\frac{x}{m}$ is the amount of chemical adsorbed per unit mass of adsorbent when in equilibrium with a solution of concentration c; K and n are constants.

Figures 1 to 5 show the logarithmic plots of $\frac{x}{m}$ against c for each of the compounds used. The figures illustrated here refer to soils 1 - 4, and cation exchange resin Fig. 5. The plot for asulam and cation exchange resin has been omitted as it is identical to that obtained with asulox.

The slopes $(\frac{1}{n})$ obtained when all four soils were tested with sulphanilamide, asulam and asulox vary, with two exceptions, between 0.8 and 1.0 (Table 2). Similar results have been reported for many herbicides.[224,512] Bryce[83] when studying the adsorption of disulfoton onto soil considered a range of $\frac{1}{n}$ values similar to those obtained here to approximate unity.

However, values of $\frac{1}{n}$ less than unity (0.8 - 0.9) can as proposed by Hance[224] be due to 1) an inherent inability of part of the soil to adsorb the chemicals and/or 2) water competition and the ability of part of the soil to adsorb water more strongly. To investigate this point in more detail one of the above soils (soil 1) was given a buffer pretreatment prior to the addition of a concentrated solution of asulam. When the results expressed as the distribution coefficient Kd,[82] where

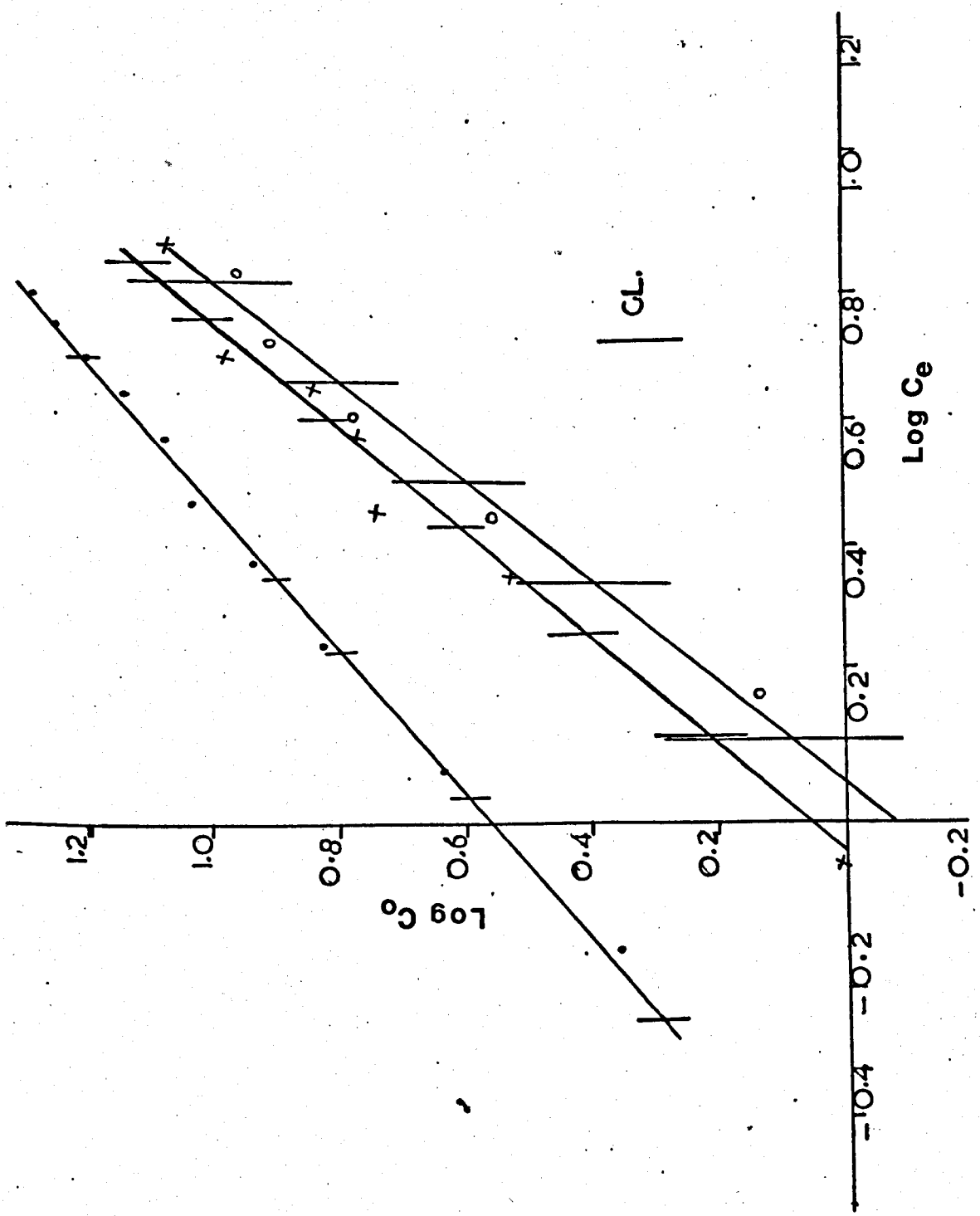


Fig. 1 Isothermal equilibrium adsorption of asulam (x) asulox (o) and sulphanilamide (•) onto soil no. 1.

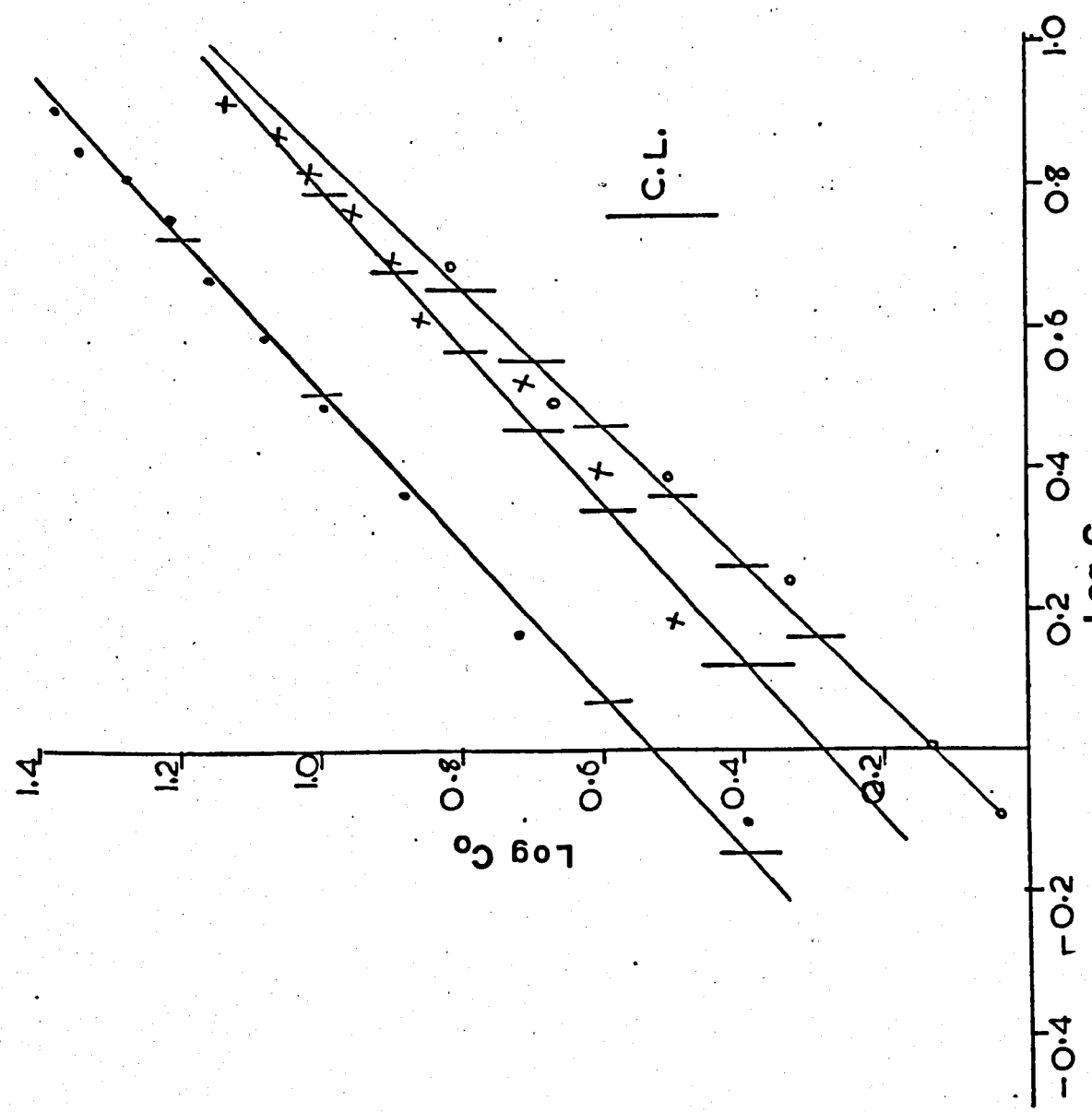


Fig. 2 Isothermal equilibrium adsorption of asulam (x) asulox (0) and sulphanilamide (•) onto soil no. 2.

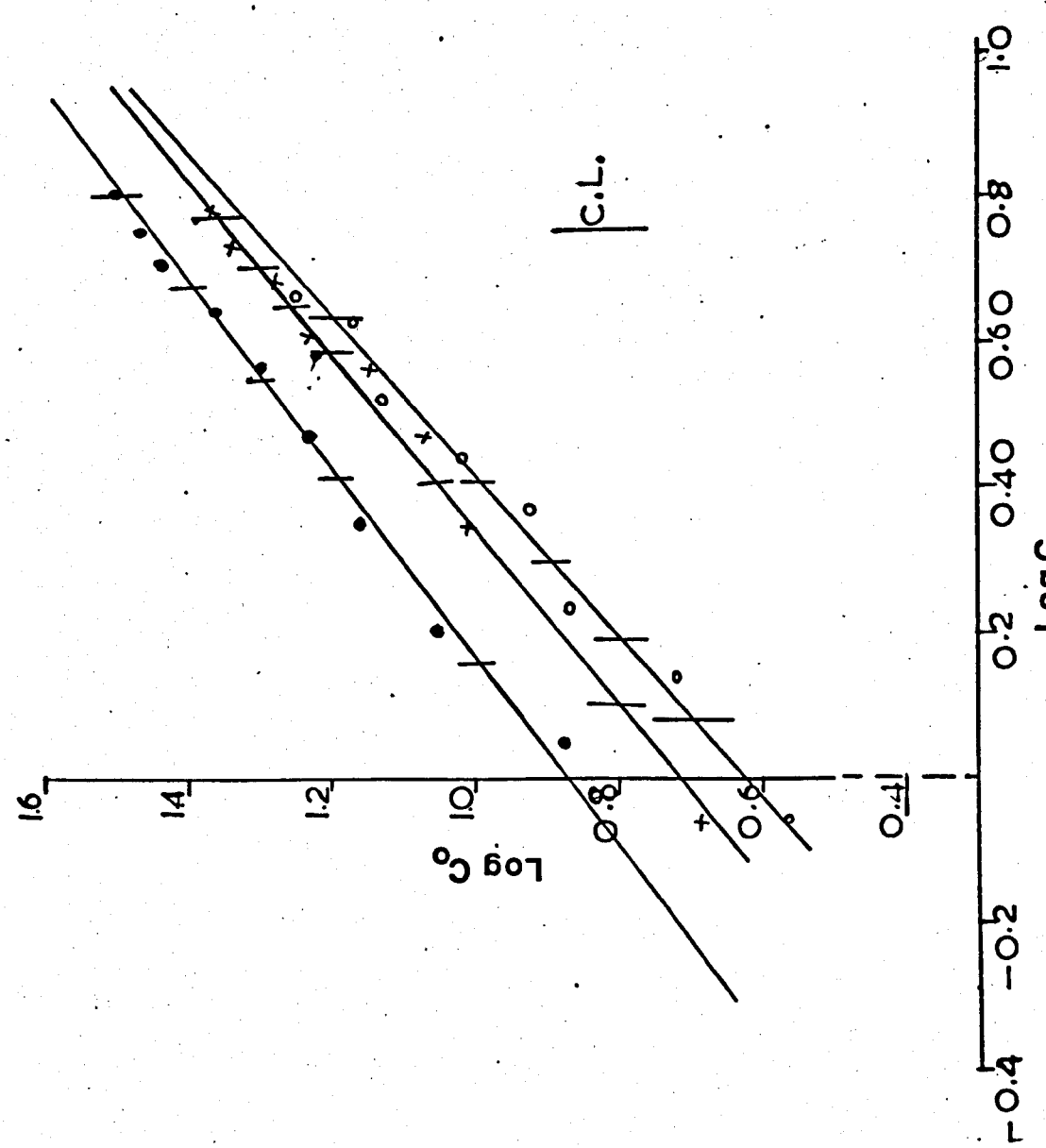


Fig. 3 Isothermal equilibrium adsorption of asulam (x) asulox (0) and sulphanilamide (●) onto soil no. 3.

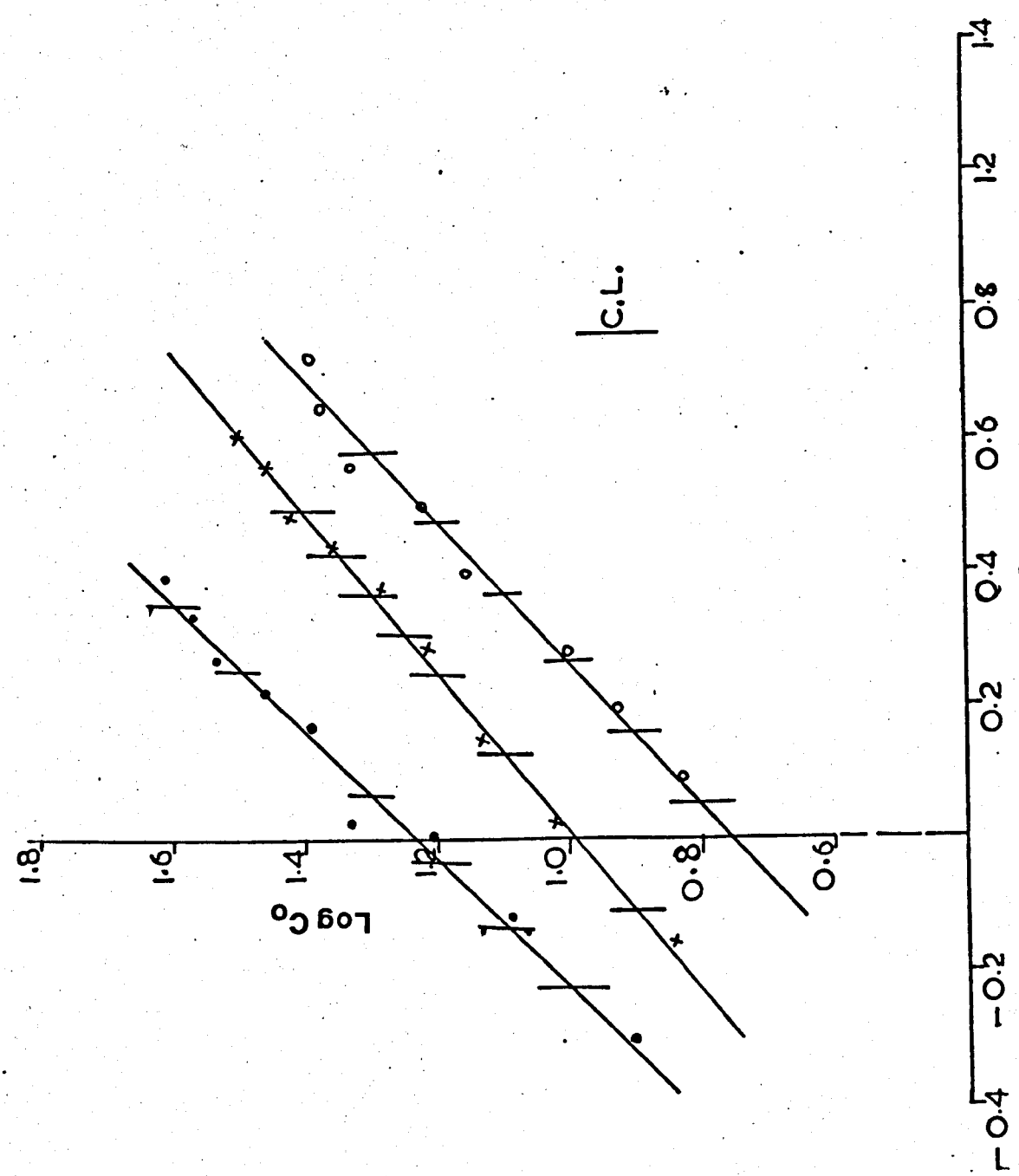


Fig. 4 Isothermal equilibrium adsorption of asulam (x) asulox (0) and sulphanilamide (●) onto soil no. 4.

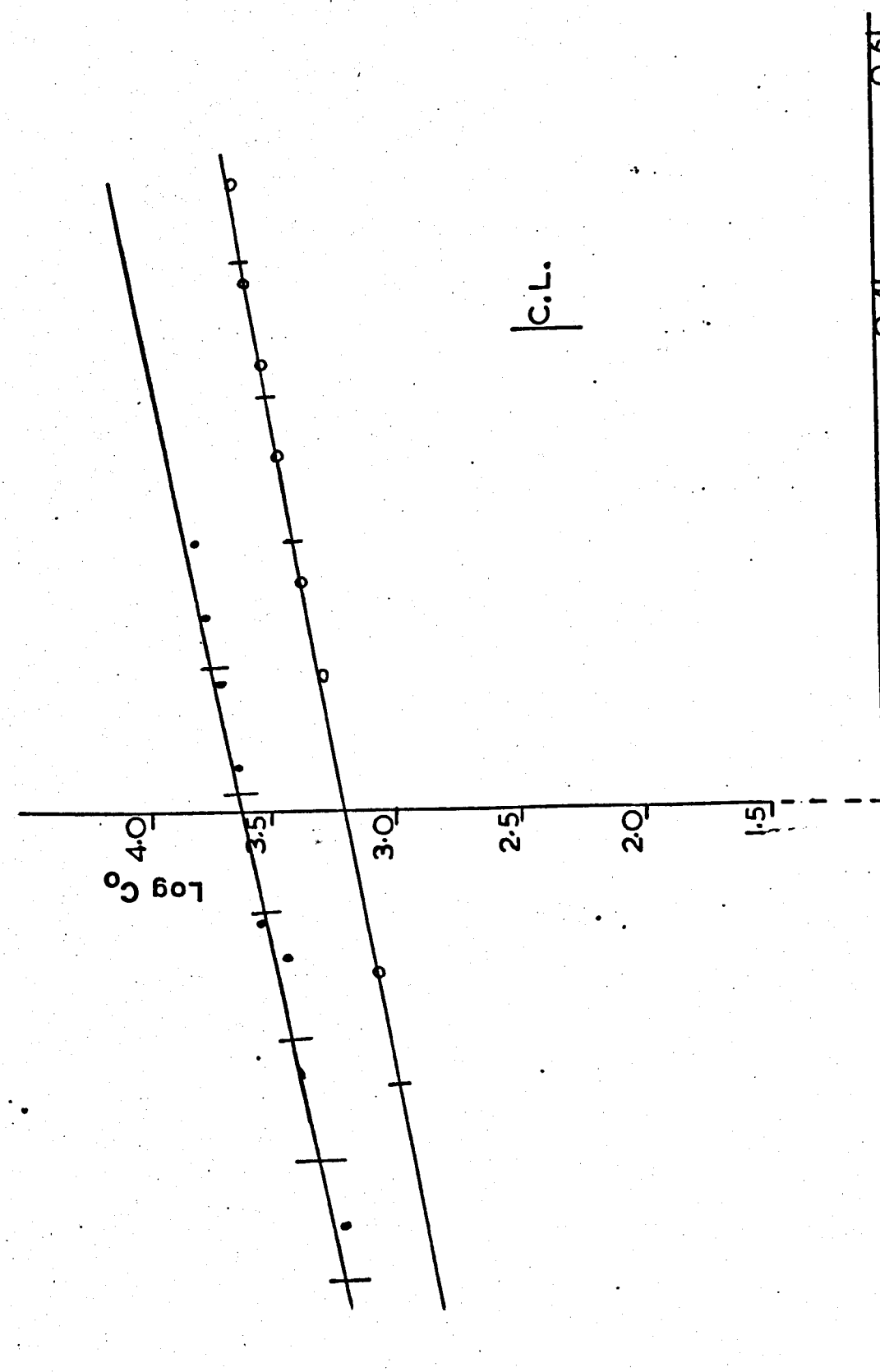


Fig. 5 Isothermal equilibrium adsorption of asulox (O) and sulphanilamide (●) onto Zeo Carb (SRC 16).

$$\underline{Kd} = \frac{\text{Amount adsorbed per unit weight of soil } (\mu g/g)}{\text{Concentration in solution at equilibrium } (\mu g/ml)}$$

are compared with those given a pretreatment with a concentrated solution of asulam followed by the buffer [Fig. 6], it is evident that less chemical is adsorbed below pH 6 onto the buffer pretreated soil. The difference is

Table 2 Adsorption of asulox, asulam and sulphanilamide onto soils, charcoal and cation exchange resin.

Adsorbent	Asulox		Asulam		Sulphanilamide	
	$\frac{1}{n}$	K	$\frac{1}{n}$	K	$\frac{1}{n}$	K
Soil 1	1.3	0.9	1.2	1.0	0.9	3.7
Soil 2	1.0	1.4	0.9	2.0	0.9	3.4
Soil 3	0.9	4.2	0.8	5.2	0.8	7.5
Soil 4	0.9	5.7	0.8	9.5	1.0	13.3
Charcoal	0.2	26,330.0	-	-	0.6	26,120.0
Cation exchange resin	0.9	2,093.0	0.9	2,100.0	1.0	2,127.0

negligible at pH 6 and above but progressively increases on lowering the pH. In this connection, Lambert[308] suggested that under the slurry conditions employed for the determination of soil sorption isotherms using high solvent ratios, the water may be an effective competitor for sites on the organic matter and that the sorption phenomena could be suitably described as a replacement reaction. Thus the reduced adsorption noted with pretreated soil at low pH (Fig. 6) could be due to the difficulty of removing water already present on the soil surface or else due to a diffusion controlled adsorption.[212] The levelling off of these differences at high pH could be brought about by changes in the relative affinities of solute to solvent, solvent to adsorbent and solute to adsorbent.[30]

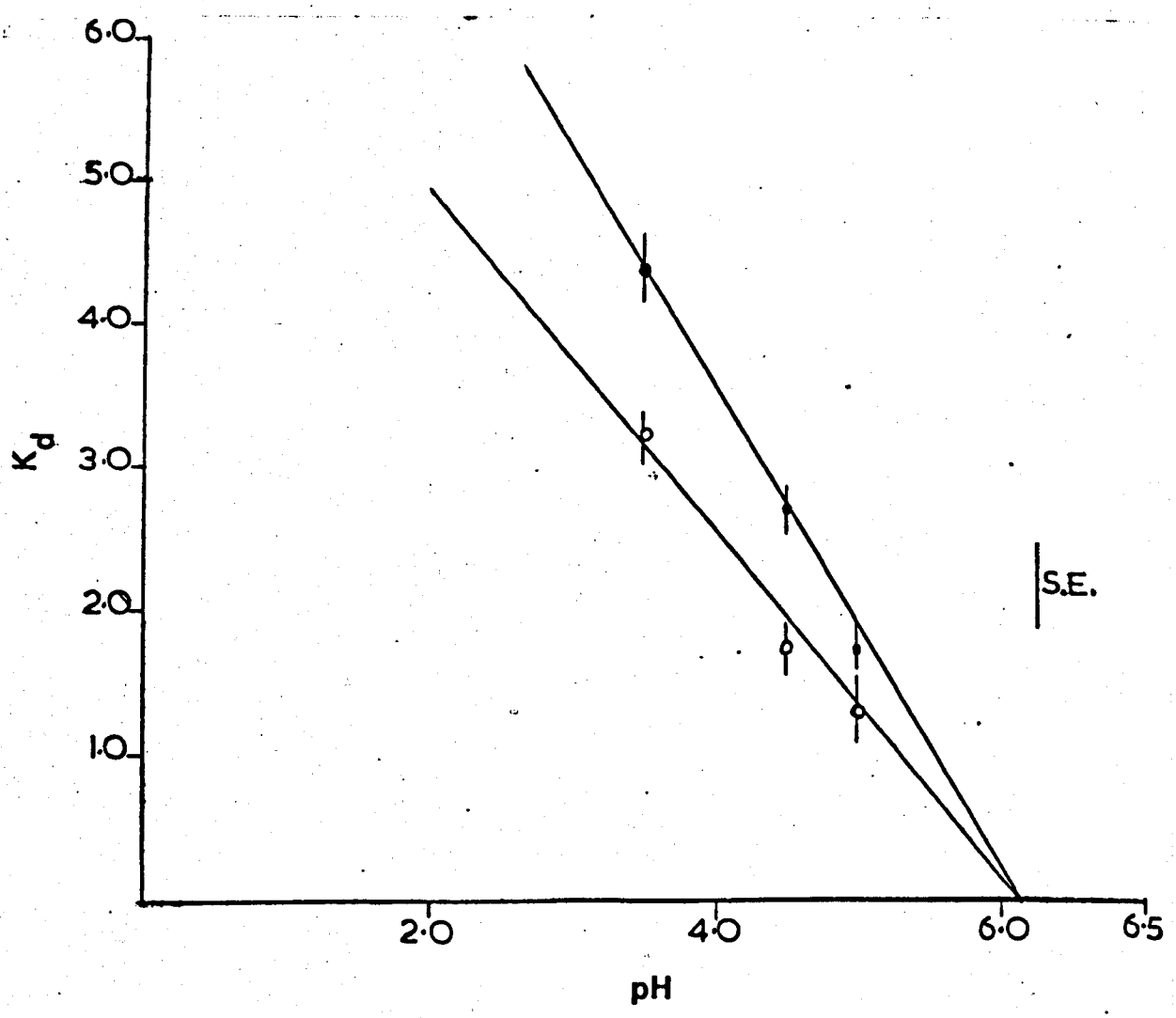


Fig.6 Effect of pH on asulam adsorption,after buffer (0) and asulam (●) pretreatment.

Changes in the hydrophilic-hydrophobic balance of asulam occur with changing pH. This was illustrated by shaking 10ml of 6.8µg/ml asulam dissolved in buffer solution with 10ml of n-octanol for 2h on an end-over-end shaker (Table 3). Such behaviour can be predicted on the grounds that the anionic species (asulam pKa 4.82) will favour the aqueous phase due to hydrogen bonding with water.[295] The changes in the hydrophilic-hydrophobic balance and the repulsion of the anion by the predominantly negatively charged soil colloids may be responsible for the observed decrease in asulam adsorption with increase in pH.[2] The net result will be that at high pH

Table 3 Effect of pH on the partition coefficient of asulam in n-octanol-water system.

pH	Asulam concentration (µg/ml) Aqueous phase			Mean	Standard deviation ±	Partition coefficient n-octanol / water	Buffer used [a]
3.0	2.6	2.6	2.6	2.6	0.0	1.6	glycine, NaCl, HCl.
4.1	3.2	3.2	3.2	3.2	0.0	1.1	sodium acetate, acetic acid
4.9	5.1	5.1	5.1	5.1	0.0	0.3	sodium acetate, acetic acid
6.6	6.8	6.8	6.8	6.8	0.0	0.0	Na_2HPO_4, NaH_2PO_4

[a] Prepared after Coggins and Crafts.[105]

any differences noted between wet and dry soils disappear as only negligible amounts of asulam are retained in any case.

A-3-1- Adsorption of asulam, asulox and sulphanilamide :-

Adsorption onto soils follows the order sulphanilamide, asulam and asulox (Table 3). The relatively higher adsorption of sulphanilamide can possibly be explained in terms of its net positive charge below pH 7; asulam has a net negative charge down to pH between 3.0 and 3.9.[26] Although more or less the same pattern of adsorption was observed for all three chemicals with the cation exchange resin (Table 2) the differences noted between them need not be solely due to charge. The possibility of differences in the physicochemical properties of these chemicals influencing adsorption cannot be ruled out.[29]

The reduced adsorption noted for asulox compared to asulam (although not always significant as indicated by the overlap on occasion of confidence limits calculated after Snedecor and Cochran)[444a] may be due to the greater solubility of the commercial formulation,[430] or the presence of the surface active agent[213,250] or other additives which could act on the chemical, or on the soil itself (surface active agents have been used to modify soil wettability)[194] thereby reducing adsorption. However asulam and asulox are more or less adsorbed to the same extent by the cation exchanger (asulam has a slightly higher $\underline{K}$ value Table 2). It may be worth noting in this connection that asulam is an ampholyte.

With regard to the role of the organic matter fraction in the adsorption of asulox, asulam and sulphanilamide onto soils [soil 4 contains charcoal so is omitted from the comparison (see A-3-2)] soil 3 with an organic matter content of 27.9% clearly exhibits the highest $\underline{K}$ values(Table 2). The situation with regard to soils 1 and 2 is not so clear cut. Although soil 2 has marginally higher $\underline{K}$ values for asulox and asulam (1.4 and 2.0 µg/g) compared with soil 1 (0.9 and 1.0 µg/g) it has less organic matter (14.3% compared with 17.8%). On the other hand for sulphanilamide the

respective K values follow the organic matter pattern in the three soils. The anomalous behaviour noted for asulam and asulox could be due to differential involvement of the organic fractions present in the soil[4,215,224,307,455,493] (see B-3-2) and/or due to differences in pH between the soils involved (Table 1) thereby bringing about differences in the extent of the ionisation.

$$AH \rightleftharpoons A^- + H^+ \qquad \text{(see C-3)}$$

The ionisation of asulam (pKa 4.82) and its sodium salt (asulox) should be affected in such a pH range (4.2 to 4.6) while that for sulphanilamide (pKa 10.7) should not.[26] This seems to be in line with what has been mentioned above about their observed adsorption behaviour onto soils 1 and 2.

A-3-2- Effect of charcoal on adsorption :-

Asulam, asulox and sulphanilamide exhibit their highest K values with soil 4 (Table 2) which has less organic matter than the other soils used (Table 1), but it contains substantial quantities of charcoal, the presence of which can be accounted for by the practice of burning regularly the vegetation cover. Organic matter determinations by the usual wet oxidative methods presumably do not account for the charcoal (vegetation charcoal) content.[249] . A rough estimation of the carbon content was obtained by igniting the soil. The loss on ignition was 19.6%. Of this loss, the organic matter accounts for 10.1% only. The significance of adsorption to charcoal was confirmed by three separate experiments:-

1. The adsorption behaviour of sulphanilamide and asulox onto charcoal was assessed by means of the empirical Freundlich isotherm (see A-3 and Fig.7). The Freundlich Constants (K) for asulox and sulphanilamide are 2.61×10^4 and 2.63×10^4 µg/g and the $\frac{1}{n}$ values are 0.2 and 0.6 respectively (Table 2). Such low $\frac{1}{n}$ values have been observed in similar studies with other herbicides and charcoal.[514]

2. The effect of added charcoal (Norit A, 0.00 - 0.85 µg/g) on adsorption

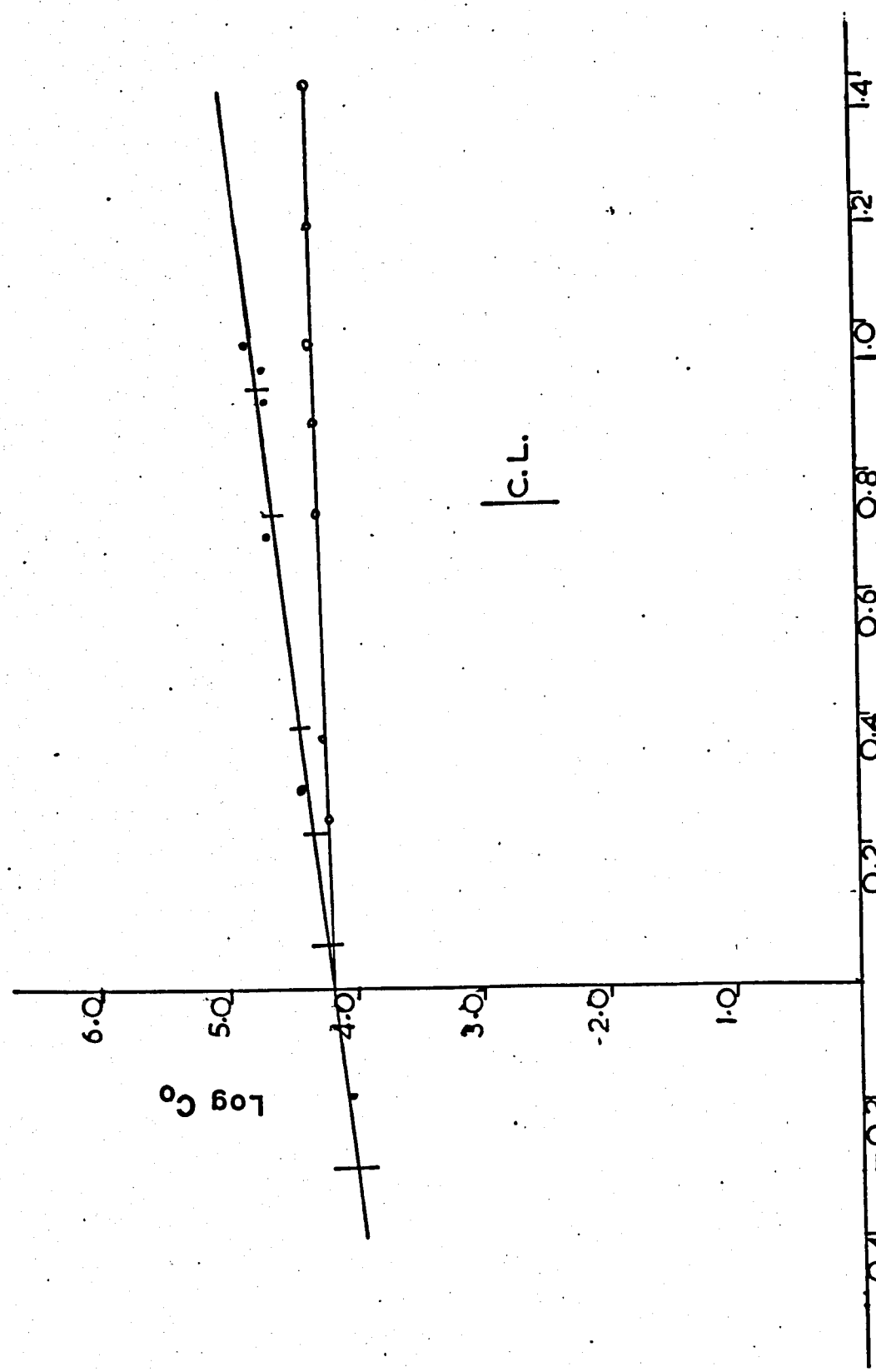


Fig. 7 Isothermal equilibrium adsorption of asulox (○) and sulphanilamide (●) onto Norit A charcoal

of asulam onto soil 2 was compared with asulam adsorption onto soil 4. Two g samples were shaken with 10ml buffer solution containing 5.0µg/ml asulam (see B-2). The results were expressed as the distribution coefficient Kd (see A-3). A plot of Kd against pH (using the method of best fit) could be expressed as a straight line in all cases (Fig. 8) fitting the formula :

$$Kd = \alpha pH + \beta$$

The terms α and β are constants. It is evident from Fig. 8 (which refers specifically to soils 2 and 4 and soil 2 in the presence of 0.85µg/g added charcoal) that 1) charcoal added to the soil can affect adsorption of asulam appreciably particularly at low pH. 2) Norit A charcoal is more efficient for adsorbing asulam than bracken charcoal and 3) negligible adsorption occurs at pH values of 5 and above.

Adsorption also increases linearly with increase in charcoal added to the soil. This is shown when the amount of added charcoal (µg/g) is plotted against the corresponding β value (Fig 9).

Table 4 Effect of charcoal on asulam toxicity to maize. Growth medium perlite. Asulam added as asulox (pre-emergence).

Treatment	Plant fresh weight (g)			Mean	Standard deviation ±	% Reduction relative to corresponding control
1) Growth medium without charcoal.						
a)	3.2	3.2	3.4	3.2	0.1	-
b)	0.0	0.0	0.0	0.0	0.0	0.0
2) Growth medium + 2.5% charcoal.						
a)	2.3	3.5	3.2	3.0	0.5	-
b)	2.0	2.5	2.1	2.2	0.2	26.7

a. Control sample b. Treated sample

3. Asulox at 10.3mg a.i. added to 15g perlite arrests maize seedlings emergence. The addition of charcoal to perlite 2.5% (w/v) greatly reduces this effect (Table 4).

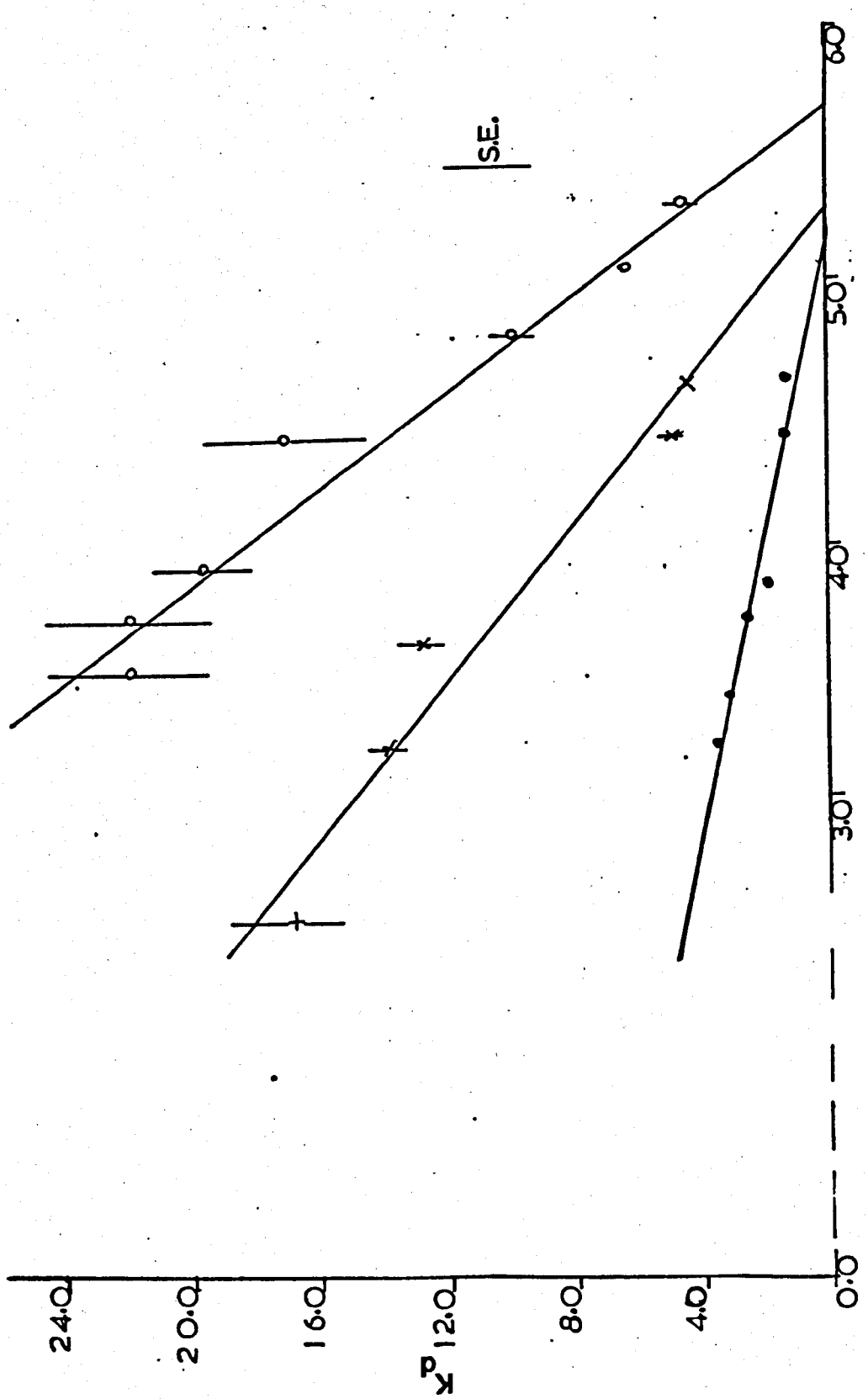


Fig. 8 Effect of added charcoal on asulam adsorption, soil 2 (●), soil 2 + 0.8%E/g charcoal (o), soil 4 (x).

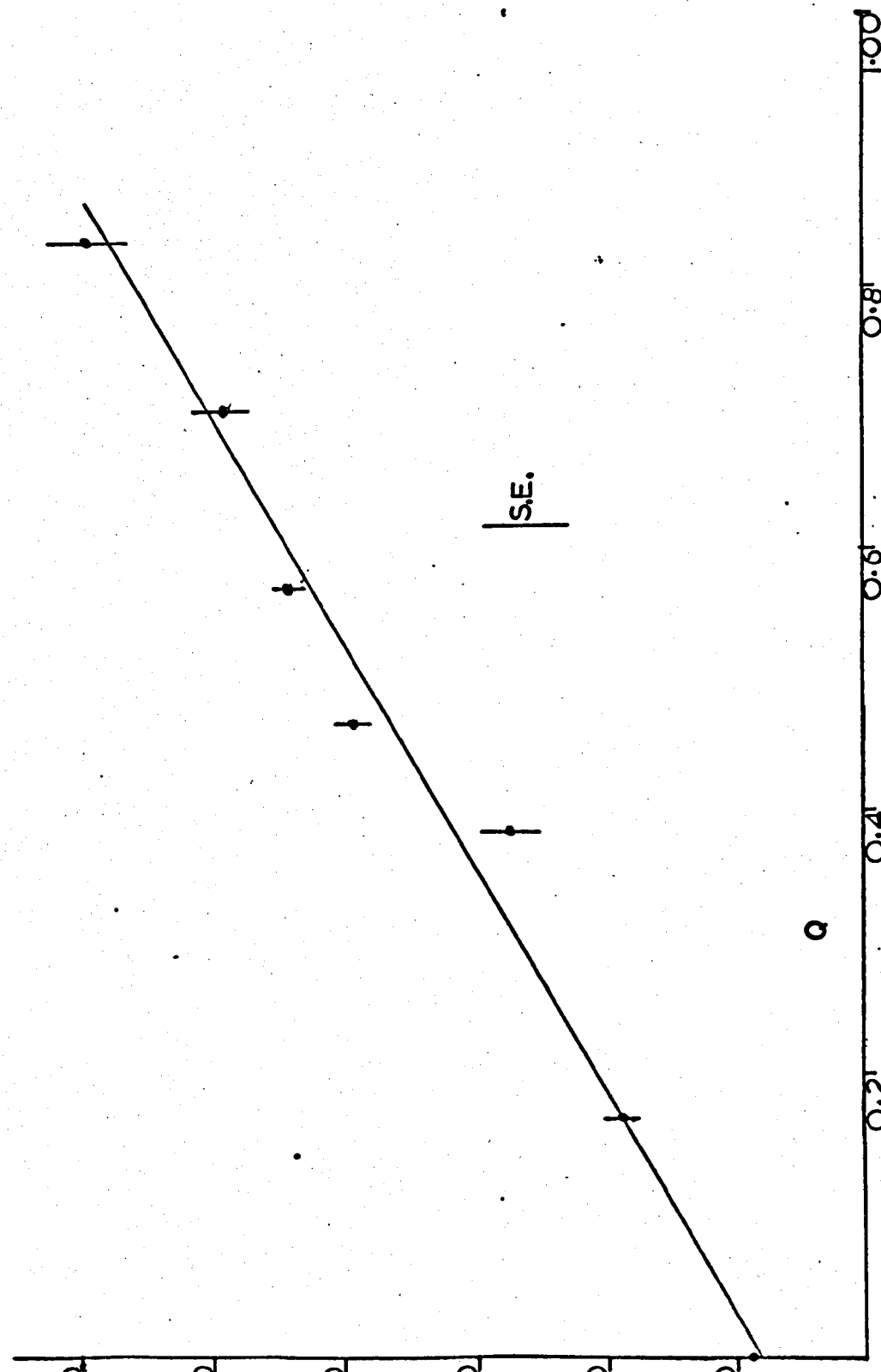


Fig. 9 Variation of β value with charcoal content of soil.

B- Adsorption of asulam onto soils as influenced by soil depth, soil composition and pH:-

B-1- Introduction :-

Studying asulam adsorption onto soils collected from different depths down the soil profile was considered necessary for the following reasons:-

1) As mentioned previously (1-2-b) such a study should reflect the adsorption behaviour of asulam onto soils of varying composition ranging from organic rich soils to mineral rich soils.

2) The adsorption behaviour of asulam down the soil profile is of interest when asulam is used as a pre-emergence herbicide, where the locus and availability of the herbicide in relation to the zone of uptake by the plant may be critical.[26,212,287] Also as a soil contaminant where adsorption will affect movement of the chemical and its dissipation.[26,314,194]

B-2- Experimental :-

The soils used in this series of experiments, unless mentioned otherwise, were as described previously (1-2-b). For treatments prior to adsorption studies see A-2-. Soil analytical data is shown in Table 1. Other adsorbents were prepared as follows :-

1- Commercial samples of montmorillonite and kaolinite were sieved through a 300 mesh BS sieve prior to use for adsorption studies.

2- The humic acid fraction was isolated from a peaty soil by the method of Hance.[223]

The following buffers were used to maintain the slurry pH (range 2.0 to 5.6) KCl - HCl, Na acetate-acetic acid and Na_2HPO_4 - NaH_2PO_4. The buffers were prepared from 0.2M stock solutions as described by Gomeri.[205]

Adsorption, unless otherwise mentioned, was studied by shaking 2g air-dried soil with 10ml aliquots of 5.0µg/ml solution of asulam at 18°C for 16h on an end-over-end shaker. The rest of the procedure was as described in A-2.

In the case of humic acid, 0.5g samples were inserted into dialysis

bags and suspended in 10ml of asulam solution (10.0µg/ml). A further 10ml of the same solution was placed outside the bag. The dialysis tubing was given a hot water treatment prior to use, to minimise adsorption onto the dialysis membrane. Controls were included to account for any asulam adsorption onto the dialysis membranes or for the presence of interfering substances.

Table 1 Soils from under grass. Analytical data.

Soil	pH	C.E.C.	% Sand	% Silt	% Clay	% Organic matter
5	5.9	55.2	17.2	47.6	13.8	21.4
6	6.1	50.0	18.8	44.7	20.0	16.8
7	5.9	27.9	17.2	37.7	28.5	16.6
8	5.7	27.4	19.6	44.7	25.2	10.5
9	5.7	30.6	27.9	20.4	43.9	7.8
10	-	52.1	-	-	34.9	15.9
11	-	41.0	-	-	23.0	4.5
12	-	35.4	-	-	22.0	2.2

Soils 10 to 12 were kindly supplied by Dr. H. Fullerton

For the clay samples, 0.1g of air-dried material was shaken with 10ml aliquots of asulam solution (2.0µg/ml) as described before.

The results were expressed as the distribution coefficient Kd (see A-3).

All measurements were carried out at least in triplicate. Appropriate blanks were included and the filtrates were checked by t.l.c.[168]

B-3- Results and Discussion :-

The adsorption of asulam, judged by Kd values, is inversely correlated with pH. The correlation coefficients (γ) for 8 soils range between -0.99 and -0.92, all significant at $P < 0.02$ (Table 2). A plot of Kd against pH could be expressed as a straight line in all 8 cases (Fig. 1 and 2) fitting the formula :-

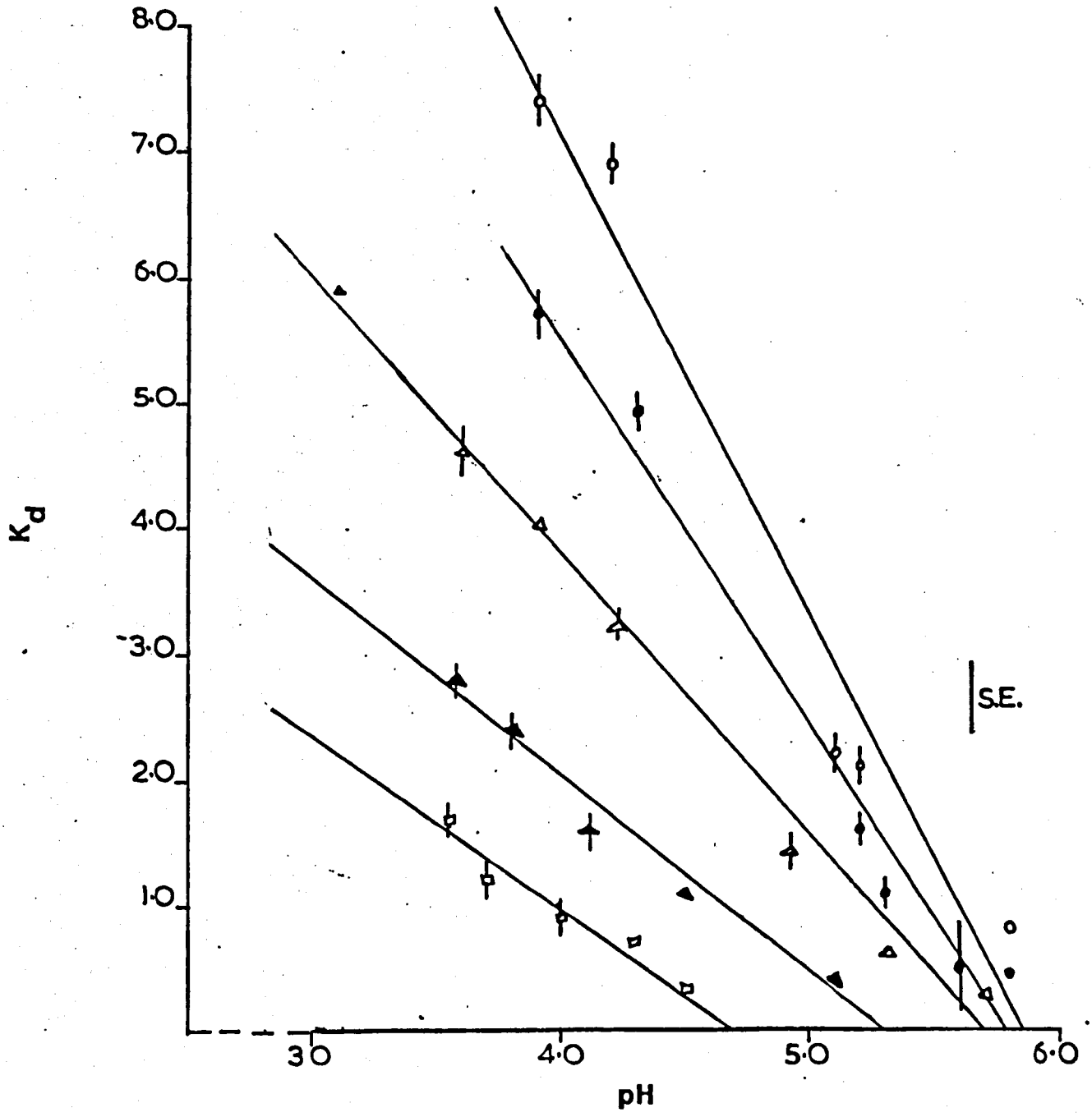


Fig 1 Effect of soil depth on asulam adsorption, 0 - 2" (0), 2 - 4" (●), 4 - 6" (Δ), 6 - 8" (▲), 8 - 10" (▫).

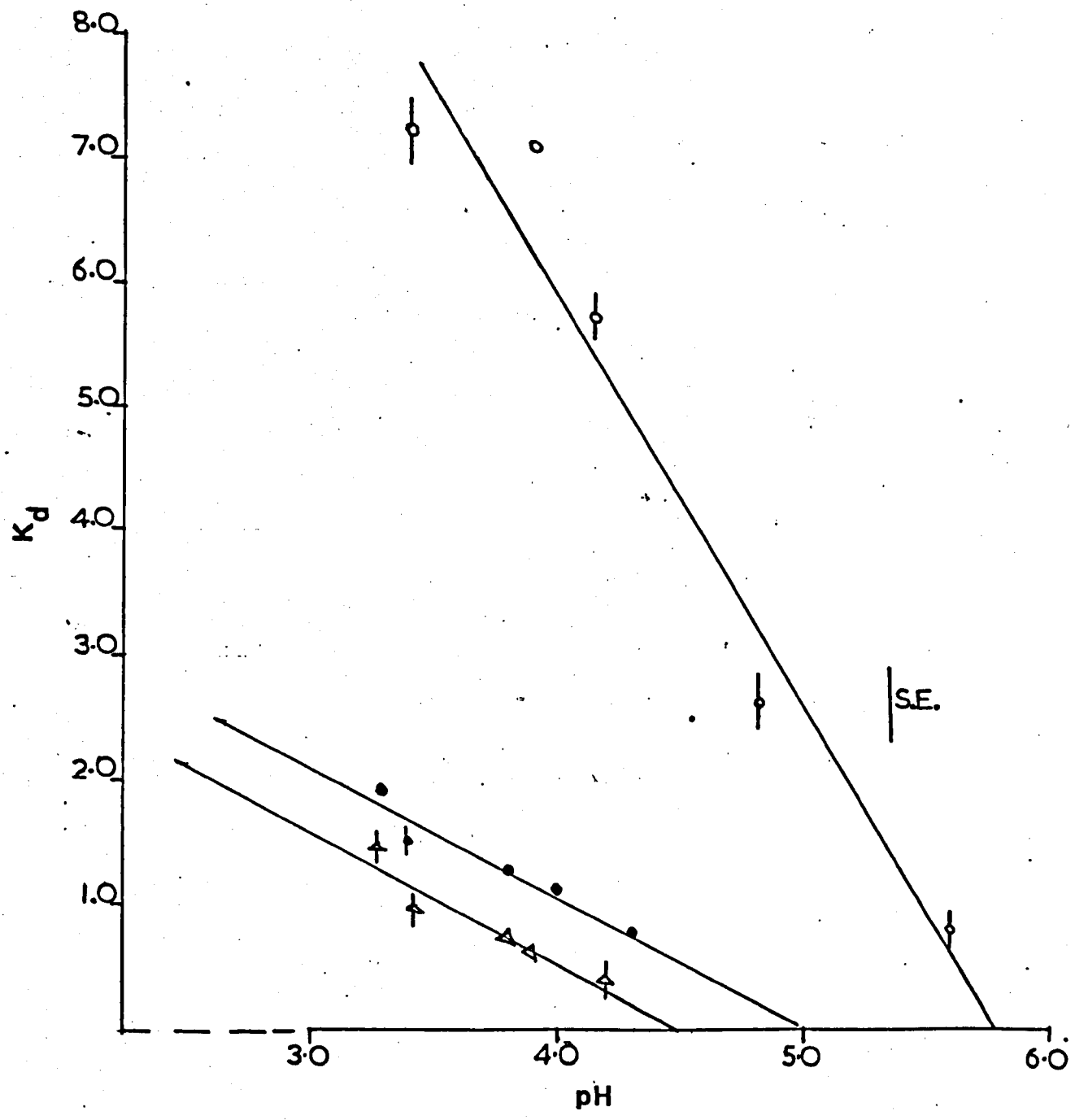


Fig. 2 Adsorption of asulam onto discrete soil horizons. A_o (0), B_{2g} (●) and B_{3g} (Δ).

$$Kd = \alpha pH + \beta$$

The terms α and β are as described before (A-3-2).

Table 2 Effect of soil depth, composition and pH on asulam adsorption.

Soil	Line formula Kd =	Correlation coefficient $(\gamma)^a$	pH where Kd = 0
5	-3.86 pH + 22.55	-0.98	5.54
6	-3.04 pH + 17.64	-0.99	5.50
7	-2.25 pH + 12.71	-0.99	5.65
8	-1.54 pH + 8.20	-0.98	5.32
9	-1.24 pH + 5.94	-0.96	4.79
10	-3.26 pH + 18.90	-0.97	5.80
11	-1.00 pH + 5.05	0.94	5.05
12	-0.97 pH + 4.50	-0.92	4.64

[a] All significant at $p < 0.02$

B-3-1- Effect of soil depth on adsorption :-

Adsorption as expressed by β values tends to decrease with increase in soil depth (Table 2). The β values range between 22.55 and 5.94 for the surface and bottom samples respectively of the Darleith series (soils 5 to 9). The changes in the β values with soil depth are more pronounced in the case of the discrete soil horizons (Dunlop series soils 10 to 12). β values of 18.90, 5.05 and 4.50 were obtained for the A_o, B_{2g} and B_{3g} horizons respectively (soils 10, 11 and 12). The pH at which negligible adsorption occurs also decreases with soil depth.

B-3-2- Effect of organic matter on adsorption :-

Many herbicides have been shown to be more adsorbed by topsoils than by their respective subsoils. Such behaviour is attributed to the high organic matter content of the topsoils.[249,318,512] Correlation analysis between β values, obtained as described above and organic matter, C.E.C., and clay content, shows that β is highly correlated with the organic matter

content ($r = 0.94$ significant at $P < 0.01$), to a lesser extent with C.E.C. ($r = 0.67$ significant at $P < 0.1$) and not at all with the clay content.

The relationship between organic matter and adsorption is best illustrated by plotting % organic matter against β value (Fig. 3).

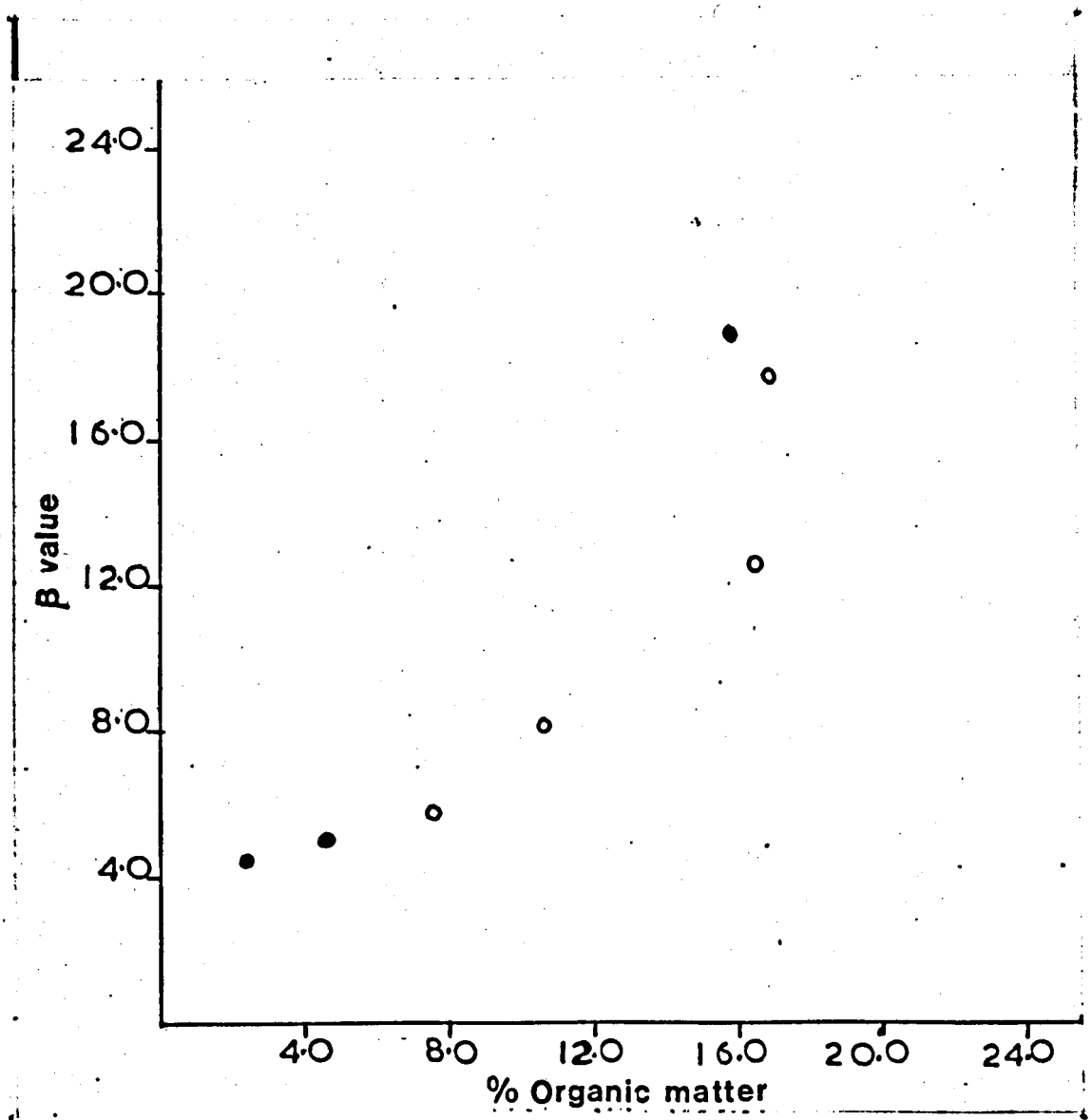


Fig. 3 Variation of β value with organic matter content of soil. Soil 5 - 9 (o), Soil 10 - 12 (●).

Examination of Fig. 3 reveals that the relationship between % organic matter and β value is not perfectly linear. Such deviations from linearity could be expected if all the organic matter (as determined by the carbon chemical analysis) does not participate in adsorption,[307,309] and could be

due to :-

1- Piling up of the organic matter. This is claimed to cause less adsorption of herbicides in some organic soils by limiting the adsorbing surface per unit weight of the organic matter.[215] The increase in asulam adsorption with increase in shaking vigour of the soil suspension (Table 3) taken in conjunction with the non-significant adsorption onto the mineral fraction (see B-3-3) seems to support this argument.

Table 3 Effect of shaking on asulam adsorption. Soil 6 was used. 2g soil were shaken for 24h with 10ml (5.0µg/ml) asulam solution.

Treatment	Kd value			Mean	Standard deviation ±
Intermittent hand shaking.	0.8	0.6	0.6	0.7	0.1
End-over-end shaker.	1.3	1.1	1.1	1.2	0.1
Reciprocating shaker.	1.8	1.8	1.5	1.7	0.1

Increase in adsorption following vigorous shaking has been reported by some workers and is attributed to an increase in availability of adsorption sites.[82]

2- Differences in adsorption could arise due to differences in the stage of decomposition of the organic matter.[483] Published evidence shows that during decomposition of plant materials cellulose and hemicellulose disappear and there is a progressive increase in the proportion of lignin like materials.[468] Hance[224] showed that diuron was strongly adsorbed by non-polar surfaces, but weakly taken up by cellulose or chitin. Similar evidence is shown here with asulam, high adsorption occurs onto humic acid (Kd 50 ± 10) but negative adsorption occurs onto cellulose (Table 4). This could be due to preferential adsorption of water by cellulose.

Table 4 Adsorption of asulam onto cellulose powder; 0.25g cellulose powder shaken with 10ml aliquots of asulam solution.

Asulam concentration (µg/ml)	Kd value			Mean	Standard deviation ±
1.6	-2.4	-2.4	-2.4	-2.4	0.0
2.0	0.0	-1.9	-1.9	-1.3	0.9
2.4	-1.6	-1.6	-4.4	-2.5	1.3
2.8	-2.9	-2.9	-2.9	-2.9	0.0
3.2	0.0	-1.3	-1.3	-0.9	0.6
3.6	-4.0	-4.0	-4.0	-4.0	0.0
4.0	-1.9	-1.9	-1.9	-1.9	0.0
6.0	-1.3	-3.3	-3.3	-2.6	0.9
8.0	-3.0	-3.0	-3.0	-3.0	0.0

An additional source of deviation could, as pointed out by Lambert,[307] be due to unknown anomalous characteristics exhibited by the soils in question.

B-3-3- Adsorption onto the mineral fraction:-

B-3-3-1- Adsorption onto ignited soils :- Significant reductions in Kd values occur after igniting the soil at 800°C for 18h (Table 5). Percentage reductions of the order 85 to 100 occur when adsorption onto ignited soil samples is compared to that onto the non-ignited controls. The size of these reductions must be viewed with some caution, as the side effects of ignition treatment on the mineral part of the soil have not been evaluated. Nevertheless the magnitude of the reductions is such as to indicate that the organic matter is quantitatively the most important site for asulam adsorption.

B-3-3-2- Adsorption onto commercial clays :- The minor role of the mineral fraction is confirmed by adsorption studies employing commercial clay samples. No adsorption occurs onto montmorillonite (Kd 0.0) and non-significant adsorption occurs onto kaolinite (Kd 0.26 ± 0.27) from deionised water

Table 5 Effect of igniting the soil on asulam adsorption.

Soil	Slurry pH	Kd value			Mean	Standard deviation ±
1 a)	4.0	2.6	2.9	1.8	2.4	0.5
b)	4.0	0.3	0.2	0.0	0.2	0.1
2 a)	3.8	2.9	2.9	2.9	2.9	0.0
b)	3.8	0.5	0.5	0.3	0.4	0.1
4 a)	3.9	8.7	8.8	8.8	8.8	0.0
b)	3.9	0.0	0.0	0.0	0.0	0.0
5 a)	4.5	5.2	6.4	6.4	6.0	0.6
b)	4.0	0.5	0.3	0.0	0.3	0.2

a) soil not ignited b) soil ignited

(pH 5.1 to 5.3). Similar adsorption behaviour has been reported for 2,4-D onto these two clays.[419] Apparently the dry clays adsorb water in preference to asulam anion. The preferential adsorption of water and/or the lack of contact with the clay particles due to the water film around the clay particles[31] and the repulsion of asulam anion by the negatively charged clay particles may be responsible for the negligible adsorption encountered. However a slight positive adsorption onto montmorillonite which increases with decrease in pH occurs (Table 6). Such behaviour has been reported by some workers for other anionic herbicides.[186,235,419]

Table 6 Adsorption of asulam onto montmorillonite as influenced by pH.

pH	Kd value			Mean	Standard deviation ±
3.2	0.6	0.6	0.6	0.6	0.0
3.6	0.2	0.2	0.2	0.2	0.0
6.6	0.2	0.0	0.0	0.1	0.1
7.3	-0.4	0.2	0.2	-0.3	0.1

C- Leaching of asulam:-

C-1- Introduction :-

In practice the leaching of herbicides is important because it results in the placement of the herbicide at some point at which it may exhibit specific characteristics and/or lead to certain consequences.[468] Movement out of the surface layer of the soil may result in diminished or enhanced weed control or increased crop injury.[11,318,443,468] Failure to move from the surface may have specific consequences some of which may be favourable and some unfavourable[230,303,468] (see General Introduction). Movement into deeper soil horizons may be a fortunate occurrence due to the removal of the herbicide from the root zone of susceptible plants which are to be grown subsequently or such movement may result in unfavourable accumulation of the herbicide in the subsoil and/or contamination of the underground water.[21,26,314,468]

The main parameters influencing herbicide movement in the soil are

1) Adsorptive relationships between the herbicide and the soil.[430]
2) The solubility of the herbicide in water.[471,472]
3) The amount of water passing through the soil.[430]
4) Chemical and biological transformations.[244]

A thorough knowledge of how herbicides leach in a soil can provide a basis for regulating their use and placement.[468]

C-2- Experimental :-

Glass columns of dimensions (2.5 cm i.d. x 15 cm) were filled with 50g air-dried soil (soil 1). After slow saturation with a buffer solution from the bottom upwards to minimize channelling,[452] 500.0ug asulam in a total volume of 0.5ml were applied to the top surface of the soil layer. Each column was eluted with 100ml acetate buffer at pH 5.0 and 4.2 and ionic strength 0.01, prepared according to Coggins and Crafts.[105] Aliquots

(5ml) were collected and asulam was estimated as previously described (see Ch.2, B). The experiment was carried out in triplicate.

C-3 Results and Discussion:-

This experiment demonstrates that asulam is very mobile. Mobility, as measured by the concentration maximum (Fig. 1) and the total amount eluted in 100ml at the two pH levels (401.5µg and 317.5µg at pH 5.0 and 4.2 respectively), is affected by pH.

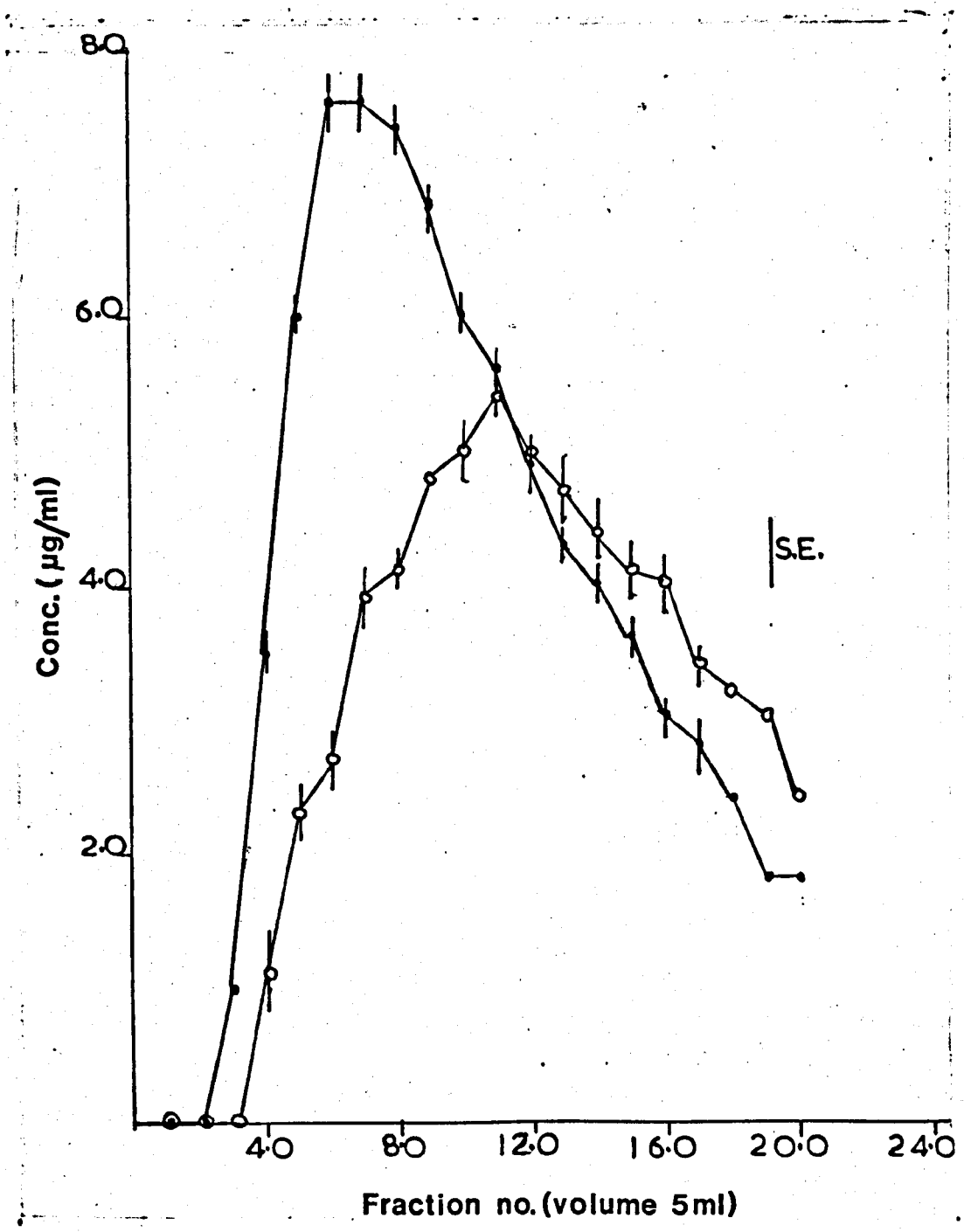


Fig. 1 Leaching of asulam through soil columns at pH 5.0 (●—●) and 4.2 (○—○).

The noted rapid movement and response to pH are in line with the adsorption behaviour of asulam described in the previous sections of this chapter (A and B). These results indicate that like many ionizable acidic herbicides, the equilibrium $AH \rightleftharpoons A^- + H^+$, where AH stands for undissociated asulam, will influence the leachability of asulam and that factors which influence this equilibrium e.g. soil pH and the proton supplying power of the soil colloids will be of paramount importance in determining the mobility of asulam.[2,440] This observation is particularly relevant with regard to bracken where the average soil pH is 5.5 (range 3.6 to 7.6).[100] This factor (soil pH) taken in conjunction with the electrophoretic behaviour of asulam (see ref. 26) or the pKa value (4.82) indicates that asulam should be very mobile in soil on the basis of the high water solubility of the commercial formulation and its anionic nature.[159, 296, 440, 471] The latter property should discourage attraction to the predominantly negatively charged soil colloids.[2]

It could be infered from the above discussion and the elution pattern for asulam (asulam eluted at the solvent front) that downward movement of water and the factors controlling it (e.g. soil permeability) will be the most critical factors regulating asulam mobility in the practical soil pH range.

As soils under bracken are generally freely drained and have good structure,[351] substantial quantities of asulam may find their way to subsoil and/or drainage water. However, the rate of transformation (chemical or biological), may reduce the amount and the depth to which the chemical leaches under practical field conditions.[244]

D- Asulam degradation:-

D-1- Introduction:-

When a herbicide reaches the soil some understanding is needed of the factors which control its degradation and/or transformation, both from the

viewpoint of 1) weed control and 2) pollution and crop damage. The latter aspect is of particular importance in the case of highly mobile herbicides for the following reasons :-

i) The rate of transformation (biological or non-biological) affects herbicide mobility.[244]

ii) Herbicides which are moved to lower depths in the soil are potentially more persistent than they would be if they remained in the topsoil and they therefore constitute a pollution hazard.[214,232]

iii) Herbicides leached down a soil profile might exert undesirable effects on susceptible crops when changes in soil moisture potential cause the solution to rise towards the surface.[194]

Asulam by virtue of its high mobility[26,331] and use both as a pre-emergence and a post-emergence herbicide[8,34,77,78] may come in contact with soils of different properties and different environmental conditions (see 1.2.b). Such variables (soil characteristics and environmental conditions are known to affect the decomposition of herbicides.[214,232]

The objectives of this series of experiments are therefore to study the effect of different factors viz, temperature, moisture, soil depth, soil composition and various additives, supplied to a subsoil and pure sand on asulam disappearance.

D-2- Experimental:-

The soils used in these experiments were sieved as before (see A-2-) but they were not - unless mentioned otherwise - air-dried. Asulam or asulox was added to the soil in a sealed container and mixed by hand. The recovery factor and the uniformity of distribution were determined by extracting soil samples and assaying the extracted asulam chemically. The treatments were carried out at least in duplicate with the inclusion of appropriate blanks. The extracts were further checked by t.l.c. (see A-2). The moisture content of each sample unless mentioned otherwise was maintained by adjusting the

sample to constant weight every other day.

D-2-1- Effect of temperature:-

(a) Incubations were carried out under non-leaching conditions in 100ml capacity bottles, each of which was restricted at the neck with a cotton wool plug. The incubations were carried out at 5, 18 and 25°C. Asulam residues were assayed 14 and 28 days after treatment.

(b) The soil was treated and incubated at 25°C as above. Asulam residues were assayed 1, 2, 3, 4, and 6 days after treatment.

D-2-2- Effect of soil moisture:-

The soil was maintained at three different moisture levels viz 4.8% (air-dried soil), 22.8% and 42.6%. The soil was treated as above. The bottles were kept in a closed container on the laboratory bench. The temperature was not controlled.

D-2-3- Effect of sodium azide treatment:-

The soil was treated with asulox, 189.5µg/g a.i., mixed as above and then divided into two equal samples (w/w). One soil sample received sodium azide 130µg/g in deionized water, while the other sample received an equal volume of deionized water only. The bottles were kept in a closed glass container on the laboratory bench as in D-2-2 above.

D-2-4- Effect of soil depth in the field:-

Asulox (7.2mg a.i.) was added to 90g air-dried soil in a sealed container and the moisture content adjusted to 16% (w/w). After mixing as above, the mixture was transferred to a column of dimension 0.75cm by 30cm. The column was then embedded firmly in undisturbed soil after removing a core of soil of similar diameter to the column. Three columns were recovered after 15 days and the columns of soil were divided into 5cm segments. The soil in each segment was hand mixed and samples (ca 2g) extracted and asulam determined as described below (see D-2-9).

D-2-5- Asulam persistence in soils collected from down a soil profile:-

The soils used in this study were soils 10, 11 and 12 collected from the A_o, B_{2g} and B_{3g} horizons (see 1-2 b). The soils were treated with asulam at 20.1µg/g and were incubated at 30°C. Asulam residues were assessed 5, 10 and 18 days after treatment.

D-2-6- Influence of various additives:-

Soil 12 (see 1-2 b) was used in this experiment. The soil samples were first incubated for 15 days in covered beakers with the following additives:-

(a) glucose.

(b) NH_4NO_3.

(c) NH_4NO_3 + glucose.

(d) NH_4NO_3 + glucose + yeast extract.

The samples were aerated daily. Deionised water was added daily to compensate for evaporation. Asulam at 20.6µg/g was added and mixed with the soil as above. The soil samples were then incubated at 30°C.

D-2-7- Influence of asulam concentration:-

Soil 12 after mixing with 0.33% (w/w) yeast extract and incubation as above (D-2-6) was treated with asulam at 5.0, 10.0 and 20.0µg/g. The soil samples were then treated as above (D-2-6).

D-2-8- Influence of soil-sand mixtures:-

Sand (acid washed) and soil (soil 10) were mixed in varying proportions from 100% soil to 100% sand and then adjusted to ca. 68% of field capacity (the moisture content of the soil when collected from the field). Asulam at 11.0µg/g was added and the soil samples were mixed and then incubated at 20°C for 17 days.

D-2-9- Extraction and asulam estimation:-

In the experiments described above a slight modification in the extraction procedure and a modified method of asulam estimation were introduced.

i) Experiments D-2-1- to D-2-4 :- In these experiments soil 1 was used (see 1.2a). Asulam residues were extracted with deionised water (May and Baker Ltd., private communication). Soil samples (ca. 2g) were shaken with 25ml water for 3h. Asulam was estimated as described previously (see Ch.2, B).

ii) Experiments D-2-5 to D-2-8 :- In these experiments soils 10, 11 and 12 were used (see 1-2-b). Soil samples (ca. 10g) were shaken with 100ml acetate buffer pH 5.6 (see Ref. 205) for 3h. Asulam in 10 to 25ml aliquots was acidified diazotised and coupled as previously described. The Bratton-Marshall colour was then concentrated by extraction into n-butanol prior to spectrophotometric determination. This modification was introduced to increase the sensitivity of the procedure and to allow for the use of asulam at low dose rates. It was used originally by some workers [107] for the determination of sulfadimethoxine in animal tissues, and was adopted here for asulam estimation after a thorough investigation. The recovery factor attained by both extraction procedures ranged between 70% and 100%.

D-2-9- Bio-assay:-

D-2-9-1- Growth of maize:- Maize seeds (3/pot) were germinated in a growth room on perlite (negative adsorption of asulam onto perlite was noted). The plants were supplied with nutrient solution and maintained at 25°C for 21 days employing a 16h day length. The shoots were harvested and the dry weight measured. A range of asulam concentrations were applied to the growth medium prior to germination.

D-2-9-2 Tetrazolium test :- Whole root systems of maize seedlings from the bio-assay were immersed in tetrazolium chloride prepared as described by Duffy [152] and the effect of asulam on root development and viability was assessed by visual inspection of the intensity of the colour produced.

D-3- Results and Discussion:-

D-3-1- Effect of temperature:-

Degradation of asulam occurs in the soil at all three temperatures

adopted (Table 1). At 25°C 16.6% and 2.6% remain after 14 and 28 days respectively. Appreciable quantities of asulam only persist in the case of the 5°C treatment (31.3% after 28 days). Rapid disappearance of asulam occurs at 25°C.

Table 1 Effect of temperature on asulam persistence. Asulam applied as asulox at 80.0µg/g (on oven dry weight basis).

Temperature °C		Amount of asulam persisting (µg/g)		Mean	Standard deviation ±	% Persistence
5	a)	42.2	42.3	42.2	0.0	53.8
	b)	24.8	25.2	25.0	0.2	31.3
18	a)	21.3	20.8	21.1	0.3	26.4
	b)	6.1	6.3	6.2	0.1	7.6
25	a)	13.3	13.3	13.3	0.0	16.6
	b)	2.0	2.1	2.1	0.1	2.6

a) 14 days after treatment b) 28 days after treatment

There appears to be no lag phase or at most a very short one. 24.2% of the added asulam (80.0µg/g) disappears in 24h (Table 2)

Table 2 Effect of time on asulam persistence. Asulam applied as asulox at 80.0µg/g (on oven dry weight basis).

Time in days	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
1	61.7	58.8	61.4	60.6	1.3	75.8
2	46.9	44.3	44.6	45.3	1.2	56.6
3	43.4	42.1	41.3	42.3	0.9	52.2
4	36.2	36.2	35.4	35.9	0.4	44.9
6	30.9	29.1	30.6	30.2	0.8	37.8

However, the rate of disappearance decreases with time as only 7.1% disappears between the 4th and the 6th day after treatment (Table 2). This decrease could possibly be due to formation of inhibitory products. Such behaviour is not uncommon and it points to the possibility of participation of a biological route in asulam disappearance,[38] (see D-3-3).

D-3-2- Effect of soil moisture:-

A slight disappearance of asulam occurs from air-dried soil ca. 6.4% in 15 days. However, appreciable disappearance occurs at the other two moisture levels as 67.1% and 61.2% asulam disappear at 22.8% and 42.6% moisture levels respectively (Table 3).

Table 3 Effect of moisture on asulam persistence. Asulam applied as asulox 80.0µg/g a.i. (on oven dry weight basis). Assessment was made 15 days after treatment.

Moisture content (% w/w)	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
4.8[a]	74.7	74.8	75.3	74.9	0.3	93.6
22.8	26.4	26.3	26.3	26.3	0.0	32.9
42.6	31.4	29.5	32.1	31.0	1.1	38.8

[a] air dry soil

Many herbicides are reported to disappear in moist soil and to persist at low moisture levels.[54] Low moisture levels have been held responsible for carry-over problems noticed with herbicides in the field and are said to be one of the many reasons associated with herbicide persistence in subsoils.[458,468] Birck and Roadhouse[54] recovered 96% atrazine from air-dried soil 55 days after treatment.

D-3-3- Effect of sodium azide :-

Sodium azide (compared to control without NaN_3) curtails the disappearance of asulam significantly over the 4 week period studied (Table 4).

Table 4 Effect of sodium azide on asulam persistence. Asulam applied as asulox at 189.5µg/g a.i. (on oven dry basis).

Time interval in weeks		Amount of asulam persisting (µg/g)						Mean	Standard deviation ±	% Persistence
1	a)	103.8	109.1	103.3	109.1	103.0	109.1	106.5	2.7	56.2
	b)	75.1	65.1	71.5	69.1	71.5	73.6	71.0	3.2	37.5
2	a)	67.3	67.3	67.3	67.3	72.5	75.5	69.0	2.4	36.4
	b)	38.0	46.7	38.2	38.2	38.0	41.2	40.1	3.2	21.2
3	a)	55.1	47.0	47.0	56.0	56.0	52.5	52.3	3.9	27.6
	b)	23.6	23.6	23.5	29.8	29.8	29.3	26.6	3.0	14.0
4	a)	21.0	21.0	21.7	21.0	21.0	18.8	20.6	1.2	10.9
	b)	12.4	12.4	12.4	12.4	12.4	13.6	12.6	0.4	6.6

a) with sodium azide

b) without sodium azide

However it does not prevent the disappearance of asulam completely. This could be attributed to two possibilities:

1. The sodium azide used was not enough to sterilise the soil completely bearing in mind that the soil has a high organic matter content and presumably a high microbial activity. Generally microbial activity is said to increase with increasing organic matter content.[93] The encountered interference of NaN_3 with asulam residue estimation precludes the use of high rates of NaN_3.

2. That asulam disappearance is mediated by biological and non-biological routes.[160]

D-3-4- Effect of soil depth :-

The assessment of the persistence of asulam in the soil columns taken from the field is complicated by the upward movement of water. The moisture content at the start of the experiment was 16.6% while after 15 days it ranged from 26.2% at the top of the column to 35.7% at the base (Table 5a).

Table 5 Effect of soil depth on asulam persistence. Asulam applied as asulox 80.0µg/g (on oven dry weight basis).

Soil depth in.	Soil moisture content at sampling time	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
a)							
0-2	26.2	43.8	41.1	45.2	43.4	1.7	54.3
2-4	27.1	41.1	50.6	49.3	47.0	4.2	58.8
4-6	31.7	46.9	46.2	46.9	47.7	0.3	58.4
6-8	35.6	33.5	36.7	36.8	35.7	1.5	44.6
8-10	36.3	42.3	34.4	39.0	36.7	3.2	48.3
10-12	35.7	19.3	16.0	21.8	19.0	2.4	23.8
b) Average persistence in the soil columns (µg/g)							
		Soil column					
		1	2	3			
		37.8	37.5	39.8	38.4	1.0	48.0

On the whole the effect on asulam disappearance is comparable to that produced in the laboratory at 5°C (Table 1 and 5b). Soil temperature averaged 7°C at the top of the column and 5°C at the bottom at the time of sampling. The experiment was carried out in January 1974. The possibility of upward movement is suggested by the concentration profile of asulam in the soil columns (Table 5a). The work of Harris[229] indicates that upward capillary movement is very efficient in moving herbicides and that even minor water movement by capillarity may be important in the movement of herbicides.

D-3-5- Asulam persistence in soils collected down a soil profile:-

Rapid disappearance of asulam occurs in the topsoil (soil 10); 21.0%, 7.0% and 0.0% remain 5, 10 and 15 days after treatment. However a very slow disappearance occurs in the B_{2g} and B_{3g} samples (soils 11 and 12) (Table 6).

Table 6 Asulam persistence in different soil horizons. Asulam 20.1µg/g (on oven dry weight basis).

Soil horizon		Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
A_o	a)	4.2	4.2	4.4	4.3	0.1	21.0
	b)	1.4	1.6	1.3	1.4	0.1	7.0
	c)	0.0	0.0	0.0	0.0	0.0	0.0
B_{2g}	a)	16.2	15.9	17.4	16.5	0.6	82.1
	b)	16.4	14.9	17.0	16.1	0.9	80.1
	c)	14.2	13.6	15.7	14.5	0.9	72.1
B_{3g}	a)	18.9	17.8	19.0	18.1	1.6	90.0
	b)	19.3	17.8	19.0	18.9	0.6	94.0
	c)	14.5	17.3	18.8	16.9	1.8	84.0

a) Assessment made 5 days after treatment
b) Assessment made 10 days after treatment
c) Assessment made 18 days after treatment

Many herbicides are reported to undergo rapid disappearance in topsoils and

to persist longer in subsoils.[89, 214, 370, 419, 468] In the field low temperature, low oxygen and high carbon dioxide in the subsurface horizons would be expected to further reduce their capacity to dissipate asulam,[214] (see Ch.1, 7-3-1).

D-3-6- Effect of various additives on asulam persistence:-

No appreciable disappearance of asulam occurs from the soil alone (soil 12) or the soil in the presence of glucose or NH_4NO_3 or their combination. However appreciable disappearance (26.7%) occurs when yeast extract is added to the glucose and NH_4NO_3 combination (Table 7).

Table 7 Effect of various additives on asulam persistence. Asulam at 20.6µg/g (on oven dry weight basis). Assessment was made 7 days after treatment.

Treatment (Additive expressed as % w/w)	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
Soil	18.7	22.0	19.5	20.1	1.4	97.6
Soil + 0.4 glucose	21.5	19.6	16.9	19.3	1.9	93.7
Soil + 0.1 NH_4NO_3	20.9	20.2	20.6	20.6	0.3	100.0
Soil + 0.4 glucose + 0.1 NH_4NO_3	19.8	20.9	20.6	20.4	0.5	99.0
Soil + 0.4 glucose + 0.1 NH_4NO_3 + 0.2 yeast extract	14.8	15.8	14.8	15.1	0.5	73.3

D-3-7- Effect of asulam concentration:-

The disappearance of asulam appears to be affected by asulam concentration in this treatment (B_{3g} + 0.33% yeast extract). 56.0%, 47.0% and 26.0% of the added asulam (5.0, 10.0 and 20.0µg/g) disappear 15 days after treatment (Table 8). The effect of asulam concentration on disappearance could be taken as a further indication that soil microorganisms are

involved in asulam dissipation. The effect of yeast extract on asulam persistence (see D-3-6 and D-3-7) taken in conjunction with the results of Hill et al.[248] with substituted urea and Wood[508] with sulphanilamide (both workers used yeast extract) is further evidence for the involvement of microorganisms in asulam disappearance.

Table 8 Effect of asulam concentration on asulam persistence. Yeast extract 0.33% (w/w) was mixed with soil. Assessment of residues was made 7 days after treatment.

Asulam concentration (µg/g)	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
5.0	2.4	2.4	1.8	2.2	0.3	44.0
10.0	5.7	5.7	4.5	5.3	0.6	53.0
20.0	14.4	16.7	13.2	14.8	1.5	74.0

D-3-8- Persistence of asulam in soil-sand mixtures :-

No disappearance of asulam occurs in pure sand, however addition of fresh topsoil (soil 10) leads to considerable asulam disappearance 17 days after treatment (Table 9).

D-3-9- The bio-assay experiment:-

The maize bio-assay shows that at 25°C up to 16 ppm. asulam, applied as asulox, has no deleterious effects on the growth of maize plants. A slight reduction which is not statistically significant is noted in the 20 - 32 ppm range (Table 10).

An assessment of root viability employing the tetrazolium test reveals several gradations in colour of the root at 12.5, 25.0, 37.0 and 50.0 ppm asulam. The primary roots are less coloured while the lateral roots are highly coloured, suggesting that asulam concentration is decreasing

Table 9 Influence of soil-sand mixtures on asulam persistence. Asulam at 11.0µg/g (on oven dry weight basis). Assessment was made 17 days after treatment. Temperature 20°C.

Soil-sand mixture (% w/w)		Moisture content at field capacity	Amount of asulam persisting (µg/g)			Mean	Standard deviation ±	% Persistence
soil	sand							
100.0	0.0	53.3 ± 0.1	0.6	1.1	1.0	0.9	0.2	8.2
80.0	20.0	46.7 ± 0.3	1.0	1.0	0.7	0.9	0.1	8.2
60.0	40.0	41.6 ± 0.4	1.3	1.5	1.6	1.5	0.1	13.6
20.0	80.0	28.7 ± 0.6	2.6	3.7	2.7	3.0	0.5	27.3
0.0	100.0	21.2 ± 0.2	10.7	10.7	10.9	10.8	0.1	98.2

with time, thereby exerting less influence on root activity. The decrease in asulam concentration may result from plant uptake and/or changes in microbial population associated with plant roots. At the higher concentrations employed feeble colour development takes place in the roots. In addition root branching and shoot development are severely curtailed (Fig.1)

Table 10 Effect of asulam on dry weight of maize shoots. Asulam applied as asulox.

Asulam concentration in ppm.	Dry weight of shoots (g)			Mean	Standard deviation ±	% of control
1.0	0.27	0.27	0.31	0.28	0.02	108.9
2.0	0.28	0.26	0.28	0.27	0.01	105.0
4.0	0.33	0.35	0.31	0.33	0.02	126.9
8.0	0.30	0.37	0.25	0.31	0.05	119.2
12.0	0.26	0.24	0.30	0.27	0.02	103.8
16.0	0.22	0.28	0.30	0.26	0.03	100.0
20.0	0.20	0.23	0.23	0.22	0.01	84.8
24.0	0.24	0.26	0.25	0.25	0.01	96.2
28.0	0.24	0.25	0.23	0.24	0.01	92.3
32.0	0.23	0.18	0.18	0.20	0.02	76.9

Of interest is the stimulation of maize growth brought about by low asulam concentrations (Table 10). Stimulation of plant growth has been observed with herbicides possessing growth regulating properties [304] (see Ch.4). The practical significance of this finding is that low asulam concentration (resulting from a slow penetration, impaired translocation, or removal of asulam from the zone of uptake) may result in growth stimulation. Similar effects have been reported with 2,4-D and some thiocarbamate herbicides.[304]

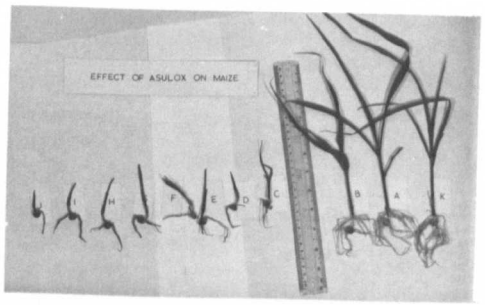


Fig. 1 Effect of asulam, applied as asulox, on maize growth and development.

Treatment	A	B	C	D	E	F	G	H	I	J	K
Asulox conc. ppm a.i.	12.5	25.0	37.5	50.0	75.0	100.0	125.0	150.0	175.0	200	0.0

The following points could be drawn from this series of experiments (A to D) :-

1) Neither asulox, asulam nor sulphanilamide are adsorbed to any marked extent by soils.

Comparatively higher amounts of asulam are fixed by topsoils than by subsoils. The adsorption which takes place is negatively correlated with pH and it would appear that little asulam (if any) will be retained by soils in the practical soil pH range. Adsorption onto soils will be lessened if the chemical is applied to a wet soil.

2) The presence of charcoal resulting either from burning bracken litter or added to the soil increases the adsorption of asulam. This indicates that if the area of bracken to be sprayed has at sometime previously been burned to remove surface vegetation or the bracken litter (see Ch.5), the residual carbon present will increase the adsorption capacity of the soil for asulam and this could be of practical significance in acidic soils.

3) Adsorption onto carbon (Norit A) is quite effective in removing high concentrations of asulam (applied as asulox) from aqueous solutions. This

could provide a very effective means of decontaminating water supplies in cases of spray drift and/or from contamination of drainage or run-off water.

4) A high degradation rate occurs under conditions conducive to increased microbial activity. Therefore it would appear that the capacity of the soil to degrade asulam rather than its capacity to adsorb asulam is the determining factor in accounting for the overall behaviour of asulam in the soil. However under conditions of high rainfall or overhead irrigation asulam may leach down to the subsoil. If the leaching is excessive it may disappear beyond the reach of all roots and may be lost as far as biological effects are concerned. This will of course depend on the intensity and frequency of precipitation. Such a possibility could explain at least in part the short term pre-emergence activity of asulam observed in the field.[77] However under dry conditions particularly in soils of low organic matter content e.g. some African soils[216] slow degradation of asulam may occur. The natural wetting-drying cycles which occur in the soil may bring about a uniform distribution of asulam as is the case with many mobile herbicides.[244] Therefore under these conditions asulam may exhibit adequate pre-emergence activity.

The low retention of asulam by soil may be advantageous in cases where the organic matter is low, the soil has low permeability (high clay content)[244] and furrow irrigation is adopted. In such situations vertical movement will be reduced and low rainfall after application would be expected to activate asulam and reduce lateral movement which tends to occur under furrow irrigation and leads to unreliable performance of pre-emergence herbicides.[244] However, under such conditions the possibility of run-off has to be guarded against.[244]

5) This study in agreement with others[370] illustrates that more than one type of investigation is necessary if insight is to be gained into the complete picture of soil-herbicide interactions.

It may be worth mentioning that the assay for asulam used here and also adopted by others[34,78] depends on the presence of a primary aromatic

amino-group. In this study the identity of asulam was checked by t.l.c. and the possibility of interference from other soil constituents was catered for by including t.l.c. analysis and by incorporating suitable blanks. Under these conditions no degradation products were detected.

The degradation pathway of asulam in the soil should be a matter of concern in view of the recent work of Bartha and others on aniline-based herbicides (see Ch.1, 8-1; and Ch.4, A-1).

CHAPTER IV

IN VITRO STUDIES ON THE INFLUENCE OF ASULAM AND RELATED CHEMICALS ON THE PROPERTIES OF HORSERADISH PEROXIDASE.

1- Introduction :-

The experiments dealt with in this chapter stemmed from two observations made during the course of this study, full accounts of which are given below. These observations suggested the possibility of interaction between asulam and peroxidase involving A-peroxidation and B-IAA-oxidation reactions. The influence of asulam on both activities was considered. Horseradish peroxidase was used in both investigations because it is known to exhibit both activities[36] and has been used by many investigators in similar studies.[38, 40, 61, 252, 320] Though the two reactions have much in common, they are treated separately mainly for convenience of presentation.

A-1- Interaction between asulam, horseradish peroxidase and PABA:-

Asulam disappears rapidly in soils with high organic matter contents, at warm temperature and adequate soil moisture. It persists almost unchanged in pure sand and persists for some time in subsoil samples. Rapid disappearance is brought about by mixing the sand with fresh field topsoil or by incubating the subsoil with yeast extract prior to herbicide treatment (Ch.3, D). Such behaviour is indicative of participation of microorganisms in asulam disappearance.[248, 479, 508] However as previously mentioned in the General Introduction, the rapid disappearance of many aniline-based herbicides is found to result in the formation of polymers and complexes that have extended life and largely unknown biological properties.[58] This transformation occurs in two steps[40] :-

1. The release of the aniline moiety.
2. Oxidation of the free amino group released in the first step, followed by a series of reactions leading to the formation of azobenzenes

and/or polymeric derivatives.[40] Peroxidases which have a wide distribution in nature and occur in soil are implicated in the second transformation step.[38,39,62]

The molecular structure of asulam suggests two possible ways it could interact with peroxidase :-

1) it could behave as an aniline derivative with a free amino group so a reaction resulting in the formation of an azobenzene derivative or polymeric materials may occur.

2) it may behave as a sulphonamide type compound, in which case no reaction with peroxidase would be expected. In addition inhibition of the oxidation of PABA by peroxidase may result.[320] Horseradish peroxidase was used in this study because it is regularly used for studies of this type and similar transformation products to those formed in soil have been reported.[62]

A-2- Experimental :-

The source and purity of asulam and sulphanilamide were as previously described (Ch.2 and 3). PABA was purchased from B.DH.Ltd. and was recrystallized from hot water before use.[384] Crude horseradish peroxidase (RZ. 0.3) was purchased from Sigma London Chemical Co. Ltd. In all experiments unless mentioned otherwise 0.04M phosphate buffer (Na_2HPO_4-NaH_2PO_4) at pH 6.0 was used. For studying the pH effect, the buffer system of Miller and Golder[348] as modified by Coggins and Crafts[105] was used. The final volume of the reaction mixture was adjusted to 3ml. The reactants were allowed to equilibrate in a water bath at 35°C for 10 min. The reaction was carried out in a U.V. spectrophotometer (Pye-Unicam model SP1800). The cell temperature was kept at 35°C by circulating water. The reaction was started by adding 0.1ml of 9.68×10^{-4} g/ml of 29% (W/V) hydrogen peroxide solution freshly prepared every 5h. The reaction was followed by recording the change in optical density with time at 474nm for 5 min. The concentration of the protein in the final solution was 16 µg/ml. A PABA concentration

of 1.31×10^{-6} mole/ml in the final solution was used throughout this experiment.

Each treatment was carried out in triplicate. The optimum reaction conditions were selected from preliminary trials in which the reagent concentrations and reaction temperature were varied. Conditions which gave very fast or very slow reaction rates or led to reagent precipitation were avoided.

A-3- Results and Discussion:-

No measurable oxidation of asulam or sulphanilamide by the enzyme system occurs. This is in agreement with Lipmann's findings.[310] However, asulam and sulphanilamide differ in their effects on the oxidation of PABA by the enzyme system. Sulphanilamide gives the expected inhibition as reported by Lipmann.[310] On the other hand the optical density in the presence of asulam shows an increase.

A-3-1- The influence of asulam and sulphanilamide concentration:-

The increase in optical density is directly proportional to asulam content at low concentrations. However, this relationship does not hold at higher concentrations (Fig. 1). The inhibition caused by sulphanilamide increases with increase in concentration which is in agreement with Lipmann's findings[310] (Fig. 1).

A-3-2- Effect of pH on the optical density change:-

Of interest is the effect of pH on the activation effect of asulam and the inhibition effect of sulphanilamide. Increase in pH is always accompanied by an increase in optical density in the presence of asulam compared to the control without asulam. When pH is plotted against increase in optical density this follows closely the ionisation curve for asulam obtained by the equation of Albert and Serjant (see Hayes[241]) -

$$\% \text{ ionization} = \frac{100}{1 + \text{antilog}_{10}\,(pKa - pH)}$$

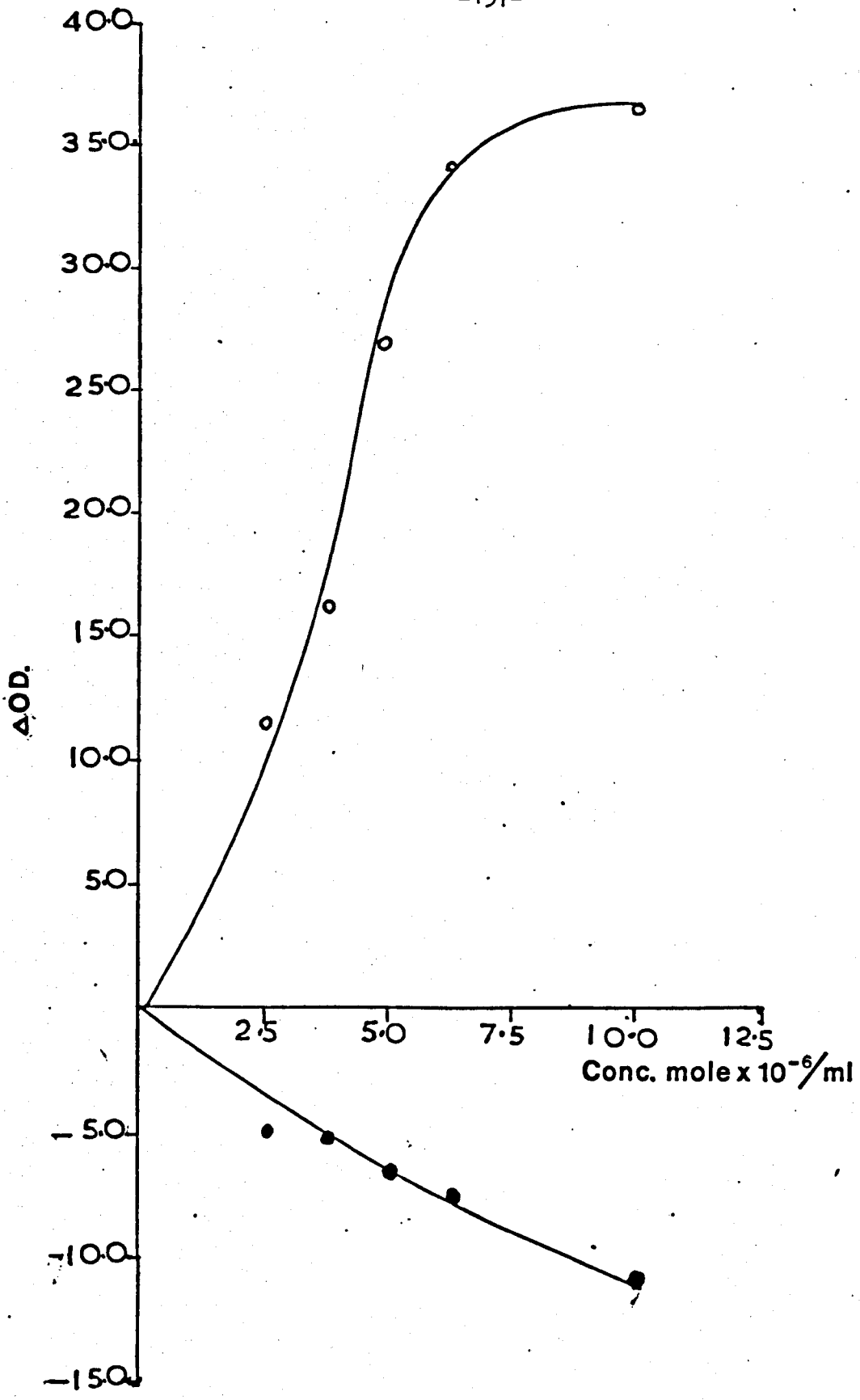


Fig. 1 Influence of asulam and sulphanilamide concentrations on the oxidation of PABA by horseradish peroxidase.
asulam (o—o), sulphanilamide (•—•).

(Fig. 2). However for sulphanilamide the inhibitory effect is at a minimum at pH 4.6 where the enzyme is highly active. This inhibitory effect rises to a constant value between pH 5.0 and 6.6 and falls again at pH 7.6 where the enzyme is least active (Fig. 2).

A-3-3- Possible causes of the interaction:-

The effect of asulam on PABA oxidation, as noted with many organic anions in similar situations, can be due to changes in the negative electrokinetic potential of the substrate micelles brought about by the anionic nature of the additive,[148] (asulam pKa 4.82; sulphanilamide pKa 10.70). The fall in the inhibitory power of sulphanilamide with rise in pH can be[188,381] attributed to changes in the hydrophobicity of sulphanilamide and/or changes in the hydrophobic nature of the binding sites of the enzyme. These changes can alter the binding affinities between sulphanilamide and the enzyme.

In the preliminary experiments where H_2O_2 was limiting the final optical density is the same irrespective of the amount of asulam present (Fig. 3), thereby indicating that asulam in the enzyme system acts solely as an activator. However, the possibility that asulam does participate in this reaction cannot be ruled out altogether at this stage as it is not inconceivable that the RNH• radicals which are known polymerisation initiators[62,485] and are produced during the enzyme phase of PABA oxidation could act on asulam directly.

A-3-4- Effect of asulam and sulphanilamide combination:-

In the case of asulam and sulphanilamide combination (Table 1) a slight non-significant decrease in optical density at the highest concentration of sulphanilamide and lowest concentration of asulam was noted. At all other concentrations an increase in optical density was observed. The effect of this combination is of interest despite the fact that no detectable hydrolysis of asulam to sulphanilamide occured in the soil. However, traces of sulphanilamide were detected in the commercial formulation and in plants treated with asulam[297] (see Ch.3.).

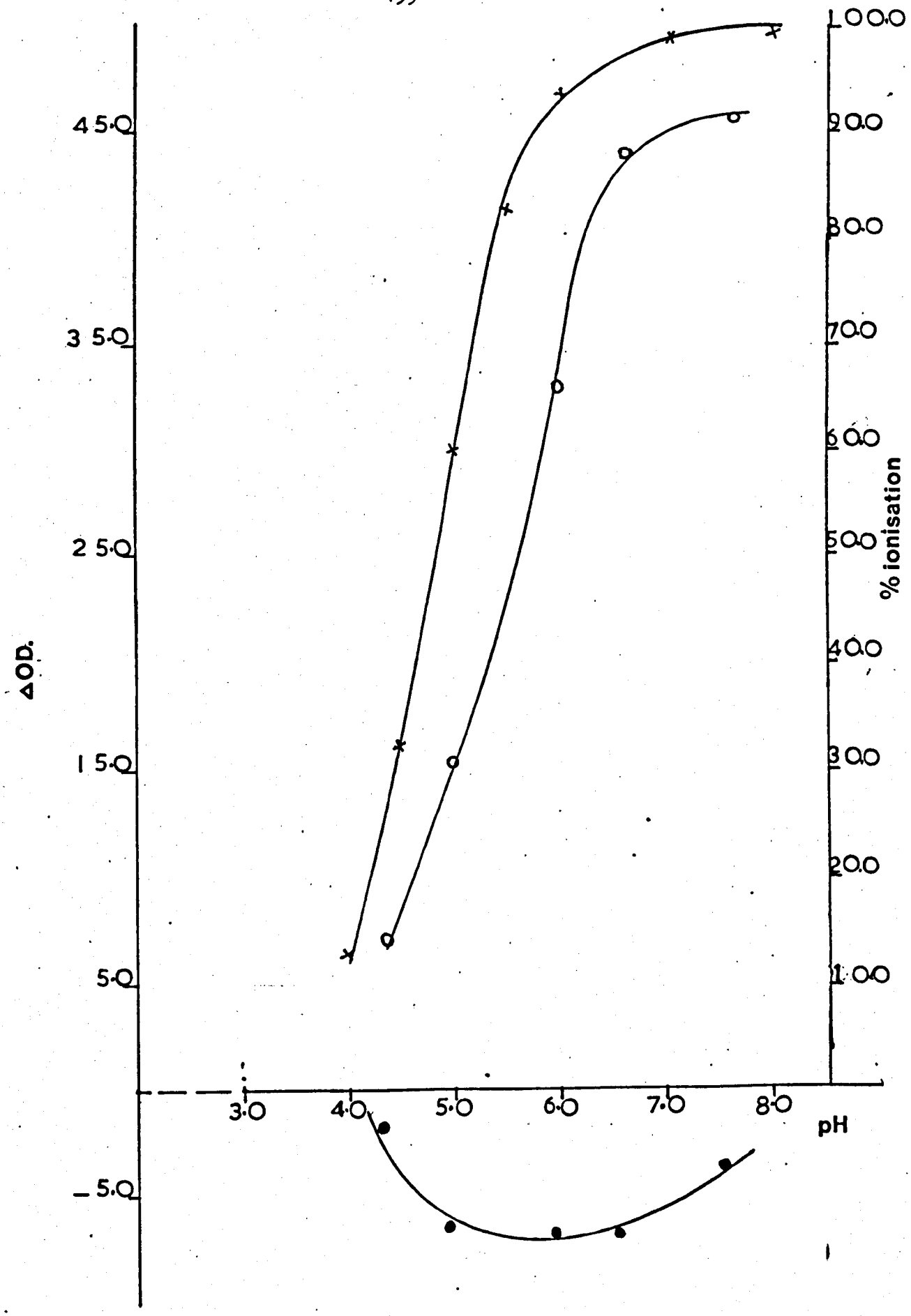


Fig. 2 Influence of pH on the oxidation of PABA by horseradish peroxidase. asulam (o—o), sulphanilamide (•—•), % ionisation (×—×).

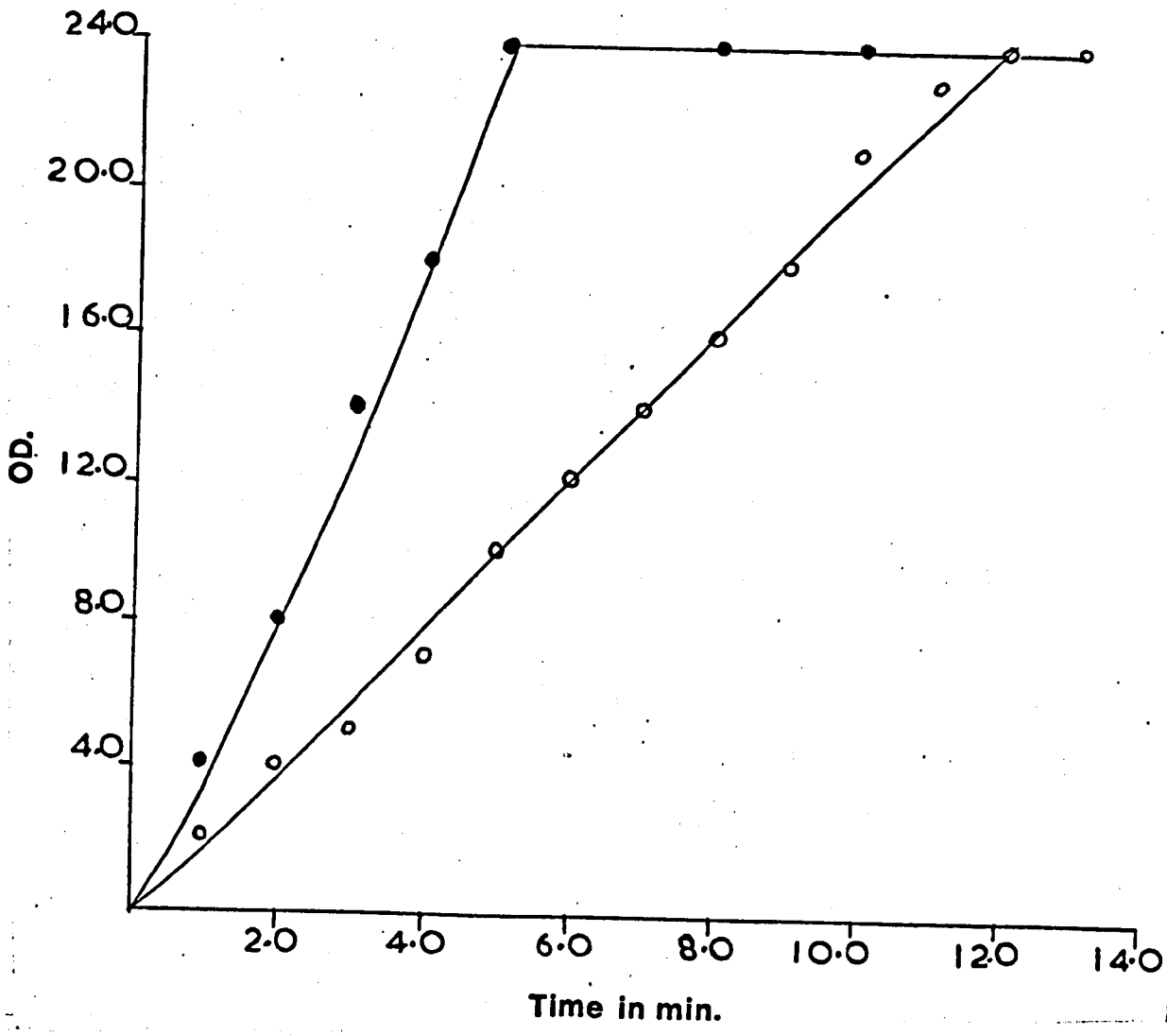


Fig. 3 Influence of asulam on the oxidation of PABA by horseradish peroxidase, with asulam (•—•), without asulam (o—o).

Table 1 Influence of asulam and sulphanilamide combination on PABA oxidation by horseradish peroxidase

concentration mole x 10^{-6}/ml		Optical density change			Mean	Standard deviation ±
Asulam	Sulphanilamide					
0.00	0.00	21	21	23	21.7	0.9
1.25	5.00	20	21	20	20.3	0.5
2.50	3.75	28	28	29	28.3	0.5
3.75	2.50	35	40	38	37.7	2.1
5.00	1.25	46	46	45	45.7	0.5

A-3-5- Possible biological significance of the interaction:-

The role of peroxidases in the soil, at present, is a matter of some speculation.[58] Recent reports on fungicidal, bacteriocidal and virucidal effects of peroxidases[48,62,300,301] open up the possibility that they may be agents of microbial antagonism.[58] They detoxify some aromatic compounds including many herbicides by polymerisation.[58] Their possible role in microbial ecology was highlighted by the interaction observed between the two soil fungi Penicillium piscarium and Geotrichum candidum (a peroxidase producing organism) when propanil is added.[61] The two fungi together bring about an efficient degradation of propanil and the mycelial yield is greater than the control, without propanil. On the other hand propanil is toxic to either fungus alone.[61] The anti-bacterial activity of peroxidases has been reported to be enhanced by some anions e.g. halides.[300] It is not known how exactly peroxidases perform this role, but one of the possibilities is that the antibacterial effect is mediated by oxidation of an intermediate substance.[300] The possibility that PABA , a known essential metabolite for many bacteria and some fungi,[12,166] and a hydrogen donor substrate for peroxidases, may play a role in such an interaction cannot be ruled out at this stage.

The reconciliation of the asulam-peroxidase interaction with 1) the rapid disappearance of asulam under conditions favouring microbial proliferation and 2) the susceptibility and resistance in vitro of some fungi to asulam[330,331] needs further study. The importance of the asulam-peroxidase interaction is evident when it is borne in mind that asulam is incorporated into herbicidal mixtures for both pre-emergence and post-emergence[8] application. Of particular interest is the mixture between asulam and diuron,[8] a substituted urea herbicide. Many herbicides of the latter group are known to form azobenzenes and polymeric mixtures.

Horseradish peroxidase has been used by many workers for investigating herbicide transformations in soil.[38,40,59,60,62] Similar end products have been identified in *in vivo* studies and in *in vitro* studies using horseradish peroxidase or peroxidases isolated from the soil.

The diversity of origin of soil peroxidases from both micro-organisms and plants,[316] and the variation in substrate specificity among peroxidases for their hydrogen donor substrates,[316] make it rather difficult to draw firm conclusions from this series of experiments alone. Nevertheless the possibility is pointed out that herbicides may affect the activity of peroxidases towards other substrates. These substrates can be present in nature e.g.PABA or added by man e.g. herbicides. The effects of interactions of the type studied here viz asulam-peroxidase have to be borne in mind when herbicidal mixtures are proposed particularly if one of the components of the mixture or a breakdown product can form azobenzene or polymeric products. However further studies with asulam and a range of peroxidases from different sources and different hydrogen donors should help to clarify the situation and make firm predictions more feasible.

B-1- Interaction between asulam, horseradish peroxidase and IAA:-

This aspect of the study had its origin in the findings that asulam at higher concentrations curtailed maize growth while at lower levels stimulated the growth of maize plants (Ch.3, D-3-9). Many chemicals, including some monohydric phenols,[386] some herbicidal triazines[118,154,208,388] (atrazine, ametryne and simazine) and maleic hydrazide,[208] show similar behaviour. This has been attributed to the increase in activity of IAA-oxidase brought about by these chemicals.[118,154,208,386,388] Such an effect has been observed both *in vitro* and *in vivo* for monohydric phenols[386] and *in vivo* for the triazine derivatives.[118] The effect due to maleic hydrazide is of interest because it has been the subject of investigations by many distinguished scientists[12,20,313] and opinions are still divided. The following

modes of action for maleic hydrazide have been suggested:

1. it acts as an antiauxin.[12] This is suggested by its in vitro stimulatory effect on IAA-oxidase activity which is said to be similar to the behaviour of certain monohydric phenols. The similarity between maleic hydrazide and monohydric phenols is said to be both in structure (maleic hydrazide in solution exists only as the enol structure) and in biological activity.[12]

2. it acts as an antigibberellin.[20] Conflicting evidence exists in the literature on this point.[12,20]

3. its action may be at a deeper more general level, underlying all growth processes and not at any site specific to one or other hormone.[20]

Though the latter possibility cannot be readily denied the complexity of factors governing the distribution and metabolism of IAA in plants,[86,139,252,383,387] the participation of gibberellins in IAA synthesis,[474] the distribution and variation in response of IAA-oxidases in the same plant and different species,[148] coupled with other complications, (see B-3-3) might have masked the in vivo effect of maleic hydrazide.

B-1-1- The role played by the oxidative destruction of IAA in plant growth:-

The discovery that the oxidative degradation of IAA plays a role in growth regulating functions of IAA[252,360] confirms the observation made by early workers that an inverse relationship exists between the activity of IAA-oxidase and plant growth.[86,195,383,387] The stimulatory and inhibitory effects of IAA in simple and in higher more complex species can be traced to a common biochemical origin which resides in the oxidative destruction of IAA.[360]

B-1-2- The oxindole pathway of IAA:-

An oxindole pathway has been found in plants and bacteria[360] (Fig. 1). Intact plants and peroxidases of plant origin as well as fungal peroxidases oxidize IAA to 3-hydroxymethyloxindole which in turn is readily dehydrated

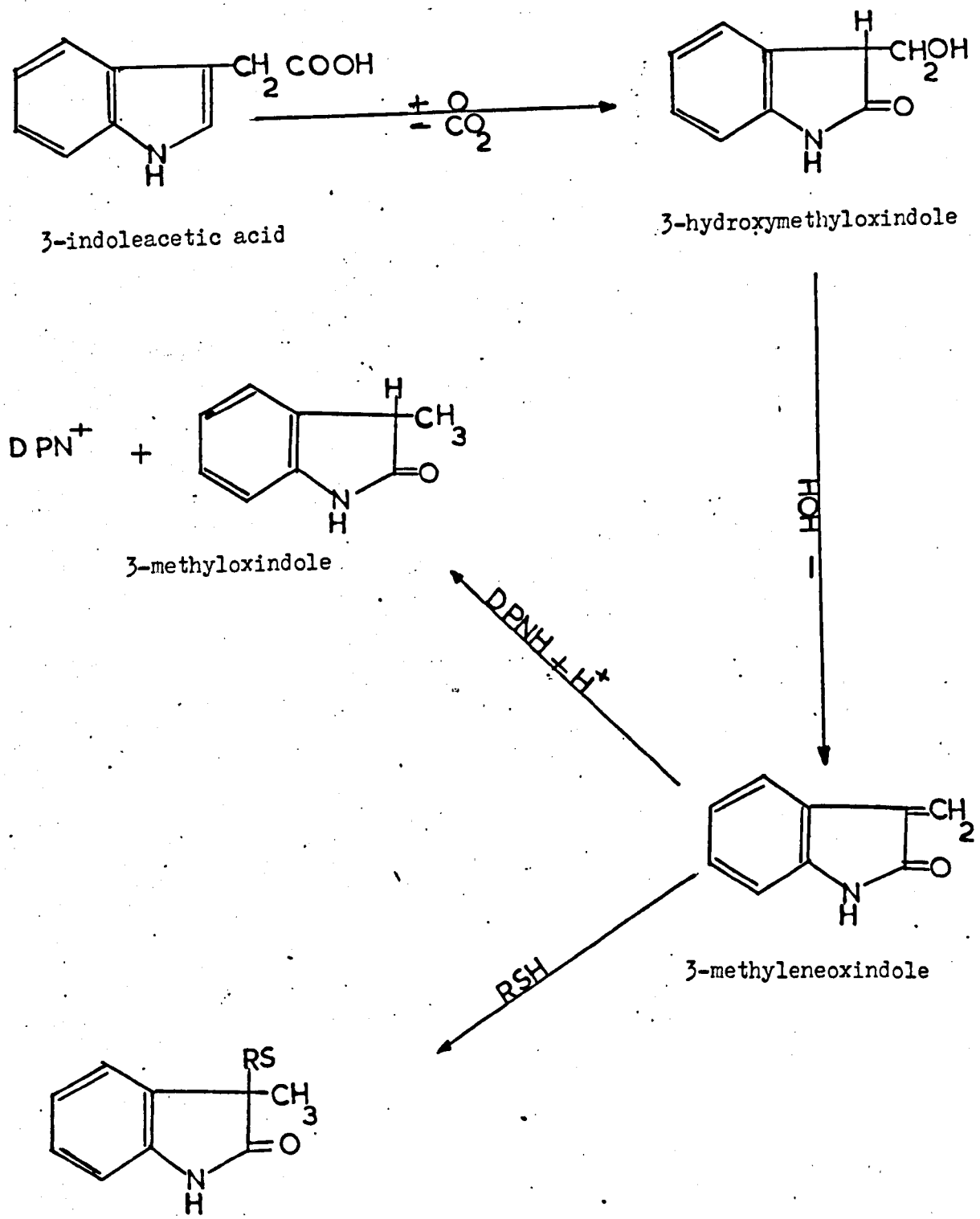


Fig 1 The Oxindole pathway of 3-indoleacetic acid metabolism (see ref. 360)

to 3-methyleneoxindole at physiological pH.[360] This non-enzymic reaction is accelerated in solutions of comparable ionic strength to that found in biological systems.[360] 3-Methyleneoxindole reacts rapidly with sulphydryl groups including sulphydryl enzymes.[360,446] The biological activity of 3-methyleneoxindole in higher plants and microorganisms is attributed to this reaction.[360] 3-Methyleneoxindole like other sulphydryl reagents is capable of releasing regulatory enzymes from sensitivity to feed back control[302,327,360,460] and thus has the potential for accelerating metabolism at relatively low concentrations and inhibiting at high concentrations.[360] Such paired effects are frequently observed in nature with the parent compound IAA.[360]

B-1-3- Detoxification of 3-methyleneoxindole:-

Detoxification by enzymatic reduction to 3-methyloxindole occurs in both microorganisms and higher plants.[360] Auxin like herbicides inhibit this reduction and so lead to accumulation of 3-methyleneoxindole.[360] Another detoxification pathway is through complex formation between these oxidation products and macromolecules (t-RNA).[292,302] Such complexes were once thought to be responsible for the growth regulating effect of IAA oxidation products.[252]

B-2- Experimental:-

Asulam, sulphanilamide and the enzyme unless stated otherwise were as described in the previous experiment. IAA was obtained from Aldrich Chemical Co. Ltd. and was dissolved in an equimolar solution of warm sodium bicarbonate. All other chemicals were dissolved in 0.04M phosphate buffer at pH 6.0. Six ml of the IAA stock solution (52µg/ml ca. 2.97×10^{-7} mole/ml) were used throughout. The final volume of the reaction mixture was 15ml (20.8 µg/ml ca. 1.16×10^{-7} mole/ml in the final solution). For studying the effect of pH on IAA destruction 5ml of a concentrated acetate or phosphate buffer at pH 4.2 and 5.2 were used. The concentrations of these buffers were calculated so as to have a final concentration per ml similar to the buffers used by Coggins and Crafts.[105] The reaction mixture was allowed to equilibrate in a water bath at 35°C for 10 min. The reaction was started by

adding 0.1ml of the appropriate enzyme concentration. Two ml samples of the reaction mixture were added to 2ml of 0.05M $NaAsO_2$ to stop the reaction. 1AA was determined by the method of Tang and Bonner.[456]

Each treatment was carried out at least in duplicate. The reaction conditions were selected from preliminary trials in a manner similar to that described in the previous experiment.

B-3- Results and Discussion:-

With only one exception (Table 1 i)) addition of sulphanilamide and asulam enhances the rate of 1AA oxidative destruction by horseradish peroxidase when compared to the control.

Table 1 Influence of sulphanilamide concentration on the oxidative destruction of 1AA by horseradish peroxidase. Protein 16.0 (µg/ml)

Sulphanilamide concentration mole x 10^{-6}/ml	1AA destroyed (µg/ml)		Mean	Standard deviation ±	M-C[b]	% increase over control
i) 10 min						
0.00	5.3	4.2	4.8	0.6	-	-
0.25	5.3	4.2	4.8	0.6	0.0	0.0
0.75	5.9	5.3	5.6	0.3	0.8	16.7
1.25	5.9	5.9	5.9	0.0	1.1	22.9
2.50	6.4	6.4	6.4	0.0	1.6	33.3
ii) 20 min						
0.00	7.5	7.0	7.3	0.4	-	-
0.25	10.3	8.1	9.2	1.1	1.9	26.0
0.75	11.4	11.4	11.4	0.0	4.1	56.1
1.25	11.4	12.0	11.7	0.3	4.4	60.3
2.50	12.5	12.5	12.5	0.0	5.2	71.3

M-C[b] = Mean treatment - Mean control

For each experiment a control was included to cater for possible changes of peroxidase activity produced by light. Light affects peroxidase activity[434] and was not controlled in this series of experiments. The increase produced

by asulam is higher than that produced by sulphanilamide (143.2% compared to 84.1% relative to control samples (Table 2).

Table 2 Influence of asulam and sulphanilamide on the oxidative destruction of 1AA by horseradish peroxidase (asulam and sulphanilamide concentration 1.25×10^{-6} mole/ml, protein concentration 16.0µg/ml). Assessed after 30 min.

Treatment	1AA destroyed (µg/ml)		Mean	Standard deviation ±	$M-C^b$	% increase over control
Enzyme	4.4	4.4	4.4	0.0	-	-
Enzyme + asulam	11.0	10.4	10.7	0.3	6.3	143.2
Enzyme + sulphanilamide	8.1	8.1	8.1	0.0	3.7	84.1

$M-C^b$ = Mean treatment - Mean Control

B-3-1- Influence of time and asulam and sulphanilamide concentrations:-

The enhancements brought by asulam and sulphanilamide differ in the following aspects :-

1. Quite a substantial increase over the control is brought about by asulam in a short period of time (146.6% in 4 min) [Table 3]. The corresponding increase brought about by a similar concentration of sulphanilamide (1.25×10^{-6} mole/ml) is 33.3% (Table 4).

2. Sulphanilamide at the lowest concentration (0.25×10^{-6} mole/ml) shows no increase over the control in the initial 10 min (Table 1). The comparable increase for a similar concentration of asulam is 87.0% (Table 5).

3. The acceleration due to sulphanilamide increases with time while that due to asulam decreases (Table 4 and 5). However, it must be stressed that in all cases the acceleration due to asulam is always greater than that for sulphanilamide.

Similar findings to the ones observed here with asulam have been reported for p-hydroxybenzoic acid.[386]

Table 3 Influence of asulam on the oxidative destruction of 1AA by horseradish peroxidase. Asulam concentration 1.25×10^{-6} mole/ml. Protein 16.0 µg/ml.

Time in min	1AA destroyed (µg/ml)		Mean	Standard deviation ±	M-C[b]	% Increase over control
4	1.2	1.8	1.5	0.3	-	-
	3.7	3.7	3.7[a]	0.3	2.2	146.7
8	3.1	2.5	2.8	0.3	-	-
	6.1	6.1	6.1[a]	0.0	3.3	117.9
12	3.7	3.7	3.7	0.0	-	-
	10.3	9.2	9.7[a]	0.5	6.0	162.2
16	4.9	4.9	4.9	0.0	-	-
	12.2	12.2	12.2[a]	0.0	7.3	149
20	6.1	6.1	6.1	0.0	-	-
	15.3	15.3	15.3[a]	0.0	9.2	150.8

[a] With asulam M-C[b] = Mean treatment - Mean control

Table 4 Influence of sulphanilamide on the oxidative destruction of 1AA by horseradish peroxidase. Sulphanilamide concentration 1.25×10^{-6} mole/ml. Protein 16.0 µg/ml.

Time in min	1AA destroyed (µg/ml)		Mean	Standard deviation ±	M-C[b]	% Increase over control
4	1.2	0.6	0.9	0.3	-	-
	1.2	1.2	1.2[a]	0.0	0.3	33.3
8	2.5	1.8	2.2	0.3	-	-
	2.5	2.5	2.5[a]	0.0	0.3	13.6
12	2.5	2.5	2.5	0.0	-	-
	4.9	4.7	4.8[a]	0.6	1.3	52.0
16	3.7	3.7	3.7	0.0	-	-
	6.7	6.1	6.4[a]	0.3	2.7	73.0
20	4.9	4.9	4.9	0.0	-	-
	8.6	8.6	8.6[a]	0.0	3.7	75.5

[a] With sulphanilamide M-C[b] = Mean treatment - Mean control

Table 5 Influence of asulam concentration on the oxidative destruction of 1AA by horseradish peroxidase. Protein 16.0 µg/ml.

Asulam concentration mole x 10^{-6}/ml	1AA destroyed (µg/ml)		Mean	Standard deviation ±	M-C[b]	% Increase over control
a-10 min						
0.00	2.6	2.0	2.3	0.3	-	-
0.25	4.6	3.9	4.3	0.3	2.0	87.0
0.75	5.9	6.2	6.1	0.2	3.8	165.2
1.25	7.1	7.1	7.1	0.0	4.8	209.0
2.50	9.7	9.7	9.7	0.0	7.4	321.7
b-20 min						
0.00	6.5	5.9	6.2	0.3	-	-
0.25	10.4	9.8	10.1	0.3	3.9	62.9
0.75	12.4	11.6	12.0	0.4	5.8	93.5
1.25	13.7	13.7	13.7	0.0	7.5	121.0
2.50	17.6	17.6	17.6	0.0	11.4	183.9

M-C[b] Mean treatment - Mean control

B-3-2- Possible causes of the interaction:-

The enhancement of 1AA oxidative destruction by sulphanilamide contrasts with its action on the oxidation of PABA. In general terms this behaviour can be explained on the basis that both activities [i.e. 1AA-oxidase and peroxidase] reside on different sites.[268, 415, 434, 450] However as to the location of these sites three hypotheses have been mooted.[60]

1- The two types of activities lie on separate and distinct enzymes.[422]

2- The two types of activities are resident on one enzyme-peroxidase, but with two active centres.[434, 450]

3- Attention is drawn to the possibility of peroxidase isoenzymes - which are known to exist in the case of peroxidase. One member of the family of isoenzymes could be envisaged as being the primary site of 1AA-oxidase

activity.[326] However, though many isoenzymes have been isolated dual activity is found to be the case in most if not all of them.[268,326]

The observed differences in patterns as exhibited by the acceleration brought about by sulphanilamide and asulam (see above) may be due to the possibility that the sites on which the two types of activities reside are neighbouring sites. The binding of sulphanilamide to the sites involved in peroxidation reactions - sulphanilamide competitively inhibits PABA oxidation by horseradish peroxidase - might have resulted in shielding of the sites with 1AA-oxidase activity. Such shielding may then be responsible for the comparatively lower rate of 1AA destruction compared to asulam (asulam should bind less to the enzyme due to its anionic character and hydrophilicity). The shielding effect of sulphanilamide is demonstrated by following the change in acceleration brought about by a fixed concentration of sulphanilamide at varying enzyme concentrations (Table 6). An acceleration in the % increase caused by sulphanilamide occurs with time. With few exceptions the % increase is highest at higher enzyme concentrations. The discrepancy may be due to limitations in the detection method. This could be deduced as it occurs at the shorter time interval only (Table 1, 2 and 6). However, an additional activation due to the anionic nature of asulam could be responsible at least in part for the observed differences between the two chemicals. This latter suggestion seems to be supported by the increase in 1AA destruction at pH 5.2 compared to 4.2 in the presence of asulam while the reverse is true in its absence (Table 7).

The shielding effect of sulphanilamide, and the comparatively low reaction rate resulting thereof, presumably leads to a lower rate of free radical formation compared to asulam. This may have a sparing action on the enzyme, which is known to be destroyed by the free radicals thus produced.[175,360] This may be responsible for the increase with time observed for the acceleration brought about by sulphanilamide (Table 1, 4 and 6) and the corresponding decrease observed with asulam (Table 3 and 5).

Table 6 Influence of enzyme concentration on the oxidative destruction of 1AA by horseradish peroxidase with and without sulphanilamide. Sulphanilamide concentration 1.25×10^{-6} mole/ml.

Protein concentration (µg/ml)	1AA destroyed (µg/ml)		Mean	Standard deviation ±	M-C[b]	% Increase over control
1) 15 min						
20.0	4.6	3.3	4.0	0.7	-	-
	7.8	7.8	7.8[a]	0.0	3.8	95.0
16.0	3.3	2.6	3.0	0.4	-	-
	5.2	7.2	6.2[a]	1.0	3.2	106.7
12.0	3.4	2.8	3.1	0.3	-	-
	4.7	4.0	4.4[a]	0.3	1.3	41.9
8.0	2.2	2.1	2.2	0.1	-	-
	2.8	2.2	2.5[a]	0.3	0.3	13.6
4.0	1.2	1.2	1.2	0.0	-	-
	1.6	1.6	1.6[a]	0.0	0.4	33.3
2) 30 min						
20.0	7.8	7.8	7.8	0.0	-	-
	17.6	16.9	17.3[a]	0.3	9.5	121.8
16.0	6.5	6.5	6.5	0.0	-	-
	14.3	14.3	14.3[a]	0.0	7.8	120.0
12.0	5.3	5.3	5.3	0.0	-	-
	10.9	10.9	10.9[a]	0.0	5.6	105.7
8.0	3.4	3.4	3.4	0.0	-	-
	6.5	7.2	6.9[a]	0.3	3.5	102.9
4.0	2.2	2.2	2.2	0.0	-	-
	3.4	3.4	3.4[a]	0.0	1.2	45.5

[a]With sulphanilamide M-C[b] Mean treatment - Mean control

B-3-3- Possible biological significance of the interaction:-

These findings (the effect of asulam on the growth of maize and on the activity of 1AA-oxidase *in vitro*) suggest that asulam may interfere indirectly with auxin metabolism. The site of asulam action is reported to be the growing points of plants and the activity as being due to inhibition

of cell division.[331] Recent reports confirm these findings and show that a decrease in respiratory activity as well as in protein and nucleic acid contents follow.[330]

Table 7 Influence of pH on the oxidative destruction of IAA by horseradish peroxidase with and without asulam. Asulam concentration 1.25 x 10^{-6} mole/ml. Protein 8.0 µg/ml (R.Z. 0.48). Assessment made after 15 min.

pH	IAA destroyed (µg/ml)			Mean	Standard deviation ±	M-C[b]	% Increase over control
4.2	9.8	10.4	11.7	10.6	0.8	-	-
	13.0	13.0	13.0	13.0[a]	0.0	2.4	22.6
5.2	6.4	7.0	7.0	6.8	0.1	-	-
	17.6	17.6	17.6	17.6[a]	0.0	10.8	158.8

[a] in presence of asulam M-C[b] = Mean treatment - Mean control

It is very difficult in such situations to distinguish between the cause and the effect. However, it is the opinion of some workers[12,355] and it would seem reasonable, that the classification of herbicides as inhibitors of growth, cell division or photosynthesis is undoubtedly an oversimplification. Carbamates for instance, were first classified as inhibitors of cell division,[12] then it was realised that some carbamates also inhibit photosynthesis.[12] Amitrole which is considered primarily as an inhibitor of chloroplast synthesis is an inhibitor of catalase activity in animals and an inhibitor of phosphorylase activity in plants.[12] Triazines were considered originally as photosynthetic inhibitors[207,374] but growth regulator-like effects were later reported for them.[118,154,208,388]

It is reasonable to assume that the application of any herbicide would affect more than a single vital process.[12] As mentioned previously,

for a chemical to modify growth, it must arrive at the physiological sites through which its actions are manifested. A variety of environmental and edaphic forces act to alter structurally, to destroy or to remove, externally applied herbicides, rendering them unavailable to the plant. Anatomical, morphological, biochemical, and physiological factors operate to control herbicidal entry into and distribution within the plant (see previous chapters).

Internally, additional factors, such as metabolic alterations, adsorption to inactive sites, and complex formation reduce the availability of the herbicide for reactions through which phytotoxicity is expressed. A herbicide's successful arrival at the site of action is controlled in part by its structural configuration, chemical composition and physical properties (see Ch.1). It is of interest to mention here that the situation is more complicated for herbicides which interfere with auxin metabolsim. Variations in auxin content and peroxidase activities in plants occur with age and in different plant tissues.[86,139,252,383,387]

It should also be pointed out that the importance of enhancement of oxidative destruction of IAA is not restricted to plants alone. Many microorganisms are known to be able to synthesise IAA e.g. Fusarium oxysporium,[5] Pseudomanus fluorescens,[5] P. solanacearum,[5] Bacillus liquefaciens[213] and some Agrobacterium and Rhizobium species.[101]

Fungal peroxidases are known to destroy IAA as exemplified by Cephalia flavida peroxidase[395] and many microorganisms e.g. Schizosaccharomyces pombe, Escherichi coli and Salmonella typhimurium are known to be adversely affected by 3-methyleneoxindole.[360]

These findings suggest that asulam and sulphanilamide may affect microbiol ecology indirectly through their effect on the oxidative destruction of IAA. However, in a complex medium like the soil one would expect that toxicity will depend on the presence of peroxidase producing organisms as well as IAA producing organisms. Other factors such as the effect of soil

pH, salt content and their effect on the non-enzymic conversion of 3-hydroxymethyloxindole to the more potent 3-methyleneoxindole are of prime importance. Of equal importance are the effects of soil reactions e.g. sorption processes on the availability of 3-methyleneoxindole for microbiol uptake.

CHAPTER V

BRACKEN CONTROL.

1- Introduction :-

Formerly bracken was a plant of very considerable value for thatching, litter etc.(see Ch. 1, 8-2). Nowadays bracken has become too abundant, its uses have been superseded, and universely it is regarded with disfavour.[70] Its ability to thrive over a wide range of habitat conditions is one of the main reasons it has become such a serious pest.[70]

Cultural and herbicidal practices for bracken control often are prohibitively expensive and have provided inconsistent results.[285]

The heart of the problem is the extensive rhizome system of the plant which can penetrate to a depth of two feet (in fertile soils),[67] overwinters and produces new top-growth (fronds) the following season.[113] Measured by weight there is more of the bracken plant below the ground (20-40 ton/acre) than there is above it (6 tons/acre).[172] Practices which fail to destroy the rhizome system fail to provide effective control.[116]

In this chapter some of the work done on bracken control (cultural, biological and chemical) is reviewed in the hope of throwing some light on the bracken problem. The inconsistency which dominates the results is discussed in the light of the work done here (see Ch. 2) and in conjunction with the work done with some other rhizomatous perennial weeds which exhibit similar powers to resist eradication by similar control measures.

1-2- The rhizome system:-

The large leaves or fronds (the above ground portion of the plant) arise from a mass of tangled branches which constitute the rhizome system of the plant (the underground portion of the plant).[109] Conway[108] pointed

out that the branches of the rhizome system can be divided into:-

1) Thick rhizomes that run deeply into the soil and are the main agents responsible for the outward expansion of the colony. Normally they carry few fronds or frond buds.

ii) Thinner smaller branches running near the soil surface. These are the main frond-bearing rhizomes.

iii) Intermediate branches linking i) and ii) and capable of developing into either as the environmental factors act on them.

She further stated that closer examination of branches of type ii) at the end of the growing season showed that they may be divided into:-

a) Those carrying expanded fronds with one or more frond buds near the rhizome apex.

b) Other similar rhizome branches which carry frond buds but no expanded fronds.

These buds may remain in a healthy state below the ground. Hodgson[254] estimated that ca. a half million frond buds may be present in an acre of infested land, and that not more than 20 to 40% of this amount develops into frond buds in one season while the rest stays dormant. Smith[441] believed such buds to be capable of long periods of dormancy, and cited examples of buds 18 years old.

1-3- How the plant spreads:-

Most of the increase of bracken in Britain and particularly Scotland is via asexual or vegatative methods[47,110] (elongating from the tips of the underground system and the breaking up of the old congested regions).[47] Young plants grown on specially prepared troughs have been seen to produce an underground system spreading in all directions with a radius as much as six feet in the first year.[109] In the field, mature bracken grows much more slowly. Watt[490] in England stated that the growth of a colony

may be little more than 3 or 4 inches each year. However in the more fertile soils of the West of Scotland much greater extension has been seen.[109] Expansion of a bracken colony in this way can be stopped by digging a trench round it.[67]

Spreading sexually by spores (produced in astronomical numbers[172]) may occur in certain seasons and under certain conditions and it may be essential for colonisation of new sites.[109] However few records exist of the finding of young sporeling plants of bracken in the fields of Scotland.[112,113] The establishment of the young sporophyte in Scotland may be limited by 1) climatic conditions[113] (late dispersal of spores - mid to late August - in most years) 2) edaphic factors[113] (soil acidity) and 3) biotic factors[112,113] (several species of soil insects and soil fungi were reported to attack young bracken sporophytes and prothalli).[112]

2- Control measures:-

2-1- Cultural:-

2-1-1- Ploughing:- Ploughing is a very effective method, and areas which can be so treated present little difficulties.[109,167] Autumn ploughing and exposure to winter frost is usually effective.[109] The sensitivity of bracken to freezing could be exploited for bracken control if the field could be worked in the late autumn or early winter. Similar control measures are effective in controlling johnsongrass (Sorghum halpense).[339] Spring ploughing on the other hand may simply scatter the pieces of the rhizome and create numerous new-growth centres. Similar results have been reported with many other perennial rhizomatous weeds.[13,46,263,365] For summer ploughing see 2-4-2-3-1.

Ploughing is quite successful in places like the North Pacific-Coast area where it is customary to break the fronds with heavy drags and follow immediately with deep ploughing. The rhizomes are then harrowed out,

piled and burned.[401] It is however, the very large tracts of unploughable land in Scotland and elsewhere that cause concern.[116]

2-1-2- Cutting:- Cutting the frond constitutes an indirect way of attacking the rhizome. The underlying principle of assault on the frond is based on 1) recognition of the frond as the organ which provides nutrients for the growing regions and builds up food reserves by means of its photosynthetic activities[116,441] 2) during frond formation, the fronds develop at the expense of the food reserves.[116,441] So it was hoped that continued cutting at the point of low food reserves (see Ch. 1, 5-3) would exhaust the rhizome by 1) prevention of replenishment of the reserves[68] and 2) cutting may induce the development of buds that would normally remain dormant for a year or longer.[116] This may lead to further exhaustion of rhizome food reserves as well as exhaustion of the bud reserves.[116,362]

Cutting by its very nature (slow and laborious)[68,69] obviates the necessity of early start and repeated cutting.[68] Repeated cutting as stated by Jarvis[285] is a soul-destroying business, for the plant has more patience than the farmer and does not grow old.

In certain situations viz newly infected sites or on shallow less fertile land, cutting could i) lead to depletion of food reserves[285] (presumably there will not be much from the beginning) and 2) reduce the litter layer. Such effects will render the plant more susceptible to adverse weather conditions e.g. frost,[489] heat and drought.[493] This could help to explain the observations that 1) some local patches of bracken die out quickly after treatment while others do not appear to suffer as much as their adjacent neighbours[67] and 2) resistance to eradication by cutting is not the same all over the country.[68] So it would appear that cutting would not eliminate bracken in all sites but may merely subjugate bracken in the case of well established bracken stands in fertile soils.

2-1-3- Flooding :- It is well known that bracken is a plant requiring good conditions of soil drainage.[390] Rise in the water table or poor drainage may discourage spreading, check growth of bracken and may lead to the death of an already established bracken patch.[70] Similar sensitivity to flooding has been reported for johnsongrass and is considered to be one of the means for its control.[338] Flooding which has once been used to check the rapid increase of bracken could be effective on flat areas.[67] But the slightest rise in ground level above the normal or the presence of rocks may give rise to 'islands' of subdued but live bracken which could recover and establish an infection centre. Moreover flooding was found to create other problems viz an increase in the incidence of liver-fluke in sheep.[67]

2-2- Biotic factors :-

2-2-1 Cattle :- Trampling by cattle could provide a good means for bracken control. In New Zealand Robbins et al.[401] reported that many thousands of acres of bracken have been cleared mainly by heavy stocking of cattle. The manure resulting from cattle dung may stimulate bud growth. The delicate buds will be destroyed by trampling and this will lead to destruction and exhaustion of both buds and food reserves with time. However there is a grave risk of bracken poisoning.[262] Some authors believe that cattle poisoning by bracken is over-emphasised, judging probably from its sporadic occurrence.[465] Such a conclusion should be taken with caution. Though cattle do not normally eat bracken, hunger may force them to do so, and this is more likely in thick bracken stands where no other vegetation may be able to grow.[264] Moreover Braid[69] has stated that 1) even consumption of well known poisonous plants like the yew Taxus baccata is not always succeeded by death or even by discomfort and 2) toxicity varies with latitude and most plants are much less toxic in the north cooler climate than further south. On the whole the work of Professor Evans and others[68, 164, 165, 242, 436]

leaves no doubt about toxicity of bracken to cattle and that it could be fatal.[465] It will be much safer to use cattle in the follow-up treatment (see 2-4-2-3-2).

2-2-2- Sheep grazing :- It is generally acknowledged that the introduction of sheep farming to the Highlands made the bracken problem more serious.[172,262] However though bracken is normally unpalatable to sheep, persistent grazing of bracken by sheep has been observed.[197] In extreme cases this has resulted in complete clearance of bracken in 3 to 4 years from patches where it was originally so vigorous that no other herbage grew.[197] Some workers believe that grazing may not be the sole reason for the death of bracken, but the weakening of bracken which results from grazing might have encouraged invasion by microorganisms[198] (see 2-3-3). Other workers have reported that intensive grazing by sheep aided by vigorous competition from crowberry (Empetrum nigrum) leads to satisfactory control of bracken.[168a] In both cases no ill effects were reported on sheep.

However recent work has shown that ingestion of bracken by sheep causes progressive retinal degeneration.[488] Moreover sheep may loose interest in the fern before it is totally eradicated.[197]

2-3- Biological control by means of insects and pathogenic organisms :-

The possibility of biological control using insects, fungi and bacteria was considered but there did not appear to be much success in this approach. Bracken has been spreading over the world for the past few million years and it never appears to have come up against a vital biotic enemy which could threaten its existence and lead to its eradication. Epidemics are very rare.

2-3-1- Insects :- Simmods[437] reported that some European insects e.g. Hepialis fusconebulosus and other monophagus Lepidoptera are promising but the European biotic factors disfavour the build up of a large insect population and thereby lead to a lack of control under natural conditions. Though this

may be true, the work of Kaplains et al.[290] showed that bracken may contribute to the observed lack of control by insects. They were able to isolate the compounds alpha ecdysone and 20-hydroxy ecdysone (insect hormones) from bracken. They concluded that the presence of these biologically active substances in bracken may be responsible for the relative immunity of this fern to insect attack.

2-3-2- Fungi :- The possibility of biological control by the dissemination of parasitic fungi was one of the methods considered for control of bracken. A disease of Pteridium caused by the fungus Corticium anceps was first investigated in 1935 and many times since then.[329] The results were inconsistent. The disease is markedly affected by environmental conditions particularly atmospheric moisture.[329] It was then concluded that the variable climate in Britain makes the success of this treatment a remote possibility.[329] The disease was investigated again in the 1960s but it proved to be unsuccessful.[172]

In New Zealand the rhizomes of Pteridium are sometimes killed by species of Fusarium.[329] However, infection experiments gave variable results and infected rhizomes showed no sign of the fungus one year after treatment.

Another fungus Gloesporium pteridis causes a 'leaf roll' disease of bracken.[329] The diseased plants are normally stunted in growth. Drastic effects were observed only in exposed bracken.

2-3-3- Bacteria :- Bacteria causing disease in bracken has been isolated, but all attempts to infect healthy plants have failed.[172] Most cases appear to be secondary infections following insect or other damage.[172] Damage or weakening of bracken caused by sheep grazing[198] or other factors[67] was said to increase the liability of the plant to be infected by microorganisms.

2-4- Chemical control of bracken:-

From what has been mentioned above it could be seen that ploughing is not always feasible. Cutting is not of universal applicability, expensive,

laborious and could be very slow. Control by flooding, insects, bacteria or fungi gives inconclusive and inconsistent results. Trampling by cattle or sheep grazing of dense bracken stands is dangerous and may be fatal. So the need for other tools becomes obvious. As pointed out by Hodgson[254] the concept of bracken eradication by chemicals is not new and dates back to 1916 where the effectiveness of some inorganic compounds against bracken was tried but with little success. Since then a large number of herbicides (contact, foliar-systemic and soil-applied herbicides) were tried in many places in the world with varying degrees of success.[404] The rest of this review will deal with the herbicidal aspects of the problem.

2-4-1- Contact herbicides :-

Ammonium sulphamate,[67] sulphuric acid,[109] diquat[475] and sodium chlorate[109] were applied to fronds. All of these chemicals proved to be powerful agents for frond destruction but they have no obvious effect on the rhizome.[67,109] The results were not better, and sometimes inferior to scythe cutting and may be more expensive.[67,109]

Some workers in the Edinburgh School of Agriculture[155] reported a systemic action of diquat and paraquat at high dose rates 16 lb/acre a.i. on bracken which gave 56% and 89% reduction in frond density on the treated area and visibly affected bracken 9-10 feet outside the treated area in the year following treatment. Systemic action for these herbicides has been reported under very special conditions (high atmospheric humidity, low soil moisture and low light intensity).[71] However this is not always the case (see Ch. 1, 2-1-2-1).

2-4-2- Systemic herbicides :-

The results with contact herbicides were not rewarding. The discovery of systemic herbicides opens a new era in bracken control research and offers a possibility of attacking the rhizome chemically.

There are two ways in which the rhizome can be attacked chemically[219] viz:

1) By applying the chemical to the fronds, the chemical then translocates to the rhizome.

2) By applying the chemical to the soil before the fronds emerge.

2-4-2-1- Foliar-applied herbicides :-

In the 1950s and 1960s many chemicals were tried[336] and at the time appeared quite promising. Of these 4-CPA, dalapon and aminotriazole are reviewed here. The relatively recent herbicide asulam is also included in this review but the more recent herbicide glyphosate is not included due to the comparative lack of information on it with particular reference to the bracken situation. Dicamba and picloram although active when applied to the fronds (Table 5 and 6) are discussed mainly under soil-applied herbicides.

2-4-2-1-1- 4-CPA, dalapon, aminotriazole and asulam:- From the reference Tables 1-4 the following points (more or less) can be drawn for each herbicide.

1) A period of maximum susceptibility was observed. The best results were generally obtained when the majority of fronds had just completed the unfurling process. Early application or late application could lead to inadequate control.

2) Rainfall after application was found to reduce herbicidal activity (the exception was 4-CPA invert emulsion).

3) Marked variations in effectiveness of the herbicide was observed and it was more pronounced at lower rates.

4) Regeneration of bracken took place. The rate of regeneration varied a) from one site to another b) with the dose rate and c) with time of application.

These common points (major points) for the four chemicals suggest that the factors (or at least most factors) responsible for the variable effectiveness of these herbicides could be traced to common origins (differences in intrinsic phytotoxicity of individual herbicides cannot be

overlooked).

In theory the maximum susceptibility of plants to herbicides depends on several conditions being fulfilled (see Ch. I., Fig. 1);

i) Spray retention: Retention of the chemical by the fronds must be optimum. This will depend on:-

a) Weather conditions at time of spraying. Fletcher and Kirkwood[172] stated that the success of aerial applications of herbicides to bracken (using helicopters and aeroplanes) is rather dependent on the prevailing weather conditions and on the nature of the terrain. When a high wind is blowing the plane has difficulty in getting below 100 feet and much of the herbicide is dissipated over the surrounding countryside.

Heavy rain just before spraying may increase the possibility of run-off from wet fronds. However high turgidity may increase the wettability of bracken fronds (dry frond surface) particularly in exposed situations where the pinnae surface may be quite rough (see Ch. II, A). The degree of roughness of leaf surface is known to affect leaf wettability (see Ch. 1, 3-1-1).

b) Posture and stage of development of the frond : a large area for spray interception and retention will be provided by fully expanded fronds.

ii) Penetration:- Penetration of the cuticle must be optimum. This depends on 1) the thickness of the cuticle, which could vary from very thin to very thick, depending on the environmental conditions prevailing at the time of its formation (see Ch. 2; A) and the stage of growth of the frond. However, as stated by Martin and Juniper[318] all plant surfaces are subject to weathering. Soil particles blown by wind, or hail, affect the wax deposits on the cuticle and the cuticle itself, and may also damage the epidermal and mesophyll cells. Prolonged strong wind can produce similar effects. Dewey et al.[147] showed that wind, soil blown by the wind and heavy rain, damage the surface of pea plants. This results in an increased susceptibility to sprays with dinoseb due to increased spray retention and

probably increased penetration of the herticide.

With regard to the bracken plant slight abrasion of the pinnule surface was found to increase penetration of amitrole (see Ch. 2, C). Braid[68] found that (under natural conditions) the whipping of the fronds together in the wind often injured the finer leaflets. Jarvis[285] stated that the buffeting caused by the helicopter's downwash is likely to cause some damage to the cuticle and hence cut down its resistance to penetration.

The occurrence of such damage to the cuticle may be of significance in the penetration of water soluble and anionic herbicides e.g. amitrole, dalapon sodium, 4-CPA sodium or amine salts and asulam (see Ch.1 3-1-2). Humidity will also affect penetration particularly of water soluble compounds (see Ch. 2). Crafts[125] stated that all mobile compounds that are water soluble will move into the plant in greater quantity when the plant is in the saturated condition. Under drier conditions only the lipoid soluble ones enter, and these somewhat in proportion to their lipid solubility (see Ch.1. 3-1-2-2) This could help to explain in part the poor results obtained in 1959 (very dry summer) with amitrole and dalapon in various places.[254] Cook[117] in this laboratory using Phaseolus vulgaris demonstrated that under low humidity conditions no significant penetration of amitrole occurred over a period of 17h while 80 to 90% penetration took place in less than 3h under high humidity conditions (see Ch.2, C). Volger[478] recommended application of amitrole to bracken in the early morning to ensure conditions of high turgor, favourable to absorption and translocation (see Ch.1, 4, 5, and 6).

The thick cuticle which is expected under dry conditions such as those which prevailed in the 1959 growing season may also be partially responsible for the poor performance of these chemicals and for that of 4-CPA esters. Although the penetration of the latter (lipoid soluble) may not be affected in a similar manner it may be arrested by the cuticle and

its partitioning into the aqueous apoplast system may be curtailed (see Ch. 1, 6-2).

iii) Translocation:- Investigators[476,477,478] have suggested that the stage of frond development at the time of herbicide application may influence the extent to which it moves to, and damages the rhizome apices and frond buds (Table 1-4). If the translocation of these herbicides is closely associated with assimilate movement (see Ch. 1, 5 and 6) then changes in the pattern of assimlate movement in the developing frond may influence the efficiency of movement of these compounds.

Carbohydrate translocation in bracken is said to be like that in all other species of plants studied, in that the sugar transported is sucrose.[217] Each pair of young fronds goes through a series of stages the timing of which varies with the general rate of development of the frond.[499]

1) Carbohydrate is imported from the rhizome and from lower pairs of pinnae.

2) Import continues, and export to the apical parts of the frond begins.

3) Import ceases and export continues both up the rachis to the apical parts of the frond and down it to the rest of the plant.

4) Export continues in the rachis only towards the rhizome.

This pattern is repeated by each successive pair of pinnae until the whole frond has expanded, when all its parts will be exporting. A similar pattern probably occurs on small scale as the pinnules of each pinna mature.[499]

Such a pattern of carbohydrate movement in bracken, its effects on rhizome reserves and its implications on bracken control by cutting were known or speculated upon a long time ago.[441] Smith[441] reported that the amount of food reserves in the rhizome diminishes steadily from April and reaches a minimum in July after which there is an increase in food reserves.

Studies on translocation of phloem mobile herbicides in bracken showed the existence of seasonal differences in translocation and accumulation of herbicides. Volger[477,478] studying the translocation of amitrole in bracken found that during vegetative growth amitrole was translocated to areas of meristematic activity, while in mature plants radioactivity became concentrated in the rhizome. A similar pattern of movement was reported for asulam.[476]

From these translocation studies and from field trials (Table 1-4) it is clear that movement of these materials in the phloem is associated with changes in carbohydrate movement. The direction of the latter as in other perennial weeds is determined by changes in the annual cycle of carbohydrate in the storage organs (source⇌sink relationships).

As mentioned previously (see Ch. 1, 5-2) the changes in the annual cycle of carbohydrate reserves in perennial weeds is closely associated with the start of the growing season and growth of the plant. So differences due to climatic and edaphic conditions should be expected and variations between sites and different growing seasons are bound to occur. Bartley and Otto[41] have shown that the low point of total carbohydrate content in salt cedar (Tamarix pentandara) roots varied as much as 3 months in a 5-year period.

With regard to bracken, Braid[68] noticed that in some years the growth of bracken was so backward that frond emergence was delayed till June. Sometimes bracken growth could be much earlier. Unfortunately such variations were seldom taken into account. These variations could be part of the answer to the noted differences in performance of herbicides at different sites and in different seasons.

In presence of active sinks, movement of phloem mobile herbicides would be expected to be at their best if photosynthesis is active (no movement from chlorotic or senscence fronds is expected).[133] A point to note is

that among ferns only in Pteridium white fronds have been observed in shade.[150] The translocation system in bracken as stated by Whittle[500] will be capable of its maximum rate of mass transfer during the middle of a sunny day when the temperature is high (see Ch.1, 4-2-3-1). However when translocation is slow (due perhaps to one or all of the following 1) slow photosynthetic activity 2) active sinks not available and 3) as noted with many other plants a decline in the rate of carbohydrate export with leaf age; see Ch.1, 5-2) accumulation or retention of the herbicide by living cells may reduce greatly the amount and rate of arrival of the toxicant at the site of action.[133] This could be critical particularly if a detoxification mechanism is operating at or en route to the active site (see Ch.1). Accumulation or retention could be augmented if the phloem conduits could be injured by the toxicant e.g. dicamba[382] and picloram.[201] However as pointed out by Crafts and Yamaguchi[133] different herbicides may be retained differently.

These points could help to explain some observations made by some workers with regard to the performance of certain herbicides used in bracken control.

1- Farnworth and Davis[167] observed that picloram applied to fully expanded fronds exhibited extremely limited translocation to the rhizomes and associated organs.

2- Conway and Forrest[115] in Scotland and Norris[367] in England observed that severe scorching produced on young fronds (50 to 70% unfurled) following application of 4-CPA reduced the activity of the herbicide. The reduced activity was attributed to an impediment in translocation. Although this point is not disputed, scorching could have resulted from slow translocation and/or rapid penetration (penetration is said to decrease with increase in frond age [476]). This argument seems to be supported by the observation that less or no scorching occurred at later stages.

3- Arbonnier[15] and Varlet et al.[475] found that amitrole activity was reduced when applied in combination with diquat.

4- McIntyre[335] from field studies reported that :-

a) Marked reduction in translocation of 2,4-D occurred at the later stages of frond development and was associated with the increasing degree of frond maturity.

b) The translocation of 4-CPA was superior to that of 2,4-D and dalapon.

The observations of McIntyre could be due to differential cuticular penetration and/or cuticular retention because unfortunately penetration and translocation were not separated in his studies (see Ch.1, 6-2)

Basipetal translocation of herbicides which leak from the phloem to the xylem could be impeded under hot dry weather conditions (see Ch.1, 6-2 point IV). This has been pointed out as one of the possible answers to the poor bracken control noticed with dalapon in 1959.[299] However, if this was true for dalapon it could be true for many other herbicides having similar tendencies e.g. amitrole, dicamba, 2,4-D; and picloram.[16] Another effect on the efficacy of these herbicides is the possibility that they may be exuded from the subterranean plant parts when they get there (see Ch.1, 6-2 point IV)

2-4-2-1-2- Possible causes of regeneration :- As is the case with many pernicious perennial weeds,[104] the prolific production of vegetative buds from the underground storage organs (see 1-2) endows bracken with a tremendous power to regenerate and makes its eradication rather difficult.[116,187] For adequate long-term effects entry of the chemical into the buds must be optimum; this will depend on the activity of the buds at the time of spraying.[382] Decline in bud activity (late in summer; bud activity is also affected by the nature of the growing season)[299] may be responsible at least in part for the less efficient and rapid recovery noticed with late applications of herbicides (Table 1-4).

A point of equal importance with respect to regeneration is the observation made by Conway[114] (later confirmed by other workers)[285] that frond buds on rhizome branches not carrying expanded fronds will receive relatively low doses compared to those on branches carrying expanded fronds at the time of spraying (see 1-2).

The existence of wide variations between sites in the ratio of frond buds in the two types of rhizome branches (as pointed out by Conway[108] the ratio reflects the ability of bracken to regenerate after treatment) may be part of the answer to variations in control and regeneration of bracken noticed at different sites.

Some workers[504] believe that herbicides which do not affect the carbohydrate storage capacity of the storage rhizomes may not provide long-term control even if all the buds present at the time of spraying were destroyed. They attributed this to the ability of the rhizome system to initiate new buds which will eventually result in the return of the bracken canopy. Although this point is not disputed (plants that have their carbohydrate reserves depleted to some degree are more susceptible to adverse weather conditions),[493] even in cases where a decrease in reserve carbohydrate content (different plants) following a herbicide treatment was observed there was no indication that this decline was the primary cause of death of treated plants.[405]

Fryer[187] and Robocker[404] proposed that because of the extensive root-rhizome system in vigorous stands of bracken the limited herbicidal action could be due to dilution of the herbicide below a lethal amount before the entire system was adequately infused with toxic material. This could explain in part the better control and slow recovery obtained with higher doses of herbicides (Table 1, 2 and 4). Other possibilities e.g. fixation and/or detoxification in the tissue pathways <u>en route</u> to the site of action cannot be ruled out.

2-4-2-1-3- Possible ways of improving response to foliar systemic herbicides :-

It could be gathered from the above review that susceptibility of bracken to foliar systemic herbicides (apart from intrinsic toxicity) depends on phenological and physiological conditions as well as environmental ones. Better results will only be obtained when these conditions allow the interception, retention and downwards translocation of herbicides in sufficient quantities to kill the growth centres. To ensure this the following suggestions are made.

1) Improvement of spray retention:-

A- By cutting down spray drifts :-aerial spraying of bracken is essential in most cases due to the difficulty of ground spraying (land is not accessible - see 2-1-1). One of the serious limitations facing aerial application in general and in most bracken occupied sites in Scotland is spray drift[172,430] (see 2-4-2-1-1). The low volume (dictated by economic reasons) and the tendency in recent years to use the smallest possible drop size so as to obtain the greatest degree of coverage of the target surface with the minimum amount of spray[107] will further augment the spray drift problem.

Spray drift could be improved to some extent by:-

i) Modifying the type and position of the nozzles.[107] However, the drop spectrum of a spray is not controlled entirely by the equipment but can be modified by the air-flow past the nozzle.[107]

ii) Reducing the height from which spraying is carried out.[107,172] However, this may not be practical (see 2-4-2-1-1).

iii) Restricting spraying to times when climatic conditions are suitable.[107] This compromise may not be practical because 1) it will increase the cost of aerial application[107] and 2) the time of maximum bracken susceptibility may be critical (Table 1-4).

Alternatively the drift may be controlled by varying the properties

of the spray.[107] In this respect two main points have to be considered.

a) Reduction of the proportion of small drops initially present in the spray emitted from the nozzle. This could be achieved by increasing spray viscosity.[107]

b) Reduction of the evaporation of the spray droplets between their production and their deposition upon the plant surface[232] (a 100µ aqueous spray droplet could be reduced to 40µ in about 15 sec. by evaporation). This could be achieved by reducing spray volatility (see Ch. I, 3-1-1).

Both requirements could be achieved by the use of water-in-oil emulsions (W/O).[107] In addition to this, water-in-oil emulsions have been claimed to leave deposits which are rain resistant and to improve biological efficiency.[299]

However, the matter is not so simple and hence contradictory results have been obtained (Table 1). As pointed out by Colthurst et al.[107] spray retention, persistence of the deposits and improved biological efficiency are very dependent on the properties of the emulsion and of the target surface (see Ch.1, 4-3-2-2 and Ch.2, A).

The properties of the emulsion are dictated by many factors[19,461] e.g. good storage, cost limits, safety of the operator, ease of preparation or application and little attention may be paid to herbicidal activity and selectivity.

From this work (see Ch.2) and the literature (see Ch.1, 4-3-2), it is felt that more attention should be given to the effect of the emulsion on the herbicide activity and selectivity. Damage to the plant surface, the extent of which could be affected by environmental conditions (see Ch.2,D) and possible interference with herbicidal penetration and translocation should be carefully studied before making a selection of the components of the emulsion (oils and emulsifiers).

B- By incorporation of surface active agents :- although bracken is not water repellent it has not got a high affinity for water (see Ch.2, D-3-1).

Incorporation of Tween 20 in the spray solution has been found to increase spreading and the water holding capacity of detached pinnules (see Ch.2,B).

Although surfactants could contribute to wetting, spreading, emulsification and solubilisation of herbicides, the following points have to be considered.

1) The relative efficiency with which surface active agents wet leaf surfaces may differ according to the nature of the leaf surface and the surfactant used.[189]

2) When using a surfactant, the effects of the herbicide-surfactant combination on neighbouring plants has to be considered.[25]

3) Surfactants could enhance, have no effect or be detrimental to the action of foliarly applied herbicides.[66]

4) Interactions between the surfactant, herbicide and plant surface may be of more importance than the surface tension lowering or the wetting ability of herbicide surfactant solutions.[180,183,281,284]

2) Improvement of penetration:-

Penetration especially of highly water soluble herbicides e.g. amitrole, asulam (sodium salt) dalapon (sodium) amine salt of 4-CPA (Table 1 to 4) must be rapid particularly in high rainfall areas such as the West of Scotland. However, rapid penetration followed by slow translocation (see 2-4-2-1-1) may injure the leaf tissues. Depending on the rapidity and magnitude of the resulting injury penetration and/or translocation may be curtailed (see Ch.2, D). A compromise between these factors (penetration and translocation) may be necessary.

Incorporation of surfactants, humectants and various additives was found to affect penetration of asulam and amitrole to varying degrees (see Ch.2, B and C). The nature and the amount of the additive and the environmental conditions prevailing before, at, and after spraying may be quite decisive in determining the end results (see Ch.2, C and D).

In general for adequate herbicide penetration high atmospheric

humidity, maintenance of an efficient water continuum in the frond and good contact between the spray droplets and the frond surface are quite essential (see Ch.2)

3) Improvement of translocation :-

As mentioned previously (see 2-4-2-1-1) for efficient control lethal amounts of the herbicide should enter the frond buds. Dilution of the herbicide in the extensive rhizome system, inadequate translocation and inactivity of the buds were mentioned as possible barriers to achieving high concentration of the toxicant in these buds. Unfortunately there is no quantitative data on accumulation of any of the herbicides used in the frond buds of bracken.

A comparable situation could be found in many perennial rhizomatous weeds with very numerous buds, on their subterranean organs, which serve as a means of vegetative reproduction and regenerative growth.[46,173,177] In such situations although the herbicide was translocated to the underground portion of the plant the buds failed to accumulate the herbicide.[273] A similar state of affairs has been pointed out by Crafts[122] (see Ch.1, 6-1).

Treatments which stimulate the activity of buds e.g. decapitation of the shoot or increasing the nitrogen supply of the rooting medium were found to increase the amount of the herbicide accumulated by the buds.[53,175] A comparable effect of nutrition on 2,4-D translocation was reported by Crafts and Yamaguchi[133] from experiments with Tradescantia fluminensis.

Evidence that this effect by the nitrogen supply on bud activity may be of practical significance was provided by Zick and Buchholtz[514] who reported that the control of quackgrass (Agropyron repens) was increased significantly when herbicide treatments were preceded by the application of nitrogen fertilizers. These workers attributed this effect to the increased resprouting rhizome buds the growth of which was highly responsive to the nitrogen supply. Bracken was also reported to show a positive response to added fertilizers.[67]

Other workers considered the use of the plant growth regulator 2-chloroethylphosphonic acid (CEPA) which degrades to form ethylene in solution at pH 4.1 or above.[53,275] CEPA application has been reported to cause axillary rhizome buds of johnsongrass to break dormancy,[53] stimulate the growth of inactive basal buds of honey mesquite[357] (Prosopis juliflora var glandulosa) and release dormancy of the under ground bulbs of wild garlic[53] (Allium vineale) and increase basipetal translocation of dicamba in the latter plant.[53]

Stimulation of growth of inactive buds will 1) reduce the number of dormant buds from which regrowth occurs 2) result in the production of more shoot and hence a greater amount of foliage for interception of the herbicide 3) lead to greater and faster utilization of rhizome reserves and 4) act directly by promoting efficient translocation of the herbicide.

2-4-2-2- Soil applied herbicides :-

The erratic performance of foliar-applied herbicides, their failure to provide a long-term control of bracken and the spray drift hazards tempted workers to try soil-applied herbicides. The obvious advantages of soil-applied herbicides are 1) it is less hazardous to cover difficult terrains when the ground is visible[172,229] 2) the availability of granular formulations of some of these herbicides has the practical implications of minimizing drift and contamination from spray treatments and 3) their application can be carried out at times when farm labour is not occupied with pressing problems.[172,229]

In putting forward these arguments no consideration was given to the effects of herbicide-soil, environment-herbicide-soil and environment-herbicide-plant, interactions and their effects on the persistence and availability of the herbicide at the site of uptake (see Ch.1, 7).

Soils under bracken by virtue of their high organic matter content and high permeability[285] present special problems to the use of soil-applied

herbicides. Generally a soil high in organic matter is more capable of inactivating herbicides (degradation and/or adsorption) than a soil with a low organic matter content (see Ch. 1, 7).

Herbicides which can be fixed by the topsoil and/or rapidly degraded (biologically or non-biologically) can be of no practical value (see Ch.1, 7-3-1). Herbicides which resist detoxification (exceptionally high herbicidal concentrations can inhibit the detoxification process[119]) or lead to soil sterlization, are undesirable because they may interfere with the use of the land.[109] This has the disadvantages of preventing an economic return, encouraging soil erosion particularly on steep slopes and of possibly polluting nearby streams. The problem will be made worse if bracken regeneration can occur after the treatment.

On the other hand herbicides which leach readily may prove to be ineffective and may be hazardous (see Ch.3, C).

Many herbicides e.g. amitrole,[497] 4-CPA,[497] MCPA,[495] chlorthiamid,[100] sodium chlorate,[109] dichlobenil,[497] CMU,[116] dicamba[167] and picloram[167] have been tried. Only dicamba and picloram (the most promising) are included in this review (Table 5 and 6).

Dicamba and picloram are active as post-emergence and pre-emergence herbicides (Table 5 and 6). Both herbicides are known to be very persistent in soil,[91,149,167,345] their breakdown is effectively microbial and they can leach down the soil.[91,229,345] They interfere with the use of land at high rates of application, but the feasibility of using the land at low rates has been reported in certain cases (Table 5 and 6). However, in these cases the time taken by bracken to regenerate is short.

It is noticeable that in pre-emergence applications of these herbicides granular formulations are more effective than spray formulations.[167,400] This may be due to several factors. However, a primary cause is believed to be slow release which allows translocation to distant parts of the plant when it is in a relatively dormant state.[404] Recovery after an initial

control indicates a possible blockage in translocation (see 2-4-2-1-1) and/or metabolic breakdown of the herbicide.[404]

2-4-2-3- Follow-up treatments:-

It is clear from this review that at present no treatment will kill bracken outright on all occasions. Bracken can and does regenerate in most cases. If bracken recovery is to be minimized and infestation by other weeds, of the cleared area, is to be prevented a follow-up treatment should follow. As pointed out by Conway and Stephens[116] the problem of land usage should go hand in hand with bracken clearance.

As mentioned previously (see 2-1-1), from the view point of bracken control, the problem can be divided into bracken on ploughable land and bracken on unploughable land. The follow-up treatments also follow the same pattern.

2-4-2-3-1- Bracken on ploughable land:-

In such situations bracken is relatively easily dealt with (see 2-1-1) - ploughing in summer followed by heavy discing, lime and fertilizers. The land is then sown to a pioneer crop such as rape, turnips or Italian ryegrass in the late summer, grazed and then disced in December. The next summer the land should be fit for potatoes, rye or oats or for direct seeding (see ref. [457]).

2-4-2-3-2- Bracken on unploughable land:-

If true long-term control is to be achieved the period when fronds are almost absent following a successful treatment must be used to increase the carrying capacity of the land. Dead bracken foliage or litter, if deep, should be removed by fire or mechanical raking for it is difficult to establish a grazing sward on friable bracken litter. This should be followed by lime, fertilizers and reseeding.[457]

Maintenance of a reasonable grazing pressure is essential. Understocking will let the pasture deteriorate; then the bracken will spread

because of lack of competition from pasture plants and lack of trampling by the stock. But overstocking may weaken the pasture and perhaps more importantly, it can also cause hungry stock to eat bracken.[264] Bracken is poisonous to cattle and horses and harmful to sheep (see 2-1-1 and 2-2-2). Calves should be kept away from the pasture because they will eat bracken whether they are hungry or not.[264] Calves have been poisoned even by grazing bracken through a fence.[264]

From what has been mentioned in this chapter it could be concluded that much has still to be learned about bracken. A spray and watch type programme of research which has tended to dominate and cripple weed control research in the past must give way to, or at least be coupled with deeper scientific studies of the subject. Research has to be directed to a proper understanding of all factors involved. These factors are discussed in some detail in Chapter 1.

Reference Tables

Table 1, 4-CPA

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
298	5 and 10 * (aqueous and invert emulsions)	Application 4-13 July gave satisfactory control next season. Application 24-28 July only the invert emulsion gave satisfactory control in the following season.
299	5, 7.5 and 10 * nonyl ester (aqueous and invert emulsions)	Invert emulsion was more efficient than aqueous emulsion. 5 and 7.5 * invert emulsion was equivalent in effectiveness to 7.5 to 10 * aqueous emulsion. Marked differences in effectiveness of the chemical at different sites was observed. This was more pronounced at low levels.
299	5, 7.5 and 10 * butoxy ethyl ester (aqueous and invert emulsions)	The same trend of results as the nonyl ester above. July application was more effective than June application. Higher dosages resulted in more kill. Variation between sites was observed but less marked than with the nonyl ester.
299	2.5, 5, 7.5 and 10 * (aqueous emulsion)	July application was better than June application. The degree of control increased with dose. (100% was obtained at higher dose in certain cases, based on 1 year after treatment.)
286	5, 7.5 and 10 * nonyl ester (aqueous and invert emulsion)	Applied to fully expanded fronds. No differences between the two formulations. No advantage in using a non-phytotoxic oil. Results given by the same formulation applied in consecutive days at different sites varied widely from one site to the other.
157	7.5 * nonyl ester 7.5 * diethanolamine 6 * butyl ester	Gave 84% and 74% reduction in frond density 1 year after treatment. Gave 87% reduction in frond density 1 year after treatment. Comparing the three

N.B. *pounds/acre.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
		derivatives the amine salt applied at 7.5# in 1959 was more effective than the nonyl and butyl esters.
367	5, 7.5, 10, 15, and 20 # (low volatility esters)	Excellent control (1 year after treatment). Rates greater than 10# did not result in commensurately increased kill. Use of 2 or 3 applications at low rate during the same season in most cases resulted in poorer control than applying the whole dose at one time. Spraying when fronds were 50-70% unfurled gave much more severe 'leaf' scorch than spraying at later stages. The efficiency of amine formulation was much reduced by rainfall after application, under such conditions invert formulations gave much better results than oil-in-water ones.
255	5 and 10 # nonyl ester 10 # butyl ester 10 # ester	All behave similarly but were less effective than expected.
	7.5 # nonyl ester 7.5 # diethanolamine	Applied 30 June to 23 September (weekly). Optimum date for application early August. Ester gave better results than the amine salt.
498	5 and 10 #	Not effective (15% reduction in frond density, 1 year after application).

Pertinent points:-

1- The best results were obtained when spraying was made to fully expanded fronds. Spraying young unfurled fronds resulted in a severe scorch.

2- The efficiency of different formulations relative to one another was not constant and was affected by climatic conditions.

3- Marked variation in effectiveness of the chemical at different sites was observed and it was more pronounced at lower rates.

The variability in the degree of bracken control by this chemical was evident from the start. The Scottish trials laid down by A. H. Marks and Co. in Scotland in 1957-58 demonstrated this variability.[254] The degree of control in the year following treatment varied between 90% and nil.

Hodgson[254] cited a more striking example where 4-CPA at a site in Shropshire at 10 lb/acre in 1959 caused a reduction in frond density of 59% when assessed in the following year. The same dose however, applied on the same date but in 1960, gave only a 26% reduction. Similarly at another site in Wales, the corresponding reductions in density in the two years were 32% and nil. More disappointing results were reported by the Weed Research Committee on Pteridium aquilinum.[492] Moreover regeneration of bracken was reported in most if not all cases.

Table 2, Dalapon

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
408	20 to 30 Kg/ha	Satisfactory control of top growth. Stand regenerated after 3 years.
162	5, 10, 15 and 20 *	Highest rate killed the grass. No effect on bracken.
68	10 and 20 *	Significant reduction in frond density first year.
493	20 and 40 *	48% and 68% reduction in frond density (July count), 20% (September count the same year)
225	10 to 20 *	Delayed frond emergence. No marked control.
81	15 *	Not effective.
146	25 Kg/ha	Satisfactory control (1 year after application). ½ the amount was recommended for bracken under shade.
254	20 *	Satisfactory control may result in the first year following treatment. Control was seldom maintained in the second year.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
492	10 - 20 Kg/ha	Results were very irregular and unsatisfactory. Only occasional good results were obtained at 20 Kg/ha. The best results (when obtained) were when the fronds were well developed.

Pertinent points:-

1- Results were very irregular and unsatisfactory. Good results (occasional) were obtained when spraying was made to well developed fronds.

2- Frond emergence was delayed and higher rates damaged underlying grasses.

3- Regeneration of bracken was found to occur.

4- Shaded bracken may be more susceptible than exposed bracken.

Table 3, Aminotriazole

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
a-Amitrole:-		
408	20Kg/ha	Satisfactory control for two seasons
49	10 and 20 Kg/ha	Satisfactory control for 4 years
50	10 and 20 Kg/ha	Virtually eliminated bracken for 2 years
498	10 and 20 *	Significant reduction of frond density at one site (50%) and an increase in frond density in another site (1 year after treatment).
298	3 to 6 *	Application 4 - 13 July satisfactory control 1 year later. Application 24 - 28 July ineffective.
b-Amitrole-T		
49	5 - 20 Kg/ha	Hardly any regrowth was observed after 4 years.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
50	10 - 20 Kg/ha	Virtually eliminated bracken for 2 years.
498	10 and 20 *	Significant reduction of frond density. at one site (40%) and an increase in frond density in another site (1 year after application).
298	3 to 6 *	Application 4-13 July satisfactory control 1 year later. Application 24-28 July not effective.
298	6 *	Early July good control 1 year after treatment. Application mid-June poor results.
286	5, 7.5 and 10 *	Satisfactory results 1 year after treatment.
156	5 *	Reduced frond density by 46% and 36% 4 and 5 years after treatment.
157	2 *	Gave 95% reduction 1 year later.
157	5 *	Applied to fronds not fully unrolled in July 1959 gave 90, 74 and 83% reduction in the number of fronds in the following 3 years.
475	9.6 Kg/ha	Excellent control one year after treatment
15	4.8 Kg/ha	Good control one year later.
162	5, 7.5, 10 and 15 *	Gave very high degree of control 1 year later.
162	7.5 *	Outstanding results at 2 out of 3 sites (1 year after treatment).
255	10 and 20 *	Frond number (1 year after treatment) reduced by 70 to 80% at 11 out of 12 sites. Increasing the dose from 10 to 20 lb did not materially affect the degree of control. The effectiveness of the chemical was considerably reduced when applications were made at the later date (July vs. August), and variation from site to site was appreciably greater. Marked regeneration of bracken took place. The rate of regeneration varied from one site to another.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
254	10 and 20 *	Control varied with site, being 74, 59% at two sites and did not exceed 30% at two other sites.
261	2, 4 and 8 *	Gave relatively poor control (heavy drizzle followed application).

Pertinent points:-

1- Generally the best results were obtained when spraying was made to fully expanded fronds, however this was not always the case.

2- Rainfall shortly after application reduced the efficiency of amitrole greatly.

3- Variation in amitrole effectiveness at different sites was observed and it was more pronounced at later dates.

4- Regeneration of bracken was observed. The rate of regeneration varied from one site to another.

Table 4, Asulam

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
494	2, 4, 6 and 8 Kg/ha	Plants sprayed monthly from June 1970, analysed July 1971. Only those treated in the period June - August showed any response to treatment. June treatment gave 98.0 to 96.8% control. August treatment gave 63.6% at 2 Kg/ha and 86.8% at 8 Kg/ha. Nil response occurred on or near the 25th of September. Average control was 90% June treatment and 76% August treatment.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
444	4.4 Kg/ha	Gave 94.1% control 1 year after application compared to 28.4% given by 4.4 Kg/ha amitrole.
444	4.4, 3.3, 2.2 and 1.1 Kg/ha	Gave 96.4, 94.0, 94.7 and 89.5 % control in the first year after application
444	4.4 Kg/ha	Gave 90 - 95% reduction in frond density after about 12 months. Persistence of the herbicide effect has been recorded so far over two seasons at 3 replicated sites.
444	3.36 Kg/ha	Gave 90 - 95% control 1 year after treatment which fell to 80 - 85% after 2 years.
444	2.24, 3.36 and 4.48 Kg/ha	The high doses gave adequate weed control but the low dose was not reliable. Application in the first fortnight in September was less effective than those made earlier, except at the highest rate of application.
444	4 *	Gave 90% control in the first year which dropped to 70 and 50% in the second and third year after application.
444	2.2 and 4.5 Kg/ha	The low dose was virtually as effective as the higher dose in the first year but poorer thereafter.
261	4 and 8 *	Applied early August. Control was over 90% in the first year after treatment. In the second year control was still over 65% with the lower rate and over 70% with the higher rate of asulam. At one site (heavy drizzle after application) asulam was less effective. Addition of 0.1% Agral 90 boosted its activity considerably in this case.

Pertinent points:-

1- The best results were obtained when the majority of fronds had just

completed the unfurling process.

2- Rainfall after application was found to reduce asulam activity.

3- Regeneration was found to occur and the degree of regeneration was found to be affected by the time of application and the dose rate.

Table 5, Picloram

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
310	1.5 to 6#	Application at early frond emergence, or 2 - 3 weeks before emergence, better than application to fully expanded fronds. Top rate gave two season control. Lower rate might be commercially acceptable if combined with sound reclamation practices
315	2.4 #	More effective when applied to fronds which had not expanded at the time of treatment.
315	0.27 to 6 #	Post-emergence early summer or pre-emergence in the spring. 6# gave virtually complete control for 2 growing seasons irrespective of site or time of application. At lower rates pre-emergence application or early frond emergence was consistently better than when fronds were fully expanded. Rates below 1.5# suppressed bracken growth in the year following application. The pre- or early post-emergence treatment allowed grazing cattle to have access to the sward throughout the season of treatment
349	1.25, 2.4 and 3.3 #	Spraying was more effective when carried out before frond emergence than during or after frond emergence.
495	3 Kg/ha	Caused significant reduction in frond density whenever applied over a calendar year. Most effective during March - June period. Full recovery expected after 8-10 years.

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
400	2.5 lb (24.9% product) /ac.	Applied May 1967 gave 99% control in the year of treatment reduced to 50% by July 1968.
496	3 Kg/ha	Applied May had no long-term effect. 94% recovery occurred 3 years after treatment.
155	1 - 3* (liquid or granular)	Spray formulation was superior to granular formulation as post-emergence treatment. Granular formulation was more effective in pre-emergence treatments. Sprays (3*) gave most effective control when fronds were fully unrolled. Granules (3*) gave most effective control in late winter or spring before frond emergence. Residues of picloram (at 1*) 12 months after application were sufficient to kill clover and other broad-leaved plants
155	32 - 128* or mixed with 2,4-D at 21 + 77 *	Applied to fronds fully expanded gave 100% control in treated area and 50% 8-12 feet outside the treated area.
261	2*	Applied early August gave good control at first but the effect decreased somewhat in the second season.

Pertinent points:-

1- Generally application of picloram over a calendar year resulted in reduction in frond density. However it was far more effective when applied as pre-emergence or post-emergence before fronds were fully expanded.

2- The efficiency of picloram pre-emergence was found to increase with increasing concentration.

3- Spray formulation was more effective than granular formulation as post-emergence, while the latter was more effective as pre-emergence.

4- Bracken regeneration took place in all cases and was faster at lower rates.

5- Reduction in the total available carbohydrate level in the rhizome after picloram application was reported (pre-emergence application).[167]

Table 6, Dicamba

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
81	1, 3 and 4 # (granules 10%)	Pre-emergence (November, January and February), high rate, maintained control the second year. November treatment superior. No residual effects of dicamba on grasses or clover if 14 weeks elapsed between herbicide application and sowing.
81	4.5 and 9.0 Kg/ha	Pre-emergence gave good control for 4 years on infertile soil, but on fertile moist soil sites, bracken may substantially recover by the end of the second year.
81	4.5 and 6.7 Kg/ha	Application in winter (December, January and February) gave excellent control at the end of the first season, ground cover ranged between 0.0 and 10%. Damage to trees varied with date of application. Residues of dicamba take much longer to break down in winter than in summer.
80	3 and 4 #	Applied September, October and November. Excellent control but dicamba residues sufficient to kill trees were present at the time of planting.
349	4, 6 and 8 #	Satisfactory control when carried out before frond emergence.
253	2, 4 and 8 #	Applied mid-July, early and late August. 8# gave ca 90% control in all treatments. 4# effectiveness varied with time and from site to site. 2# ineffective. Frond deformities were observed in plants located several feet from the treated area,

Reference	Applied dose as active ingredient unless stated otherwise	Remarks
		indicating that dicamba or its derivatives had been translocated over greater distances.
6	4 and 6 *	Application in spring and early summer gave adequate bracken control. Sites where complete control of bracken was obtained in 1964 showed little recovery by 1967.
498	8 *	Applied in August had no long-term effects.
261	4 *	Ca 35% control 3 years after treatment. Post-emergence gave good control in two sites but poor control in a third site. The control decreased with time.
404	2.2 Kg/ha	Pre-emergence granular formulation was found to be superior to sprays.

Pertinent points:-

1- Dicamba could be active both as pre-emergence and post-emergence herbicide. It could be translocated following post-emergence application in the rhizome over greater distances. However, its effect as post-emergence varied with the dose rate, time and site.

2- As pre-emergence, granular formulation was found to be more effective than sprays.

3- Bracken regeneration took place and was faster at lower rates and in fertile soil.

4- Reduction in the total carbohydrate level in the rhizome after dicamba application was reported. [47]

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