Some properties of SENCOR (Metribuzin) and bases for variation of its effect on *Solanum nigrum* and *Solanum sarachoides*

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ABSTRACT

In Part I of this study, the relative potencies of SENCOR and its metabolites have been investigated. SENCOR has been established as a potent Hill reaction inhibitor. The observed action of SENCOR and its metabolites are discussed in terms of structure/activity relationship.

The mechanism of action of SENCOR and one of its metabolites is compared with other known Hill reaction inhibitors. A scheme is then proposed for the mode of action of Hill reaction inhibitors.

In Part II the observed variation in the field, in the response of Solanum nigrum and Solanum sarachoides to SENCOR has been established as a phenomena of species selectivity.

From studies on uptake, distribution and metabolism of radioactive SENCOR it has been established that a restriction to movement of the herbicide from the xylem to the mesophyll occurs in Solanum sarachoides (tolerant species) while the material is distributed throughout the mesophyll in the susceptible Solanum nigrum. This restriction to movement into the mesophyll in the tolerant species has been confirmed by studies on the inhibition of rate of transpiration and from effects on stomata. SENCOR also appears to be metabolised to a greater extent in the tolerant species.

This study leads to the conclusion that the protection of the active centre, the chloroplasts, through restriction to movement of SENCOR into the mesophyll, combined with partial breakdown of the herbicide in the plant may be responsible for the tolerance of Solanum sarachoides to SENCOR.

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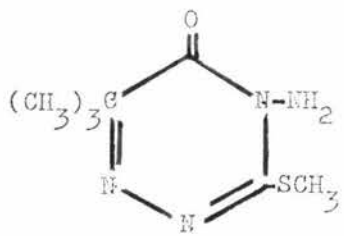
Chapter 1

INTRODUCTION

SENCOR, a pre-emergence and post-emergence herbicide is being developed by Henry H. York & Co. Ltd under licence from Farbenfabriken Bayer A.G. Its chemical name is 4-amino-3-methylthio-6-t-butyl-1, 2,4-triazine-5-one. Its chemical structure is:

SENCOR (metribuzin)



This compound has shown, effective control of many annual broad leaf and grass weeds when applied to corn, tomatoes, potatoes and legumes.(1).

Variation in control of two solanum species, i.e. Solanum nigrum and Solanum sarachoides have been observed (141). Therefore this project is an attempt to investigate whether this variation is due to species selectivity rather than an observed differential effect due to stage of growth difference.

In order to establish bases for selectivity, knowledge of mode of action and properties of the chemical is essential. As regards activity of SENCOR, only passing reference to it as having similar mode of action as uracil herbicides have been made in a technical paper (76). The metabolite "deaminated diketo" (Fig.1) of SENCOR has been alleged to possess the herbicidal activity (inhibition of photochemical reaction). This assumption is based on the observed structural similarity of this metabolite with that of uracil, a Hill

reaction inhibitor (83). However, structure activity relationship studies on inhibitors of photosystems provides theoretical support for SENCOR (a triazinon) itself to possess the herbicidal property.

Draber et.al. (50) have related the structure of triazinones with inhibitors of the Hill reaction. The chemical structure of triazinones fits into the scheme developed for herbicides with such a mode of action.

In such a case of uncertainity, it it necessary to establish the mode of action of SENCOR and also evaluate the relative potency of the primary product and the metabolites.

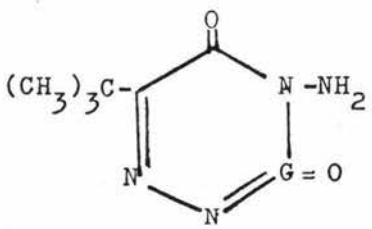
The tolerance of Solanum sarachoides to the herbicide may be due to the protection of active site (Hill reaction centre of photosystem II) by relative impermeability of chloroplast membranes.

In comparing the degree of inhibition of isolated chloroplasts of the two species, if difference in susceptibility occurs, then the selectivity must be a function of the chloroplast membrane itself. If, however, the degree of inhibition of the photosystems of the two species are similar then perhaps, tolerance is a function of some physiological or biochemical phenomena which may involve differential rate of uptake, distribution or metabolism of SENCOR. While, if differential rate of metabolism of SENCOR is evident, then it is also possible for these products to form conjugates and thus, prevention of translocation may be a basis for selectivity. Autoradiographic studies may establish evidence for such a selectivity.

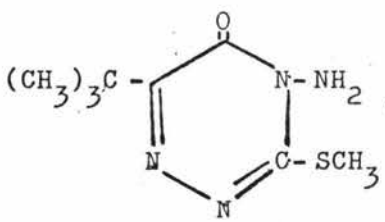
Full study of all factors will entail a detailed project of research which is almost impossible to be carried out single handed and within the duration of masterate studies; but as will be seen from this presentation, an attempt has been made to investigate some pertinent and important aspects.

Fig.1

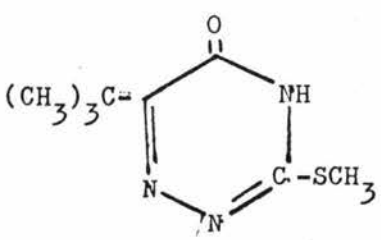
SENCOR and its metabolites



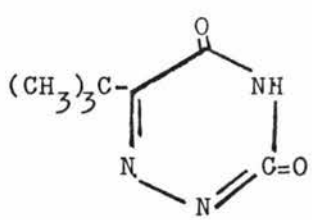
Diketo metabolite
(DK)



SENCOR active ingredient
(S)



Deaminated metabolite
(DA)



Deaminated Diketo metabolite
(DA DK)

PART I

PROPERTIES OF SENCOR

Chapter 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

In the past the term 'mode of action' and 'mechanisms of action' have been used interchangeably. In this study, however, they will be used as defined by Anon (9) and Ashton and Crafts (15).

'Mode of action' involves the total processes of physical, biochemical and physiological nature, which contributes to the phytotoxic actions of an introduced chemical. Mode of action thus covers, all processes from the time the chemical is introduced into the plant's environment, to the time it causes the death of the plant.

The term 'mechanism of action', however, is restricted to the primary biochemical of biophysical lesions leading to the death of the plant. Ashton and Crafts (15) define the 'primary biochemical site of action (lesions)' as "the single enzyme or metabolic reaction, or the first reaction affected at a given low concentration".

Many herbicides increase *in vivo* in concentration at the sub-cellular level with time (123). This increasing concentration could result in one or more less sensitive sites of action being involved. Experimentally it is difficult to separate the various physiological processes without inducing other fundamental changes. Therefore, for the purpose of this study, it is more useful to consider mode of action of herbicides rather than the restricted mechanism of action.

2.2 MECHANISM OF ACTION OF HILL REACTION INHIBITORS

2.2.1 Introduction

In order to understand the mechanism of action of Hill reaction inhibitors, which belong to such herbicide groups as triazine, ureas, anilides and benznitrile, an understanding of the photosynthetic electron transport system is essential. This system traps and converts light energy to chemical energy, through a series of redox

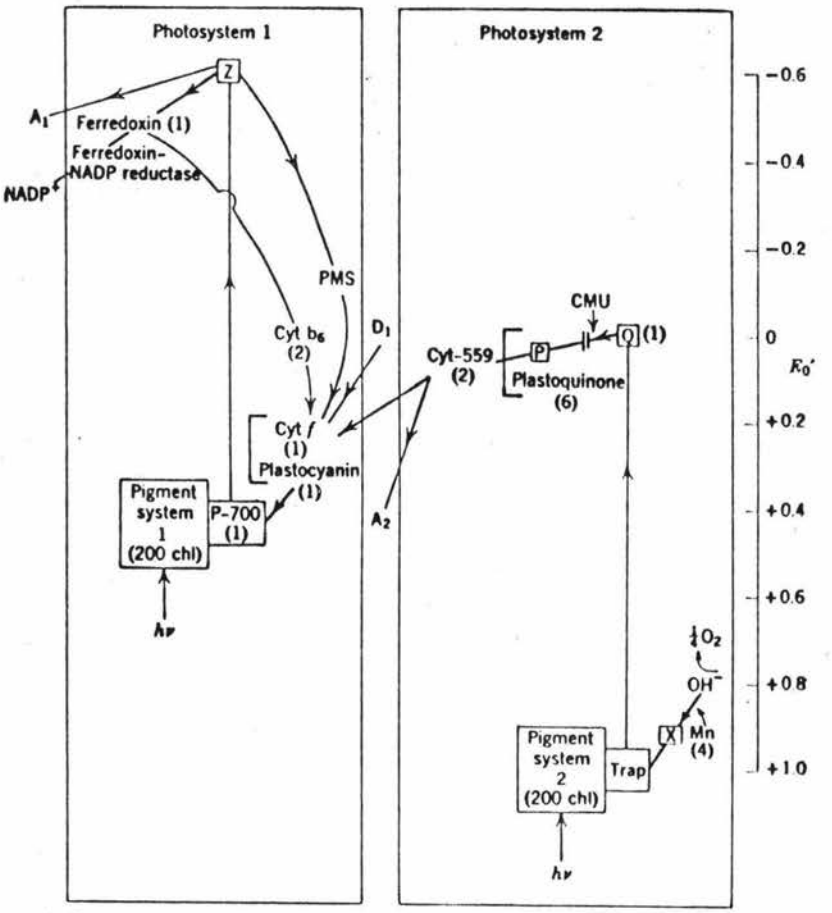


Fig. **2** Photoinduced electron flow in chloroplasts. Direction of flow is indicated by the small arrows. The pathway from OH^- to $NADP^+$ is indicated in heavier outline. The text should be consulted for detailed explanation. The figures in parentheses indicate the moles of the electron carriers per photosynthetic unit of 400 chlorophylls. Artificial acceptors (Hill oxidants) (A_1, A_2) receive electrons after photosystem 2 or after photosystem 1. Artificial donors (D_1) feed electrons into photosystem 1. A scale of redox potentials is shown on the right.

reactions. This subject has been reviewed extensively by Boardman (27) and Bishop (24).

The generally accepted pathway as proposed by Boardman and co-workers are shown in Fig.2. This scheme consists of two physically and chemically separable photosystems designated 'Photosystem I' (PSI) and 'Photosystem II' (PSII) (27). The system proposed by Arnon's group, however, differs from the above, in that, they maintain PSII is further separable into PSIIa and PSIIb (11,12,97). Although the scheme presented by Boardman (27) is generally accepted, the sequence and nature of nearly every site and the reaction sequence between the photolytic decomposition of water and PSII is not well understood.

Photosynthetic inhibiting herbicides appear to disrupt the electron transport pathway. Also photochemical reactions have been related to the lamellae structure seen in electron micrographs of chloroplasts. This subject has been reviewed by Park and Sane (135) and Gibbs (72). The nature and structure of thylakoids and other membranes play a vital role in electron transport. Some herbicides may act by disrupting these membranes.

Since the biochemistry of O_2 evolution during photochemical reaction is unknown, the exact 'lesion' or the primary site of action of Hill reaction inhibitors is also unknown. Various experimental techniques have been advanced in order to elucidate this site of action.

2.2.2 Inhibition of CO_2 assimilation

Several workers (122,179) have carried out assays of CO_2 assimilation after treatment of plants with herbicides. However, results obtained from these studies are only gross indications of the effect of these compounds on the photosynthetic apparatus. Focus of attack cannot be defined, as CO_2 fixation takes place at the end of the line in the photosynthetic apparatus. High concentration of herbicide is also required to inhibit CO_2 assimilation, and these experiments never indicate the true potential of the herbicides.

2.2.3 Distribution pattern of C^{14} labelled photosynthates

If a herbicide inhibits photosystems then carbon reduction products requiring reduced pyrimidine nucleotides should be totally blocked, i.e. sucrose value should be low, but the dark CO_2 fixation, i.e. phosphoenol pyruvate carboxylase reaction is not blocked, thus aspartic and glutamic acid will be relatively higher than sucrose. Based on this working hypothesis Zweig and Ashton (197) attempted to demonstrate the distribution pattern of photosynthates in excised bean leaf from plants previously treated with atrazine. Atrazine treated leaves and corresponding dark control leaves both exposed to $^{14}CO_2$ gave a similar pattern of low sucrose and high aspartic and glutamic acids. Since the products requiring pyrimidine nucleotides are totally blocked it is evident that only the photosystems are involved.

2.2.4 Inhibition of O_2 evolution

Several techniques have been used to demonstrate that only part of the photosystems, i.e. only PSII or the Hill reaction is inhibited. Among these are studies on oxygen evolution. The popular method is measurement of oxygen evolution using manometric techniques. Hill reaction being catalysed by ferricyanide or indophenol dyes encompases only PSII and is thought to terminate before PSI (19,75,122). The herbicide: chlorophyll molecular ratio is calculated for 50% reduction in O_2 evolution. Miklo (122) demonstrated that O_2 evolution is reduced to 50% by pyridinol chlorophyll ratio of 1. Ratios as low as this indicate that the site of action resides in the electron transport system, involving only the O_2 evolving system through to pigment system II.

2.2.5 Changes in fluorescence

Zweig and other workers have attempted to demonstrate Hill reaction inhibition using fluorescence studies.

Zweig et.al. (198) observed that photosynthesis inhibiting herbicides, atrazine, simazine, monuron, diuron and dicryl, at a concentration at which they totally inhibit oxygen evolution by illuminated Chlorella caused a corresponding increase in fluorescence. A possible explanation for the stimulation effect of herbicides on the fluorescence is hypothesized as being that the excess photochemical energy normally channelled into the carbon reduction cycle is now dissipated as radiant (fluorescent) energy (196). Zweig et.al. (198) also established an excellent correlation between oxygen evolution and fluorescence increase thus establishing the site of action to be close, if not at, that of the O_2-evolving system itself.

2.2.6 Effect on hydrogen adopted micro-organisms

Based on the work of Bishop (22,23) the primary site of action of ureas and triazines can be located with reasonable confidence in the Hill reaction centre. Bishop (22,23) demonstrated that substituted ureas and triazines had no effect on photoreduction reactions of the hydrogen adopted Scenedesmus.

2.2.7 Changes in electron spin resonance

Two overlapping electron spin resonance (ESR) signals are observed when algal cells are illuminated with light. ESR signal II appears to be related to the O_2 evolving mechanisms of green plants and ESR has been correlated with Hill reaction activity and fluorescence (4,8,104,188). When using algae cells the ratio of signal I to signal II is more important than the measurement of either signal alone. Ratio calculation is more reliable because it tends to compensate for the uncertainity in the number of cells in the sample tube during each experiment. Treharne et.al. (177) using this method were able to demonstrate an increase in the ratio of signal I to signal II in the presence of urea and triazine herbicides. Thus they established the primary site of action or lesion to involve the oxygen photolytic system.

2.2.8 Review

Even though there are several methods to elucidate the primary site of action (lesion) of Hill reaction inhibitors, the exact mechanism of action of these herbicides is still in doubt. Unless the biochemistry of O_2 evolution is worked out one will not be able to establish the mechanism of action of Hill reaction inhibitors.

As is evident from the above experiments many of the techniques employed involve studies with algae or isolated chloroplasts. Good (77) found no relationship between activity of compounds as Hill reaction inhibitors and their effective herbicidal activity. This lack of correlation is probably a manifestation of the difficulty of getting the chemicals to the appropriate sites. By studying the effect on isolated chloroplasts and algae, however, one overcomes difficulties such as root uptake and translocation. Also the aquatic environment provides a constant concentration for the test compound in which no photodecomposition occurs.

2.3 THEORIES ON THE MANIFESTATION OF PHYTOTOXIC SYMPTOMS

2.3.1 Introduction

Three theories have been proposed to account for the manifestation of phytotoxicity through the inhibition of Hill reaction or PSII.

2.3.2. Reduction of starch content or starvation theory

Typical symptoms produced by herbicides which inhibit photosynthesis are, chlorosis and necrosis of the leaves. The rationale for proposing the above theory was that, photosynthetic inhibitors block the active sites on the chlorophyll molecule. Consequently, the associated electron transfer system is inhibited and the production of ATP and reduced NADP which are utilized in the metabolism of carbohydrate are halted. Thus when the susceptible plant has exhausted its carbohydrate reserve, lack of photosynthates leads to death by starvation.

Aston et.al. (17) reported that simazine and simetone inhibited CO_2 fixation in light by Phaseolus vulgaris (bean plants). Atrazine completely blocked sucrose synthesis in light (197).

Moreland et.al. (127) showed that toxic action of simazine on barley plants (Hordeum vulgare L.) was through inhibition of activity of the chloroplasts. They were able to protect intact barley plants from lethal effects by supplying exogenous sucrose through open vascular bundles on leaf tips. These results indicate that the systems which utilise the photosynthates are not directly affected by these herbicides.

Ashton (14) treated bean plants with atrazine and kept these for 72 hours either in the dark or exposed to light. Only plants exposed to light were damaged. The weight of plants treated with either atrazine or monuron and grown under different wavelengths of light, produced by using coloured filters, indicated that the action spectrum of these herbicides corresponded to the absorption spectrum of chlorophyll. It was concluded, that the chlorophyll is the primary absorbing pigment involved in the production of toxic symptoms. Thus Ashton (14) and other workers, who found lack of toxic symptoms when treated plants were not subjected to light began to question the assumption that Hill reaction inhibitors killed by starvation.

Sasaki and Kozlowski (153) treated Pinus seedlings with atrazine and monuron and assessed the CO_2 consumption using infra red gas analysis. Atrazine caused chlorosis and necrosis at 3 weeks but photosynthesis stopped only after 4 weeks. However monuron halted photosynthesis in 11 days but no symptoms appeared within the 4 weeks. This difference in development of damage suggests monuron to be primarily a photosynthetic inhibitor while atrazine may affect other mechanisms too. For example Jordan et.al. (91) noted the inhibition of tobacco callus tissue in dark by atrazine at 10^{-7}M and monuron at 10^{-5}M. The triazine inhibited growth at concentrations that were required to block Hill reaction but higher concentrations of monuron was required.

Further evidence that development of leaf injury is not a direct result of lack of carbohydrate is provided by Davis (48) who treated seedlings of shrub live oak (Quercus turbinella Green) with fenuron. Leaf injury began prior to exhaustion of the food reserves as the acorns gave a positive starch test, and carbohydrate were still being translocated from the acorn, as root growth continued. Several flushes of leaves were formed and subsequently died. Application of sucrose delayed the development of symptoms but did not prevent eventual chlorosis and necrosis.

Banji and Krinsky (21) showed malate can act as a reductant for oxidised carotenoids. Stranger and Appleby (163) has proposed a redox reaction based protective role played by carotenoids, and they hypothesized, that sucrose served as a reducing source for carotenoids, thereby allowing them to function in protecting chlorophyll from photoxidation rather than acting as a carbohydrate source and thus preventing starvation.

2.3.3. Free fatty acid theory

Increased fatty acid concentrations have been shown to inhibit the Hill reaction (41,119,138). They associate the site of action with the photochemical reaction rather than the dark reaction. The inhibition is irreversible and evidences presented by Constantopoulus and Kenyon (41) and Smith and Wilkinson (159) show that fatty acids are firmly bound to the chloroplast structure.

Disorganization and destruction of chloroplast lamellae membrane have been shown to activate endogenous lipase enzyme systems (151,152). Spinach chloroplasts selectively release either saturated or unsaturated fatty acids depending on pH (41).

Constantopoulus and Kenyon (41) reported 70% decrease in Hill reaction actively in response to 42% increase in free fatty acids. Smith and Wilkinson's (1959) data, however, show that the 10^{-4}M atrazine induced increase in free fatty acids never exceeded 18%. This is less than half the concentration required to inhibit 70% of Hill reaction, but Moreland et.al. (125) and Moreland and Hill (126)

found atrazine at 10^{-4}M concentration to inhibit Hill reaction completely.

Inhibition of Hill reaction is directly correlated with concentration of herbicides (126) whereas no relationship could be established between increased fatty acids and concentration of herbicides (159).

Atrazine and simazine increased free fatty acids only 30 minutes after treatment (159) however it has been shown that inhibition of Hill reaction by simazine to be immediate (125,126).

Lee and Fang (102) have shown that monuron could be removed from chloroplasts by washing with concomitant restoration of activity, but free fatty acid inhibition is irreversible (41,159).

All the above arguments preclude the possibility of increases in free fatty acid concentration producing the initial Hill reaction inhibition. However, it provides evidence that the mode of action of the herbicides may involve destruction of chloroplast lamella system which may then lead to release of fatty acids through the action of endogenous lipase enzyme systems.

2.3.4. Production of toxic substances or free radical theory

The triazine, urea, and uracil compounds, though not considered to be growth regulators, have profound effect on chloroplast structure as well as exhibiting other growth effects. (7,15,42).

In two of their papers, Ashton et.al. (16,17) found atrazine to affect the chloroplast structure. Atrazine was observed to cause gross morphological changes such as precocious vacuolation of chloroplasts leading to eventual destruction in bean (16). In the latter study, (17) with the electron microscope, they observed the fine structure of chloroplasts. Chloroplasts of atrazine treated plants in the light assumed a spherical form, frets were destroyed which led to disorganization of grana. The grana swelled and eventually the granal membrane disintegrated. Starch disappeared from the lamella system. These effects did not take place in the

dark, thus, they are not results of diminished photosynthates following inhibition of photosynthesis. Ashton et.al. (16) also observed atrazine to have other growth effects. They observed treated plants to have reduced stomatal aperture and cellular air space, cambial activity ceased, sieve tubes and tracheal elements were observed to have reduced cell wall thickness. These effects only took place in the light and this led them to postulate a possible production of toxins. They proposed that the damage by atrazine in plants may be due to the reaction of atrazine and light in the presence of chlorophyll to form a free radical.

Hill et.al. (84) observed similar changes in barnyard grass (Echinochloa crus-galli) chloroplasts as those of Ashton et.al. (17). However, the changes observed by Hill and co-workers took from 2 to 8 hours and were well advanced in the first 12 hours, while those observed by Ashton et.al. (17) took 30 hours or more.

Similar effect of Hill reaction inhibitors on chloroplasts has been reported. Geromine and Herr (71) reported effect of pyridor on tobacco and Anderson and Schelling (6) reported similar effect by pyrazon on bean plants.

Sweetser and Todd (169) proposed monuron toxicity to Scenedesmus species in light and supplemented with carbohydrate was due to accumulation of toxic intermediates of photosynthesis. Davis (48) however, suggests production of toxic components in the blocked photosynthetic unit

Mitidieri as cited in Stranger and Appleby (163) hypothesised a mechanism based on above proposals and on studies reported by Krinsky (100). Krensky and co-workers have demonstrated how two forms of carotenoid pigments which underwent redox reaction in presence of light and oxygen in Euglena gracilis. Based on this he proposed a scheme through which carotenoids function to inactivate excited chlorophyll oxygen complex which would otherwise catalyse lethal photosensitised oxidations.

The carotenoid pair, antheraxanthin and zeaxanthin are thought to act as "chemical buffers" to protect cells from photoxidations.

Krinsky produced a scheme, called the epoxide cycle, which functions to protect green plants and algae against photoxidations. Chlorophyll excited by light is deactivated in the process of photosynthesis. However, some excited chlorophyll molecules combine with oxygen and this leads to photoxidations. The epoxide cycle could normally function to protect against this lethal process. In the cycle zeaxanthin will be oxidised to its epoxide derivative, antheraxanthin. An enzymatic process involving NADPH and antheraxanthin de-epoxidase regenerates the protective substrate zeaxanthin.

Mitidieri (163) hypothesized that Hill reaction inhibitors inhibit NADPH formation which is necessary for maintaining the protective mechanism and thus induce its phytotoxicity through photosensitised oxidations. Stranger and Appleby (163) tested this hypothesis and their data support the above proposal.

2.4 STRUCTURE ACTIVITY RELATIONSHIP

The effectiveness of a herbicide depends upon, its inherent toxicity and upon its ability to reach its site of action.

It has been speculated that the inhibitors act in a very lipophilic compartment or a very lipophilic enzyme or both (29,30,69 ,81,82).

General properties that will be of importance are thus:

(1) Partitioning characteristics expressed in terms of hydrophilic/lipophilic balance, which will allow penetration to active sites.

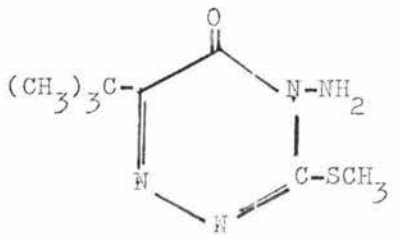
(2) Steric configuration, i.e. the ultimate attainable configuration which should control the fit of the inhibitors to the above site as well as the ease with which they might reach the active site.

(3) Electronic distribution which contributes to chemical reactivity and to binding once the inhibitor reaches the active centres in the chloroplasts.

Based on these an approach towards quantative structure activity correlations has been developed by Hansch. This model is discussed by Gabbott (69) and Hansch (81).

For Hill reaction inhibitors the degree of negative charge on the carbonyl oxygen atom is alleged to parallel its effectiveness (81). The side chain is related to the promotion of electron release to the carbonyl group. Its contribution to the lipophilicity of the molecule is not considered to be important in the expression of inhibition (50,69,81,82,124). Most Hill reaction inhibitors considered by Hansch (81) possess in addition a N-H group attached to an electron deficient sp^2 carbon atom. Triazinones differ from the above, only in the fact that a nitrogen atom carrying a positive charge takes the role of electron deficient sp^2 carbon in this class of herbicide.

Triazinones have been shown by Derber et.al. (50) as examples of another group of Hill reaction inhibitors which fit the quantitative structure activity correlation of Hansch.



They obtained a good quantitative correlation between activity in the Hill reaction test and the partition co-efficient when only the substitute in position 6 is varied. Activity of the group depends on the steric effects exerted by substitutes in position 3. The results thus indicate that position 3 is the active site and position 6 may contribute to fixation of the molecule at the active centre through a hydrophobic bonding. Position 6 seems not to

exert any steric hindrance and electronic factors play a minor role. The assumption that the NH_2 group is the toxophore in the triazinone series seems to be consistent with their data and assumptions.

Chapter 3

MATERIALS AND METHODS

3.1 INHIBITION OF PHOTOSYNTHESIS BY SENCOR AND ITS METABOLITES

3.1.1 Introduction

A method of chloroplast isolation was devised, based on those described by Avron (19) Cockburn et.al. (37) and on discussions of Kalberber et.al (93) and Walker (184).

Measurement of the reduction of indophenol dye was based on the study of the role of indophenol dyes in photoreactions by Gromet-Elhanan and Avron (75) Keister (94).

Preliminary experiments were carried out to find the optimum pH for the extraction and reaction medium, the pH tested ranged from pH 6-8. Optimum macerator speed and duration of maceration was established. Also optimised were centrifuge speed and reaction time. Of the two wavelengths tested for spectrophotometric readings 620 nm was selected in preference to 600 nm. It was also established that centrifuging before taking spectrophotometric readings produced a more consistent result. The method finally adopted is described below.

3.1.2. Materials

0.1 M phosphate buffer of pH 7.6, extraction mixture containing 0.1 M sucrose, and 0.1 M KCl in 0.1 M phosphate buffer of pH 7.6. MSE Homogeniser, mirah cloth, bench centrifuge, Philips 400 W lamp. 0.001 M dichlorophenol indophenol dye (DPIP) Hitachi 101 spectrophotometer.

3.1.3. Isolation of Chloroplasts

Leaves from Solanum nigrum plants collected from the field were washed in ice cold distilled water, dried under blotting paper and weighed approximately to 5 gm. These were cut into 1 mm strips and added to a cooled macerator bulb containing 10 ml extraction mixture

The macerator was switched on for 30 seconds at full speed. The extract was then filtered through 2 layers of mirah cloth into cooled plastic centrifuge tubes and centrifuged from rest to 10^4g. to rest. The supernatant was then discarded and the pellets resuspended gently in 10 ml cold extraction mixture.

3.1.4 Preparation of bulk reaction mixture

The bulk reaction mixture was made up immediately before use as follows:

Phosphate buffer pH 7.6	-	37 ml
DPIP	-	5 ml
Chloroplast suspension	-	8 ml

This mixture kept in the dark by wrapping the tube in aluminium foil and sealing the top with black polythene.

3.1.5. Experiment

Four ml of the reaction mixture was drawn from the bulk solution and added to the tubes containing either 1 ml blank (methanol and water) SENCOR or metabolite (dissolved in methanol and then diluted with distilled water) at various concentrations. (The final concentrations in the tubes were either 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M, methanol concentration never exceeded 1 in hundred). The tubes were shaken and placed in the dark for 1 minute, then 4 minutes in the light; the tubes were held 20 cm from a Philips 400 W lamp. A tank of water was interposed between the lamp and the tube to act as a heat filter. The tubes removed from the light were immediately centrifuged at 10^{-4} g to remove the chloroplast particles and read in the spectrophotometer.

3.1.6 Measurement of inhibition

The rate of reduction of DPIP was measured using the spectrophotometer set at 620 nm. Blank containing chloroplast in a buffer solution but lacking DPIP was also centrifuged and then used to set the zero reference on the spectrophotometer. A tube containing the

reaction mixture but no SENCOR or metabolites was centrifuged and kept in complete darkness and read as a reference to 100% inhibition.

It is possible to complete each replicate of the experiments within 30 minutes.

3.2 USE OF THE GAS-CHROMATOGRAPHIC (GLC) TECHNIQUE TO STUDY PROPERTIES OF SENCOR AND ITS METABOLITES

3.2.1 Introduction

An attempt was made to produce standard curves for SENCOR and its metabolites DA, DK, and DADK. It was hoped that the partitioning behaviour of the herbicides and its metabolites could be studied using GLC techniques to measure the relative quantities in various solvents.

3.2.2. Materials

Pye Series 104 Chromatograph, equipped with a flame ionisation detector. Column : 4 ft 1/8 inch o.d. standard wall glass column packed with either OV1 solution coated on 80-100 mesh gas chrom Q or OV17 coated on 80-100 mesh gas chrom Q. These columns were conditioned as in the Series 104 Chromatograph Technical Manual (Model 64). Double distilled methanol, chemically pure standard SENCOR and metabolites DA, DK and DADK was supplied by Bayer, Germany. Pyridine and TMCS (Trimethylchlorosilane), TMS trimethyl ($-Si(CH_3)_3$) as N,O-bis-(TMS)-acetamide were obtained from Pierce Chemical Co. and BSTFA (N,N-bis (trimethylsilyl) trifluroacetamide) obtained from Sigma Chemical Co.

3.2.3 Method

Initially the method described by Robinson et.al. (145) and Gronberg et.al. (76) for detection of SENCOR was modified to suit the equipment. However, SENCOR was not soluble in hexane as stated in the above two papers, also substantial changes had to be made to temperature and gas flow rate before a suitable peak of SENCOR was recorded. For detection of SENCOR OV1 in gas chrom Q

was suitable. Other conditions are presented with the results. For the detection of metabolites several techniques were applied in an attempt to alter the retention time and eliminate the tailing (2,137). The two types of column described above and silylation methods were attempted. The following combinations were tried out as an attempt to silylate the metabolites.

1.	Metabolite	100 ul)	
	Pyridine	40 ul)	mixed and left overnight
	TMCS	20 ul)	in refrigerator
	BSTFA	40 ul)	
2.	Metabolite	100 ul)	
	Pyridine	40 ul)	mixed and conditioned at
	BSTFA	40 ul)	50°C for 3 hours
	TMCS	20 ul)	
3.	Metabolite	200 ul	
	Pyridine	100 ul	
	BSTFA with 1% TMS	100 ul	
4.	Metabolite	200 ul	
	BSTFA	200 ul	
5.	Metabolite	100 ul	
	Pyridine	40 ul	
	BSTFA	40 ul	
	TMCS	20 ul	

Gas flow was regulated between ten to 80 ml/min.

Chapter 4

EXPERIMENTAL RESULTS AND OBSERVATIONS ON THE PROPERTIES OF SENCOR

4.1 INHIBITION OF PHOTOSYNTHESIS BY SENCOR AND ITS METABOLITES

Photoreduction of DPIP activities in an isolated chloroplast system were measured. The data were expressed as percent inhibition. The percentage values were transformed to arcsine and analysed as whole plots in a randomised complete block design. The results are presented in Fig. 4 and Tables I, II & III from which the following observations are clear.

(a) Only SENCOR and metabolite DA are biologically active at the concentrations tested.

(b) Of the four chemicals tested SENCOR inhibits DPIP reduction at the lowest concentration DA requires an higher threshold concentration.

(c) DADK seems to have a higher activity than DK.

(d) For an increase in percentage of inhibition, proportionally more SENCOR seems to be required at the higher inhibitory levels (greater than 80% inhibition). In the case of DA however, the linear relationship seems to be maintained throughout, once the threshold concentration is reached.

Table I

Relative potencies of SENCOR and its metabolites

CHEMICAL	MEAN
SENCOR	50.99
DA	29.32
DADK	4.57
DK	2.84

Table II

Relative differences between concentrations at one level of chemical

Concentration (M)	SENCOR	DA	Dark	Dk
10^{-3}	90.00	90.00	17.72	18.06
10^{-4}	90.00	53.82	11.90	2.35
10^{-5}	80.44	27.55	3.49	2.35
10^{-6}	66.26	18.46	3.49	0
10^{-7}	36.39	16.12	0	0
10^{-8}	23.87	14.30	0	0
10^{-9}	20.92	14.30	0	0
0	0	0	0	0

Table III

Relative potencies of chemicals at one level of concentration

Chemical	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
SENCOR	90.00	90.00	80.44	66.26	36.39	23.87	20.92
DA	90.00	53.82	27.55	18.46	16.12	14.30	14.30
DADK	17.72	11.90	3.49	3.49	0	0	0
DK	18.06	2.35	2.35	0	0	0	0

Bar (|) indicates means are not significantly different at 5 per cent level as measured by Duncan's multiple range test (51,58,173).

Fig.4

SENCOR and its metabolite inhibition of dichlorophenol indophenol reduction (Hill reaction) by isolated chloroplasts of Solanum nigrum.

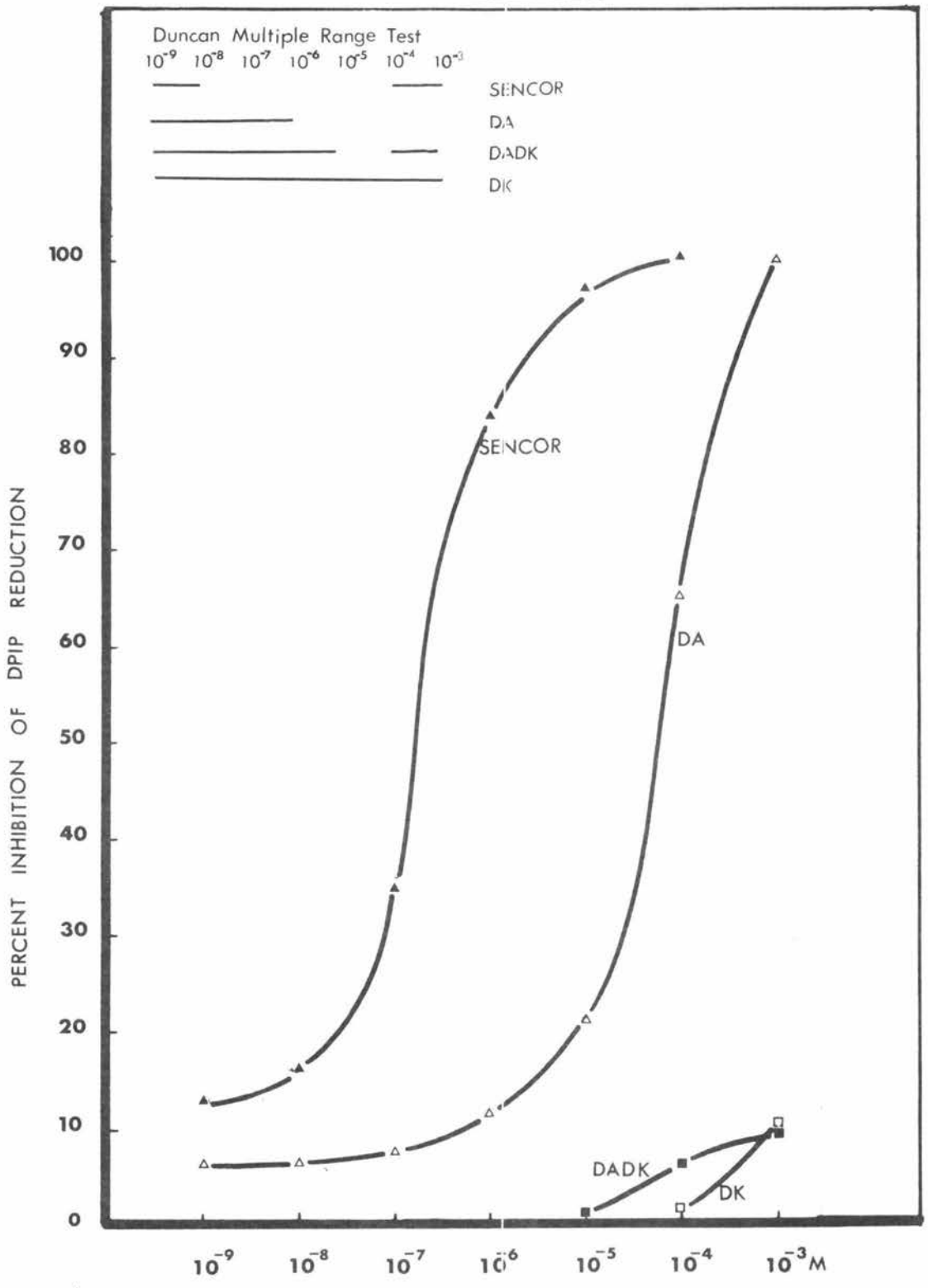
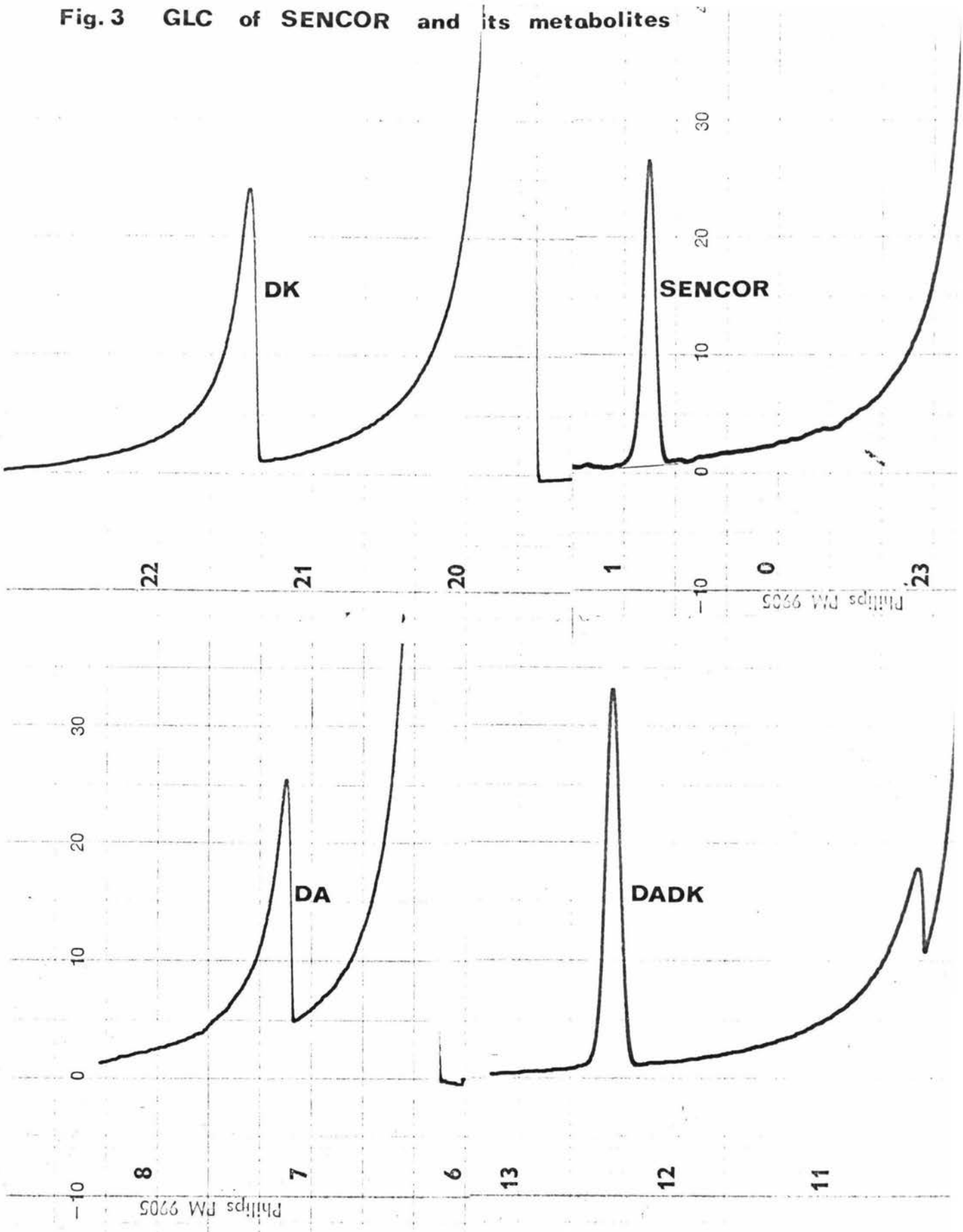


Fig. 3 GLC of SENCOR and its metabolites



4.2 USE OF GAS-CHROMATOGRAPHIC TECHNIQUE TO STUDY PROPERTIES OF SENCOR AND ITS METABOLITES

The chromatographs and conditions of operation of the GLC are presented in Fig. 3.

Attempts to produce suitable standard curves failed after 8 weeks of trial and thus the experiment to establish the partitioning behaviour of SENCOR and its metabolites was not attempted.

Though attempts were made to eliminate the tailing effect of the metabolites, especially at lower concentrations, none of the methods attempted produced improved results. It appears that GLC with control of injection port temperature may have improved the results and also a different column should be used. Recently Church and Flint (36) have been successful in producing suitable results using glass columns packed with 5% Reoplex 400 on 80/100 mesh Gas Chrom Q (Applied Science Laboratories) or glass columns packed with 3%)V-25 on 100/120 mesh Super port (Supelco Laboratories, Incorporated). This report was received after the experiment was terminated.

From Figure 3 in page 25, it is evident, however, that all chemicals, except DADK are chemically pure. DADK seems to contain an impurity with retention time very close to that of methonal, the solvent.

4.3 DISCUSSION

Little has been reported on the property of SENCOR. Of the reports published (76,50) there is an element of uncertainity as regards the mode of action of this herbicide.

In the present report, the relative potencies of SENCOR and its metabolites DA, DK and DADK is established. The observed action of SENCOR and its metabolites are then discussed in terms of structure activity relationship.

The mechanism of action of SENCOR and its metabolite DA are compared with those of monuron, isocil and simazine which belong to groups of herbicides, the phenyl ureas, the substituted uracils and the s-triazines respectively. A scheme is then proposed for the mode of action of Hill reaction inhibitors.

Figure 4 indicates that in the present study, SENCOR and DA to be biologically active at the concentrations tested. Statistical evidence from Table I supports that DK and DADK are not significantly different in their activity. The loss of the thiosulphate ($-SCH_3$) group attached to the carbon at position 3, the only common alteration to the SENCOR molecule to yield DK and DADK metabolites, renders the herbicide biologically inactive. It is thus evident that the thiosulphate group is the toxophore.

From Figure 4 the nonlinearity of the dosage probability curve is eliminated by expressing the dosage logarithmically (192). When the data of graded responses are plotted against log-dosage it yields a symmetrical curve (26). The long central section of the curves can then be used as the linear expression of the data. Percentage of inhibition is then directly plotted on log-probability graph paper to compare the mechanism of action of SENCOR and DA. It is also then possible to establish the pI_{50} valves (negative log of the inhibitor concentration giving 50% inhibition of DPIP reduction). Thus from Figure 5 it is evident that the dosage-response curves of SENCOR and DA are parallel. It has been established that for toxicants, equal slopes describe a similar mechanism of action, provided the compounds are structurally related (110). SENCOR and DA are structurally related and since the conditions of the experiments were the same, one could conclude that both SENCOR and DA have similar mechanism of action.

The experimentally established pI_{50} value of SENCOR is 6.7 and that of DA is 4.1. Thus SENCOR is a relatively more potent herbicide than DA. Also SENCOR has a lower threshold concentration than DA (Fig.4). From these observations and results in Table II and III it is clear that a higher concentration of DA is required to

Fig.5

Log-dosage probability curves of SENCOR and DA.

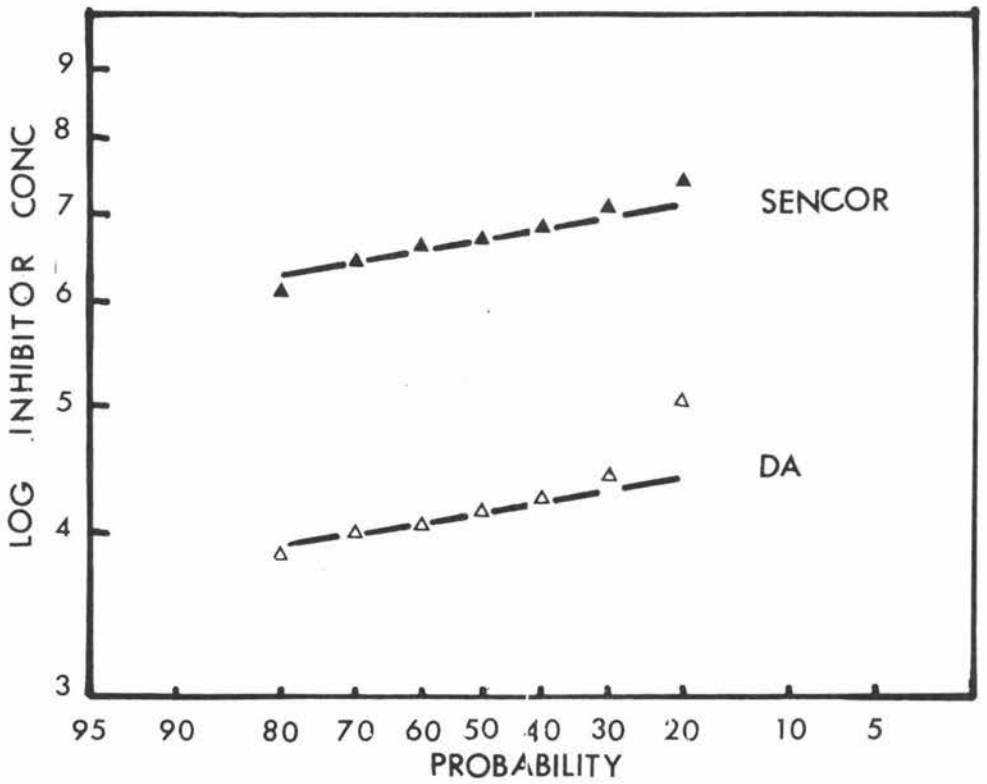
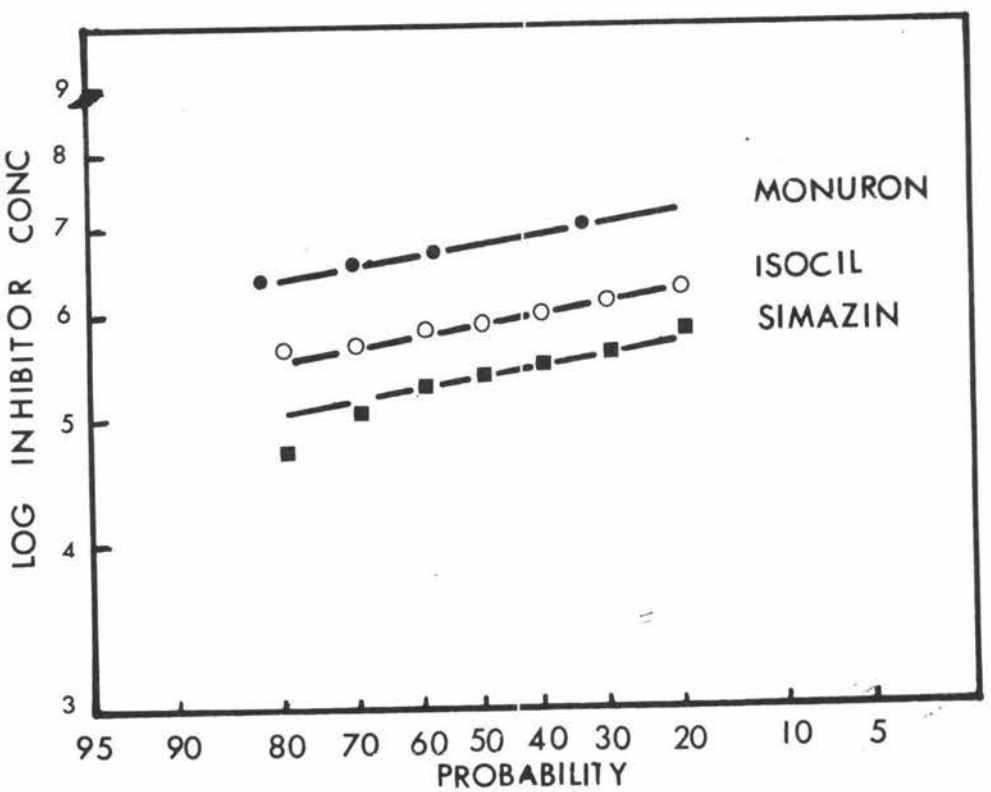


Fig.6

Comparison of mechanism of action of monuron isocil and simazin. Data transformed from (85,102,125).



produce levels of inhibition similar to that of SENCOR. The loss of the NH_2 group from SENCOR thus seems to alter the lipophilic/ hydrophilic balance or the partitioning property of the molecule such that a higher concentration is required before DA begins to inhibit the reduction of DPIP. However, once the limit below which DA ceases to be perceptible is passed, its mechanism of action and potency is similar to that of SENCOR as indicated by the parallel lines in Figure 9.

From Figure 4 visual evidence indicates that DADK to be more active than DK. This contradicts the logical deduction as well as experimental evidences (76,36), that of the metabolites tested DADK is positioned last in the metabolic degradation sequence. It is thus expected that DADK should be less active if not, at least similar in its relative potencies to those of DK. Statistical evidence indicates no significant differences between the two metabolites at 5% level. Also the slightly higher activity evident in (Fig.4) for DADK can be attributed to the impurity in the sample used. (Figure 3) as evident from the GLC study (Fig.3). Since the impurity has a retention time close to the solvent methanol, one could expect the impurity to possess some properties similar to that of methanol. Methanol has the effect of damaging chloroplasts and is used as an extractant of chlorophyll (12). It is possible that the impurity may cause similar damage to the chloroplasts. Thus the slightly higher activity of DADK compared to DK as evident in (Fig.4) is not a property of the chemical itself but may be attributed to the impurity in the sample.

SENCOR seems to be less efficient at higher concentrations (Fig.4). Proportionally more SENCOR is required for an equal increase in inhibition, e.g., at levels greater than 80% inhibition. DA, however, maintains almost a direct proportional relationship throughout. The observed property may be due to the amide side chain blocking the site of action through either steric, structural or electronic influence and thus preventing further SENCOR molecules reaching the site of inhibition. It must be emphasized, however,

that this implication is based on observations (Fig.4) alone, it is not possible to provide any statistical support from the data recorded.

The results presented in this report dispute the assumption based on the structure activity relationship studies of Derber et.al. (50) that the NH_2 group is the toxophore. Derber et.al. (50) based their arguments on substitution studies whereby various groups were substituted to the carbon at position 3 of the triazine molecule. They tested neither the triazinones lacking the NH_2 substituent nor a diketo form of the molecule.

It is also evident from this study that SENCOR is the active form of the herbicide. The allegation that DK is the active form (76) is not in accordance with the results presented here. It must be pointed out, however, that Gronberg and his associates (76) suggestion was based only on the observed structural similarity of the DK molecule and that of uracil which is a Hill reaction inhibitor (83).

Derber et.al (50) determined the partition co-efficient, in an octanol/buffer system, of several triazinones with carrying substituents attached to the carbon at position 6. They then correlate the partitioning properties of these molecules with their pI_{50} values determined from Hill reaction inhibition studies. They claim that if through substitution a more lipophilic molecule is producted, the easier it will penetrate the chloroplast membrane. However, from studies on the structure and functions of the chloroplast lamellae (135,72) the indications are that a hydrophilic/lipophilic balance is more important in facilitating penetration of the herbicide molecules to the active centre. The chloroplast lamellae, like many functional membrane systems in nature, are composed of high density lipoprotein - half lipid and half protein (106). The lipids are, however association with the interior of the protein molecules rather than exposed to the external water phase (190). It has also been established that the Hill reaction centre

and the photochemical systems are sited not on the membrane surface itself but along hydrophobic regions within the chloropast lamellae. There are several pieces of evidence that these lipophilic regions are sited mainly within the chloroplast lamellae.

Among these evidences are:

(a) Deep etching experiments which showed that the chloroplast lamellae fracture plane differs greatly in morphology from the adjacent membrane surface (134).

(b) Experiments in which the lipid when extracted or fixed with $KMnO_4$ the fracture plane was lost (28).

(c) A model experiment using ^{14}C labelled stearic acid bilayers, where, the splitting of such layers after freezing occurred along the hydrophobic region of the bilayers (49).

(d) Theoretical arguments of Brandon and Park (28), where it was predicted that loss of the restoring forces of liquid water by freezing would render the hydrophobic region of the membrane as the most weakly bonded region during freeze fracture experiments.

It is clear from above that for a herbicide, to reach its site of action it must penetrate firstly a mainly protein portion of the chloroplast lamellae and then act at a site that is embedded in a region that is lipophilic. Thus it seems logical that for a Hill reaction inhibitor a balance in hydrophilic/lipophilic property is more important in determining its ability to reach its site of action. From this study it has been predicted that the loss of NH_2 group upsets this balance and thus provides basis for the property of DA. The emphasis Derber and his associates (50) base on the lipophilic property alone determining the facility with which the herbicide molecule penetrates the lamellae system seems illogical.

From the studies of Husisige and Yamamoto (86) it is evident that DPIP accepts electrons close to the water splitting system.

Hugisige and Yamamoto (86) however, propose PSII to include two different photoreactions similar to those proposed by Arnon's group (97). This proposal differs from the generally accepted scheme of Boardman and co-workers (Fig.2). It is important to note however, that Husisige and Yamamoto's proposed system differs from that of Arnon's in that NADP reduction does not occur through co-operation of the two PSII (IIa and IIb). Arnon's proposal that NADP reduction is carried out by PSII besides PSI has been the point of controversy that led to their scheme being less acceptable than that of Boardman's group. If Husisige and Yamamoto's proposals are accepted then DPIP reduction is a close and convenient measure of Hill reaction activity of isolated chloroplasts.

In (Fig.6) data obtained from studies of Lee and Fang (102) on monuron, Hoffmann et.al. (85) on isocil and Moreland et.al. (125) on simazine are plotted directly as a log-inhibition, probability curve (Fig.6). The phenylureas, substituted uracils and triazines have been established as Hill reaction inhibitors (191,125,123,77,126,78, 83,140). It is evident that the mechanisms of action of these herbicides are similar since the plotted curves are parallel (Fig.6). It is stressed here, that it is incorrect to make any comparison of relative potencies of these compounds from figure 6.

Conditions of experiments in the 3 papers from which these data were drawn differ! Figure 6 is exclusively used here to establish the similarity in the mechanism of action of these compounds. Figure 6 when superimposed on (Fig.5) provides further support that SENCOR is a Hill reaction inhibitor, since all the curves are parallel.

The 50% inhibition concentrations (expressed as pI_{50}) determined with isolated chloroplasts and artificial electron acceptors for the best of Hill reaction inhibitors are in the range of 10^{-7} moles/l (i.e. pI_{50} is greater than 6) (124). The pI_{50} value of SENCOR is 6.7, this is within the above range. Thus even though the experimental conditions in this study differs from that of

Moreland (124) it is evident from the pI_{50} value that SENCOR is a Hill reaction inhibitor.

The above discussion:

(a) on the site at which DPIP accepts electrons in the photosystem.

(b) the graphical analysis of mechanism of action of SENCOR and known Hill reaction inhibitors (Fig.5 & 6), and

(c) pI_{50} value of SENCOR being in the range of other known Hill reaction inhibitors,

provides substantial support for mechanism of action of SENCOR to involve inhibition of the Hill reaction.

From the above discussions it is not apparent how the phytotoxic symptoms are manifested. From the literature reviewed in this study (2.3) the scheme proposed by Mitidieri and cited by Stranger and Appleby (163) is the most logical. However, this scheme does not account for all the symptoms and the eventual senescence of the plant treated with Hill reaction inhibitors.

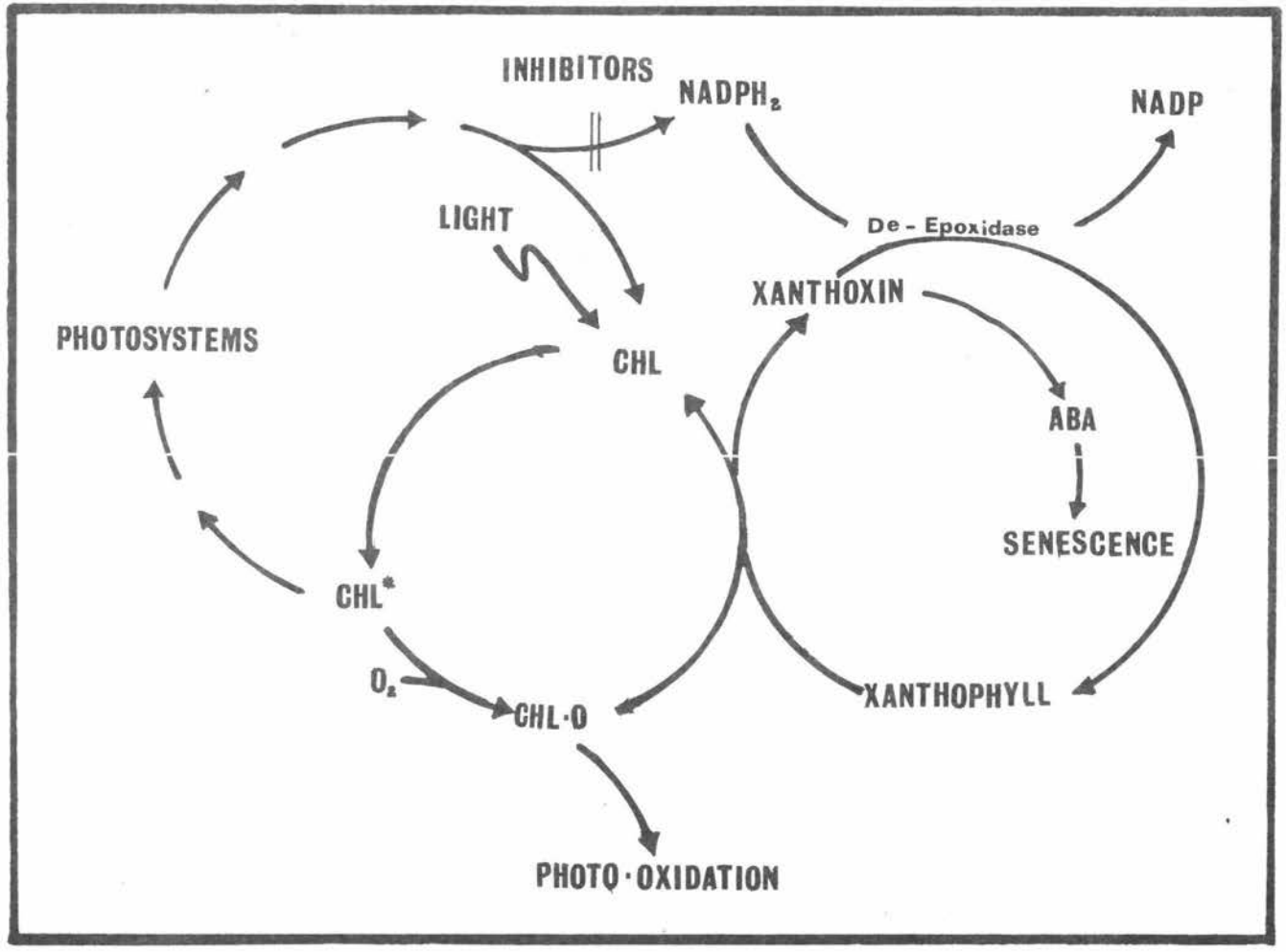
Here, the proposed scheme of Mitidieri is extended in order to develop an explanation to account for the observed symptoms, i.e. chlorosis and senescence.

Butler and Simon (31) have published a detailed review of senescence and Anderson and Thomson (7) have established, through meaningful comparisons, similarities between herbicide induced and natural senescence.

Butler and Simon (31) and Phillips (139) have cited several papers to suggest that senescence in plants may be regulated by changes in the plant hormonal level. It is apparent from these reviews (31,139) that no one hormone may have overall control of senescence, the balance in hormonal levels seem more important. However, it is evident (185) that much of the senescence symptoms can be induced by an increased level of the plant inhibitor abscissic acid (ABA). Perhaps ABA is the trigger mechanism involved in

Fig.7

Schematic representation of the mode of action of Hill reaction inhibitors.



effecting senescence.

There is conclusive evidence that ABA is synthesized from the plant carotenoids. Certain leaf xanthophylls can be oxidatively degraded to yield xanthoxin which then, acts as a precursor in the biosynthesis of ABA (171,170,172). Xanthoxin has also been established as naturally occurring in plants (61).

Based on the foregoing reports, the scheme presented by Stranger and Appleby (163) can be extended to include the synthesis of ABA through xanthoxin production which occurs due to photo-oxidation of xanthophylls. ABA then acts as a trigger for senescence. This scheme is presented diagrammatically (Fig.7).

If this scheme is acceptable, then the observations that plant treated with Hill reaction inhibitors and placed in the dark failed to produce phytotoxic symptoms (2.3) is readily explained. In the dark chlorophyll is not photo-oxidised. The xanthophylls are not involved in the epoxide cycle and thus they are not oxidised to xanthoxin and further degraded to ABA. Since the level of this senescence triggering hormone is not raised, senescence is not effected. Also in the dark the accumulation of betaxanthin has been shown to be slow (73). Thus the level of xanthoxin and ABA could be assumed to be similarly lower. Thus ABA level may not be high enough to trigger off senescence.

The delay in phytotoxic symptoms when sugars are supplemented after treatment with herbicides (2.3.2.) can also be explained through this scheme. Several of the ABA induced physiological changes, e.g., the closure of stomata are reversible on the removal of ABA (47). The reversal cannot be attributed to the catabolic degradation of the hormone (46) but may involve removal of the hormone through conjugation to sugars and other plant constituents or to sites where it cannot manifest its actions. It is possible that, the supplied sugars apart from functioning as a reductant of oxidised carotenoids (163) may be involved in the inactivation of ABA through the formation of conjugates. In fact the first products of ABA catabolism has been established as the glucose ester of ABA (abscisyl-B-glucopyranoside) (99).

Lichtenthler (105) has shown that the breakdown of chloroplast thylakoids in a number of species is accompanied by an increase in the leaves of carotenoids, but, xanthophyll levels remain constant. This implies that no further ABA production occurs! This observation of Lichtenther however, does not dispute the proposed scheme for the manifestation of phytotoxic symptoms through production of ABA. It is argued here, that once the senescence is triggered and the structural and functional degradation reaches the irreversible phase further production of ABA is not necessary for the continuation of the degradation processes. At this stage chloroplasts may be ruptured and this will activate the lysosomal enzymes (114,180,115). Also latent acid phosphatase (28) and lipase enzyem (151,152) present in the chloroplasts may be involved in the further breakdown once initial degradation processes had been induced.

PART II

BASES FOR SELECTIVITY

Chapter 5

PHYSIOLOGICAL AND BIOCHEMICAL BASES FOR HERBICIDAL SELECTIVITY

5.1 INTRODUCTION

Selectivity is a phenomenon, where, a chemical affects one type of organism without unduly harming another when both types are in contact or in close proximity. It is a relative rather than an absolute phenomenon. The living matter which is to be injured or destroyed is usually an "uneconomic species" and that which is to remain unaltered is usually an "economic species".

Mechanism of selectivity involving either physiological or biochemical aspects may be classified into groups based on three main principles. Thus the fundamental bases for chemical selectivity may involve;

(i) accumulation of the toxicant, principally by the uneconomic species at the site of influence

(ii) utilisation of comparative biochemistry whereby the chemical may injure a physiological or biochemical system important to the uneconomic, but not to the economic species.

(iii) it may react exclusively with a cytological feature that exists only in the uneconomic species

Selectivity through accumulation is sometimes only a matter of form and gross morphology. Thus, the comparative hairiness, of weeds in a crop of grain, or a comparative large surface area brings about greater retention of sprayed material by the uneconomic species. In other cases selective accumulation is achieved in a positive way.

Though there are remarkable physiological and biochemical similarities between organisms, striking differences have been found in many of the processes. Such differences are found not only in the degradative process, but in the choice and biosynthesis of enzymes

and smaller substances used in growth and division. Since the smallest changes in chemical structure of a selectively toxic agent often leads to an enormous change in its biological activity, these biochemical differences offer bases for selectivity.

Selective action of a toxicant can also be based on cytological differences within species. It has long been known that plants and animals have outstanding cytological differences. With the help of electron microscopes, it has been found that a cell itself is full of component parts, the organelles. It is also known that each kind of these components display strong species differences; also there are differences between cells from different tissues in the same species.

Often these principles function together and are not separable. In other cases one mechanism may predominate.

From the above it is apparent, that in contrast with ecological selectivity, such as physical separation, the chemical and biochemical selectivity involves activation or inactivation of herbicides or biochemical variation in the nature of the active site itself.

Most activation or inactivation reactions are enzymic reactions, however, examples of non-enzymic or chemical inactivation also occur. As an example the replacement of chlorine with a hydroxyl group in chlorotriazines may proceed via a primary reaction involving an enzyme or it may involve reactive plant constituents such as the oxazinones (109).

MCPB and 2,4-DB are typical examples of selective herbicides designed to take advantage of the difference in the activation mechanisms of weeds and crop plants (181).

Several works on metabolism of s-triazines could be cited to provide evidence, that chemical transformations alter the hydrophilic/lipophilic balance of a chemical. Such transformation provides a basis for tolerance since transformations may prevent the toxicant reacting at its site of action.

The inactivation mechanisms both, degradation of herbicide and conjugation with some kind of metabolites could be considered as mechanisms attributing to selectivity of amiben. The formation of N- glucosylamiben has been connected with the inactivation of amiben (164).

A more typical example will be the selectivity of propanil in rice plant (Oryza sativa L.). The tolerance of rice plants to this herbicide has been explained by the existence of a special enzyme which can hydrolyse propanil into 3,4-dichloroaniline (66).

Physiological differences which cause lack of movement of herbicides, resulting from such factors as, conditions detrimental to photosynthesis or assimilate translocation, anatomical or physiological barriers, fixation to cellular or metabolic components may all contribute to the selective action of herbicides.

With the aim of illustrating a particular mechanism of selectivity, where possible, only one example of herbicide/plant relationship is considered in detail. An attempt is then made to cover all factors that contribute to such a mechanism. However, it must be emphasized that other herbicides may follow similar mechanisms of selectivity.

It is hoped that from this review of literature, the three principles involved in physiological and biochemical aspects of selectivity will be apparent. It will be obvious also that it is difficult to separate the various processes that contribute to selectivity.

5.2 ENZYMES IN RELATION TO SELECTIVITY

The tolerance of rice plants to propanil through enzymic actions is the best illustration of the existence of degrading (hydrolysing) enzymes in plants. The difference in the level of this enzyme in various plants has been illustrated as a basis for selectivity.

Propanil shows high selectivity between rice plants and weeds, especially barnyard grass (Echinochloa crus-galli L.) (116). The

mode of action of this herbicide has been established as inhibition of photosynthesis. (Hill reaction) (118).

Adachi et.al. (3) studied the distribution of the inactivating activity in the higher plants (see Table IV). It is evident from their studies that both rice plants and crab grass (Digitaria sanguinalis (L). Beauv) have high degradative activity while barnyard grass has a very low activity. They also established that hydrolysis of propanil (N- (3,4-dichlorophenyl)-propionamide) into 3,4-dichloroaniline and proponic acid had an optimum pH of 8.4 and the boiled homogenate was inactive. This provided indirect support from the existence of a degrading enzyme.

Table IV

Inactivation of propanil by homogenates of various plants. (Adachi et.al. 3)

Source	% Hydrolysed in 24 hours
monochoria (Monochoria vaginalis)	0.0
smartweed (Polygonum sp.) (willow weed)	5.5
barnyard grass (Echinochloa crus-galli L.)	7.0
crab grass (Digitaria sanguinalis L. Beauv)	58.4
rice plant (Oryza sativa L.)	69.6

Ishizuka and Mitsui (87) had the opinion that even barnyard grass had a high hydrolysing activity. Their opinion was based on the hydrolytic activity on other anilids in barnyard grass. Thus, they alleged that the difference of activity in the case of propanil is not based on the existence or absence of an hydrolysing enzyme but rather on the difference in the specificity of the enzymes for substrate.

Between the rice plant and barnyard grass one could expect many histological, physiological and biochemical differences, and if comparisons of tolerance due to special histological differences or to localization of enzyme are made, the mechanisms of tolerance will be mixed and confused with each other.

Matsunaka (117) fortunately found a rice plant mutant susceptible to propanil. An artificial mutant of rice plant was induced by chemical and isotopic radiation. To elucidate the cause of selectivity, the susceptible mutant was compared with the original variety of rice plant (Norin No. 8) and susceptible barnyard grass.

Matsunaka (117) found the mutant to be highly susceptible to propanil. For example at the treatment of 0.05% of propanil there were almost no symptoms in the original variety Norine No. 8, while the mutant No. 408 and barnyard grass were killed. Comparisons of the effect of treatment of 0.1% propanil emulsion on the photosynthetic activity of the mutant and the original, showed that the CO_2 fixation process of the original rice plant variety was at first inhibited by the treatment but it recovered perfectly after 3 days. On the other hand in the case of propanil treated mutants there was no recovery.

A study of the activity of enzymic hydrolysis of propanil in the mutant and tolerant variety was then carried out (117). The amount of metabolite (3,4-dichloroaniline) produced was compared after incubation of the enzyme solution (rice leaf homogenate) and substrate (propanil) for just one hour. The original variety showed high activity of the enzyme, on the other hand the homogenate from the mutant produced a trace amount of 3,4-dichloroaniline. The mixture (1:1) of both homogenates showed just half of the original activity. These results may indicate a lack of propanil hydrolysing enzyme in the susceptible mutant.

Yih et.al. (195) showed two steps to be involved in the hydrolysis of propanil to 3,4-dichloraniline. It is however not clear from Matsunaka's (117) investigations which of these steps is blocked in the mutant variety.

Frear and Still (66) succeeded in partially purifying an enzyme, arylacylamidase (arylacylamine amidohydrolase) from rice plants. This enzyme hydrolysed 3,4-dichloropropananilide to 3,4-dichloroaniline. This enabled comparative studies on rice and barnyard grass tissues on the relative amounts of the acylacylamidase enzyme to be carried out. The enzyme was present in all the tissue studied although rice leaves were found to contain 50 times more enzyme units than barnyard grass.

The above studies provide conclusive evidence that the differential effect of propanil on rice and barnyard grass is due to the striking difference in the enzyme distribution in the tolerant and susceptible plants.

There are significant differences among various species with regards to their rate of urea herbicide dealkylation. Cotton (Gossypium hirsutum L.) seems to be particularly efficient in breaking down these compounds, whereas corn (Zea mays L.) seems to be the most inefficient of the plants investigated (161,147,132). This selective action can be attributed to the differential levels of degradative enzyme in the susceptible and tolerant plants.

Based on the structure of the dialkyl and alkylalkoxphenyl ureas one might be tempted to suggest that the classical urease type of reaction to cleave these compounds directly may occur but it is now evident that although urease is widely distributed in plants, the enzyme has an absolute specificity for urea (165). Frear (64) has partially characterized a cotton leaf microsomal oxidase system that N- demethylated monuron 3- (4-dichlorophenyl) -I,I- dimethylurea to 3- (4-dichlorophenyl) -I-methy-urea. The difference in the level of this enzyme in tolerant and susceptible plants however has not been investigated.

Induced abberations in the nucleic acid metabolism has been proposed as a mode of action of auxin herbicides.(15). Malhotra and Hanson (113) found that picloram promotes nucleic acid synthesis both in susceptible (soybean and cucumber) and resistant (barley, wheat and maize) plants. From their study they allege that the presence

of higher levels of bound nuclease are responsible for prevention of accumulation of nucleic acids in resistant species. They propose that although picloram reduces nuclease in both the resistant and susceptible species, the higher net concentration of nuclease in the resistant plant, however, maintains the high rate of degradation of nucleic acid that is synthesized. In this case again, differential levels of an enzyme in a resistant and susceptible species have been proposed as the basis of the mechanism responsible for selectivity. This proposal was based on studies on the changes of nucleic acid levels in the picloram treated susceptible and resistant varieties. Thus the proposed mechanisms is based on indirect evidence. The role of nuclease in the metabolism of the cell is still unknown and the relative concentration of this enzyme in resistant and susceptible species has not been established.

Jordan and Jollife (90) believe that the metabolism of simazine in association with citrus roots may be at least partially responsible for the tolerance of citrus to simazine when it is used as a herbicide in orchards.

The selective action of pyrazone between Chenopodium album and sugar beet (Beta vulgaris var saccharifera) (63). DNOC between Orobanche sp. and beans (Vicia faba) (174) soybean (Glycine max L.) tolerance to fluorodifen (146,53) can all be attributed to the difference in metabolic rate. Wheat (Triticum aestivum L.), barley (Hordeum vulgare L.), wild mustard (Sinapis arvensis L.) and tartery buck wheat (Fagopyrum tataricum (L.) Gaertn) tolerate dicamba in that order (35). This ranking corresponds with the ability of the plants to metabolise dicamba.

Although, one can list several cases of selectivity due to differential rate of metabolism of herbicides, the identity and relative abundance of the enzymes in tolerant and susceptible species has seldom been established.

5.3 SELECTIVITY DUE TO ACTIVATION THROUGH SIDE CHAIN DEGRADATION

Wain (181) showed that 2,4-DB and MCPB themselves to have no herbicidal activity, however when transformed to 2,4-D or MCPA by the β oxidation mechanism in plant tissues, they converted to active herbicides. Based on this it was proposed that weeds having high rate of β oxidation activity such as annual nettle, (Urtica urens L.) charlock (Sinapis arvensis L.) and californian thistle (Cirsium arvense (L). Scop.) would be killed by 2,4-DB or MCPB but clover plants, other legumes and celery plants (Apium graveolens var dulce) having low β oxidation activity would be relatively unaffected. The selective action of 2,4-DB between soybean and cocklebur (Xanthium sp.) is yet another example where such a mechanism is involved (186). Thus 2,4-DB can be regarded as an herbicide designed to take advantage of lethal synthesis within the plant by means of β oxidation. By this reaction specific β oxidation enzymes in plant tissue attacks the β carbon or the organic acid and oxidises it, removing two carbon atoms from the chain. Thus as shown in (Fig.8) the inactive butyric form is oxidised to the herbicidally active acetic acid form.

Selectivity exhibited by MCPB (4- (4-chloro-2-methylphenoxy) butyric acid) and 2,4,5-TB (4 (2,4,5-trichlorophenoxy) butyric acid) follows a similar mechanism (183).

Wain (182) has shown that cereals and legumes are able to oxidise the butyric acid side chain of 2,4-DB only at a slow rate and thus are able to escape the herbicidal effects. Most weeds on the other hand carry out β oxidation rapidly and are thus killed due to the lethal rate of production of the active acetic acid derivative 2,4-D. The apparent selective action of 2,4-DB was thus attributed to the differential quantity of oxidase enzyme in the plants.

The most direct evidence that β oxidation is the primary mechanism of conversion of 2,4-DB to the toxic form was provided by Webley, Duff and Farmer (189) who found β hydroxy acids after

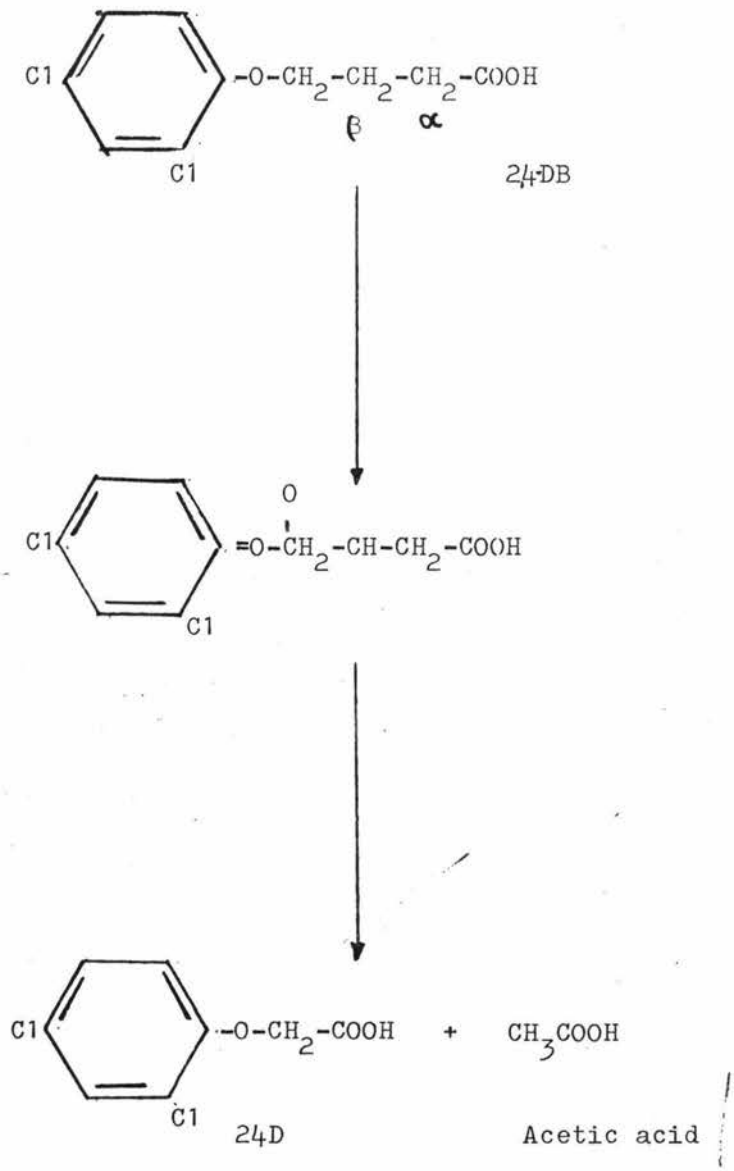


Fig 8 Beta oxidation of 2,4-DB

exposure of chlorophenoxybutyric acids to Nocardia. Using a homologous series of ω phenoxy acids Fawcett, Ingram and Wain (55) obtained chemical evidence of β oxidation by treating flax seedlings (Linum usitatissimum) through the roots. Harmless phenols were produced only where the acids possessed an even number of side chain methylene groups. Whereas the lethal active acid derivative was formed if the side chain contained an odd number.

Findings that γ - (2,4,5 trichlorophenoxy) butyric acid and corresponding caprionic and octanic acids are highly active in one test and not in others indicated that specific β oxidase enzyme systems may be present in different plant species. Evidence for this was obtained by exposing solutions of the 2,4,5-trichlorophenoxy homologues first to wheat coleoptile tissue and then examining them in the pea test. They showed typical alternations in activity indicating that the enzyme system present in the wheat tissue had degraded alternative homologues to the acetic derivative, active in the pea test. Further evidence that the enzyme in the wheat tissue were affecting β oxidation of the side chain was obtained by treating wheat coleoptile tissue with solution of γ - (2,4,5 trichlorophenoxy) butyric acid and demonstrating by chromotography that conversion to the corresponding acetic acid had taken place (183).

2,4-dichlorophenoxy acids were metabolised in wheat coleoptile and pea stem tissues and the products subjected to chromotographic analysis have shown that the homologue with an odd number of methylene groups are the only members of the series which can yield the highly active acetic derivatives (56). Studies of Fawcet et.al. (57) showed that ring substitution played an important part in influencing the degree to which β oxidation occurred at the butyric acid step, hindrance being associated with electronic and steric factors. Linscott (108) pointed out that plants may have a tolerance potential if they lack the capacity for β oxidation if the reaction proceeds too slowly or the plant rapidly detoxifies the herbicide.

There is evidence that the mitochondrion is a major site of β oxidation (103). Consequently meristamatic regions, such as root and shoot apices which contain many mitochondria may be important regions of conversion. It has also been reported that many mitochondria occur in phloem companion cells (120).

The location of β oxidation enzymes is a controversial issue. It is still uncertain whether penetration of outer or both mitochondrial membranes is necessary (143). Allmann et.al. (5) carried out enzymic analysis of membrane fractions of beef heart mitochondria. They suggest that enzymes activating fatty acids (Carnitine-long chain acyl transferase) occur in the interior part of the outer membrane enabling their products the acyl-CoA esters, to penetrate the membrane as acyl carnitine esters and so interact with the enzymes for β oxidation situated in the interior face. It has previously been reported by these workers that the outer components necessary for β oxidation and citric cycle enzymes except for succinic dehydrogenase were also located in the outer membranes while the electron transfer chain was localized in the inner membrane. It was envisaged that NADP was the mobile link between the dehydrogenases of the outer membrane and the electron transfer chain, the inner membrane is thought to be endowed with the property of limited permeability, while the outer membrane is considered to be a porous sieve imposing no restraint on the movement of the solute molecule. There is also evidence to suggest that extracellular enzymes may be responsible for β oxidation of phenoxybutyric compounds. Garraway and Wain (70) found evidence of extracellular oxidation in beans (Vicia faba) and extra mitochondrial enzyme system has been discovered in germinating peanuts (Arachis hypogaea) cotyledons which catalysed the β oxidation of palmatic (long chain) and butyric (short chain) fatty acids without the operation of TCA cycle. It is believed such a mechanism takes place in the microsomes.

Since the exact site of β oxidation is still not definite, it is not possible to speculate or infer localization of enzyme as

contributing to selectivity. It is also not possible to measure levels of enzyme at the active site and correlate selectivity to a differential level of β oxidation enzyme. Thus, the exact mechanism, i.e. whether a differential level of enzyme itself or a selective exclusion of the butyric acid derivatives from the sites of lethal oxidation within the mitochondrial membrane attributes to the selectivity is still to be elucidated.

5.4 INACTIVATION DUE TO PLANT ENDOGENOUS CHEMICAL SUBSTANCE AS BASIS FOR SELECTIVITY

The triazines, as a group have gained much attention as selective herbicides. Among the transformation mechanism the inactivation of S- triazines due to endogenous chemical substance present in plants like corn, provides an excellent example of a selective mechanism that involves a non-enzymic chemical reaction.

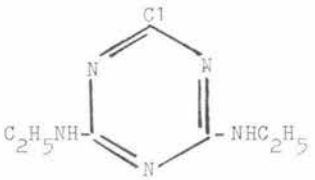
The classical example of this mechanism of selectivity is the transformation of simazine and similar halogenated triazines, with a chlorine or other halogen atom in position 2. Triazines with methyoxy or methylthio radicals in position 2 are however not involved in such a chemical transformation reaction and are active against corn plants (121).

The tolerance of corn plants to such herbicides as simazine, propazine and atrazine has been at least partly attributed to such an inactivation mechanism involving a chemical reaction. The transformation reaction has been shown to involve the chemical compound 2,4-dihyroxy-7-methoxy-1,4-benzoxazine-3 one found in corn plants (175). This compoudn, referred to as benzoxazinone is shown to transform the chlorine or other halogen atoms to the hydroxyl radical through a non-enzymic reaction. The hydroxy derivative for example, hydroxysimazine has no herbicidal activity. It has been shown by comparative studies that plants with a very low content of benzoxazinone are very sensitive to simazine (32,80,79).

Fig.9

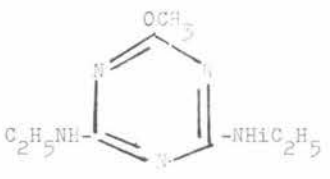
Examples of three groups of s-triazines.

Chloro triazine



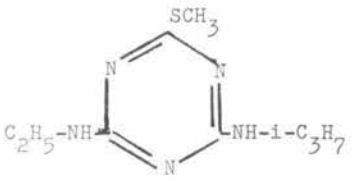
Simazine

Methoxy-triazine

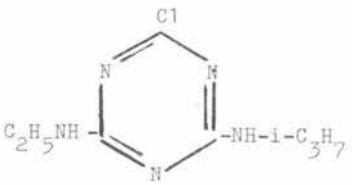


Atrat ne

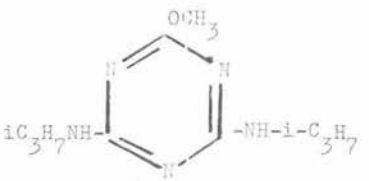
Methyl-thio-triazine



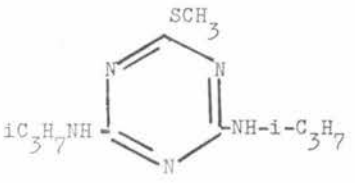
Ametryne



Atrazine



Prometone



Prometryne

Propazin

Fig.10

Transformation benzoxazinone upon boiling

Benzoxazinone

Benzoxazalone

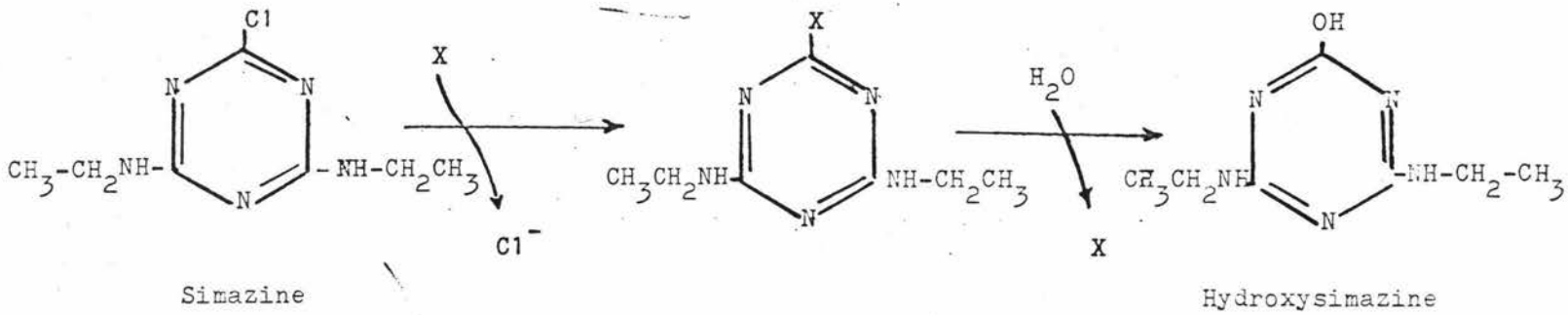
The replacement reaction where the halogen atom is replaced by an hydroxy radical was originally thought to be an enzymic one. This assumption was based on the observation that the reaction only proceeded with fresh but not with boiled pressed juice of the maize plant. It was later shown that a non-enzymic reaction of simazine with benzoxazinone (148) or its glucoside present in maize is responsible for the formation of hydroxysimazine. This benzoxazinone is not stable to heat (33) and is transformed by boiling in water into benzoxazalone, a stable compound and does not react with simazine. (Fig.10).

Simazine degrades more quickly in corn plants or in corn juice, than in corresponding preparations of wheat plants. Thus the corn plants are tolerant while the wheat plants respond to simazine. In corn, metabolism is more rapid in the roots than in the shoots. Simazine degrades on incubation with various tissues or parts of the corn plant and with particle free extracts of corn. The simazine is transformed to 2 hydroxysimazine by a dechloroination reaction (80). Since a non-particulate extract is able to transform the chemical from a halogenated form to a hydroxy form, then one could conclude no enzymic reaction is involved. This reaction has been shown to involve 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3 one, normal constitudent of ethiolated corn seedlings (79,175). A corn mutant low in this active constituent exhibits less tolerance to atrazine than one with normal benzoxazinone content. This relationship, however, does not seem to be a general phenomenon, for example, excised roots of six grass species studied by Hamilton (79) converted simazine to hydroxysimazine in relation to their content but not in relation to the degree of resistance to the herbicide. A low yield of hydroxysimazine was observed to be accompanied by a higher yield of other water soluble metabolites (79).

The presence of benzoxazinone compounds in corn is not unique, they also occur in plants which do not readily destroy simazine, suggesting that the selective herbicidal action involves other

Fig. 11

Simazine dechlorination involving benzoxazinone (X)

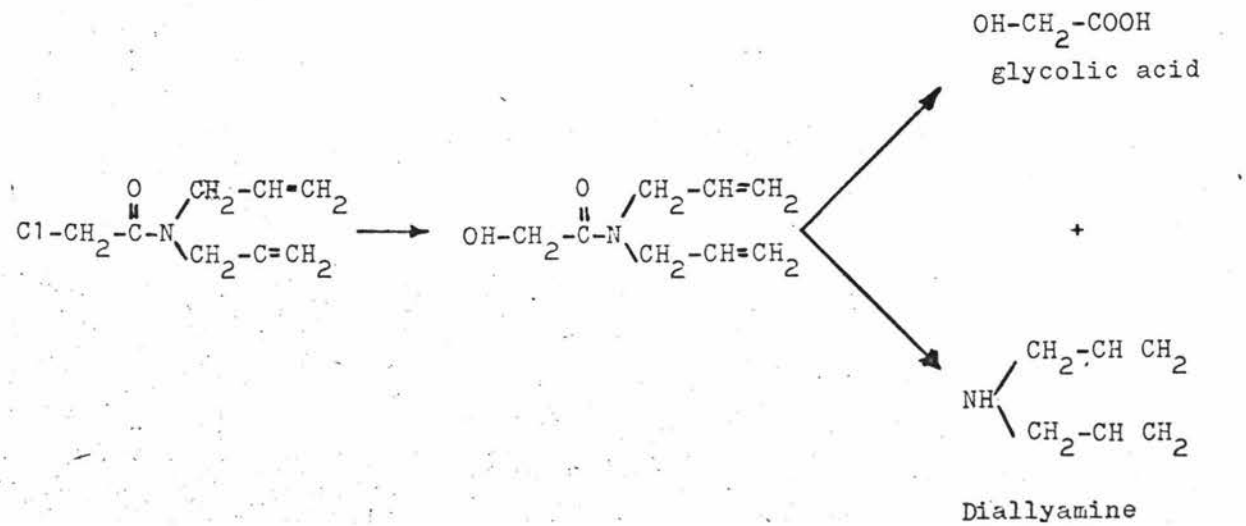


Simazine

Hydroxysimazine

Fig. 12

Inactivation of CDAA



Diallyamine

factors as well. Sorghum (Sorghum vulgare) which is resistant to simazine does not appear to contain an active resistant factor (62) however it is possible that the resistant factor was present in a conjugated form which was not extractable. Pyridine, hydroxylamine and other compounds as well as benzoxazinone mentioned above catalyse the nucleophilic attack on carbon 2 of simazine, probably producing an unstable intermediate that reacts with water to produce hydroxysimazine (156), however it appears that their potency is much less than that of benzoxazinone. Hydroxysimazine undergoes further decomposition possibly by oxidation to the keto form and cleavage to CO_2, basic amines and other compounds, some of which are incorporated into plant products (156). There is evidence of other metabolites of the 2 chlorinated triazines but in most cases dechlorination appears to be the first step. Peas, however, definitely metabolize atrazine by an alternative pathway that does not involve dechlorination. In peas an N de-ethylation at the 4 position is involved and this yields a persistent product (155).

Prometone is less sensitive as a herbicide because the methoxy carbon bond is more stable than the chlorine carbon atom (98). The prometryne ring appears to be stable in plants.

The simazine, benzoxazinone reaction can be summarized as in Figure 11.

Chloroalkylacetamides such as CDAA (2-chloro-NN-diallyl-laceta-mide) may be degraded in corn by a similar mechanism (Fig.12) (92) with subsequent hydrolyses to glycolic acid and diallylamine.

5.5 SELECTIVITY DUE TO FORMATION OF CONJUGATES

The working hypothesis of such a basis for selectivity is that the conjugates formed are either non-phytotoxic, prevented from penetration to active centre, or are held at an inactive site.

Generally glucose seems to be the sugar involved in the formation of conjugates. Glucosides of several herbicides have been investigated. Towers et.al. (176) proved maleic hydrazides form glucosides of the enol form. Hydroxylated metabolites of 2,4-D the,

4-hydroxy-2-3-dichlorophenoxy acetic acid and 4-hydroxy-2,5-dichlorophenoxy acetic acid have been found as glutamic and aspartic conjugates in soybean (60) and as glucosides in bean. Amiben (38, 164) and amitrole (68) form N-glucosides. Lamourex et.al. (101) consider the probable inactivation of s-triazine herbicides is through formation of highly polar conjugates.

Formation of conjugates, it must be emphasized does not necessarily lead to inactivation of a herbicide. In cotton there is evidence that conjugates of 2,4-D are not responsible for induction of resistance to this herbicide (43). The conjugate of 2,4-D referred to as Unknown I when injected into cotton seedlings produced symptoms similar to 2,4-D injury.

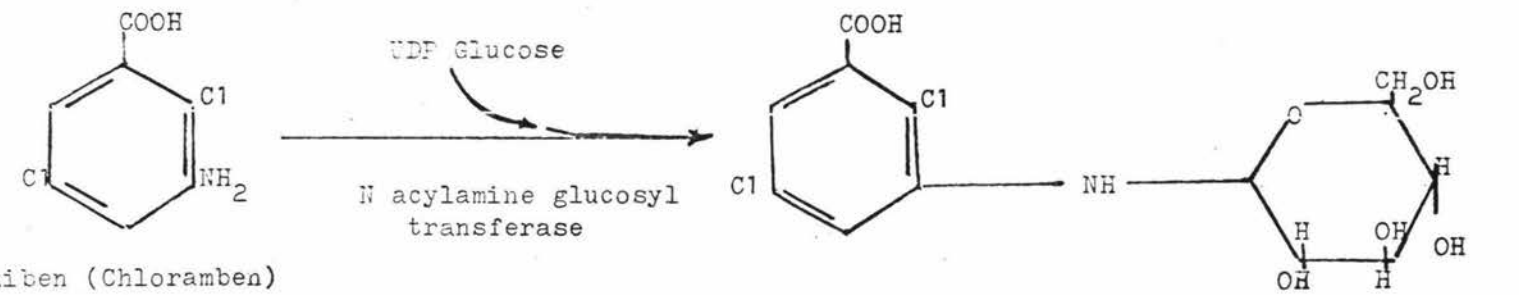
In this review, amiben is selected as a typical example to illustrate complex formation as a means of inactivation and that the rate differences in plants is attributable to selectivity.

Natural plant products in soybeans, barley and tomatoes (Lycopersicon esculentum) and possibly in cucumber and squash (Cucurbita maxima Duchesner) have been shown to be involved in the formation of conjugates. The rate of metabolism of amiben in tomato plants was studied by (Baker and Warren 20,38), suggested that the formation of N- glucoside as a detoxication mechanism is responsible for the tolerance of soybean to the herbicide. He based his suggestion on observations made on the metabolic rate of amiben in the both species produced N- glucosyl amiben it was evident that soybean formed relatively more of the derivative than did barley. Generally it is evident that there is no net loss of amiben through degradation in plants (38,39). Amiben seems to be complexed rapidly by most plants with very little subsequent transformations. Johnson grass (Sorghum halepense L.) seems to be the only species among those evaluated that contained amiben in free form (166).

Swanson et.al. (167) confirmed the presence of N- glucosyl derivatives in soybean. They established the conjugate as N-(3-carboxy-2,5-dichlorophenyl)-glucosylamine.

Fig. 13

Pathway of amiben conjugation



Amiben (Chloramben)

3 amino, 2,5 dichloro-benzoic acid

N-glucosyl amiben

N -(3 - carboxy - 2,5 - clichlorophenyl) -glucosylamine

Swanson (166) has cited several papers to show that N- glucosyl amiben was formed in roots of soybeans, tomatoes, snapbeans, corn, peas, barley and sugar beets. From many of the studies it is apparent that amiben is poorly translocated in soybeans, tomatoes and sugar beet. Distribition studies showed that absorbed amiben remained in the roots as N- glucosyl derivatives.

Distinct tissue differences as well as species differences in the ability to form conjugates of N- glucoside of amiben have been conclusively demonstrated (67). There is a relative rate difference in the formation of these conjugates. Their stability in the tissue where they are produced is note-worthy.

Partical purification and characterization of an amiben-metabolizing enzyme from soybean have been achieved by Frear and cited by Swanson (166). The enzyme N- acylamine-glucosyl transferase is specific for uridine diphrosphate 5-glucose (UDP-glucose). The tentative pathway is presented in figure 13.

Yet another mechanism of conjugation could confer resistance, i.e. conjugation with cellular constituents. Efficiency of herbicides depend on the concentrations of the chemical reacting the site of action. Any factor which reduces the rate of arrival of the molecules at the site must reduce the overall effectiveness of the herbicide. Thus it is possible that resistance to an herbicide may be conferred through an interaction, adsorption or absorption of the herbicide molecule at a non-active site on route to the site of action. Such a basis for selectivity has been demonstrated by Blackman (25) and Sargent and Blackman (150,195). Blackman (25) demonstrated that 2,4-D was absorbed by Pisum stems and Avena sections, however, it was readily released from the susceptible Pisum stem tissue but not from the resistant monocotyledonous Avena sections.

Robertson and Kirkwood (143) have cited several papers to suggest that protein or lipid complexing was involved in initiating such a mode of selectivity. Also other possible sites of inactivation such as cell vacuoles and cytoplasm are discussed by them.

Yih et.al. (195) have provided evidence that major portions of propanil metabolite 3,4-dichloroaniline to be inactivated due to conjugations with polimeric cell constituents, mainly lignin.

Conjugation prevents a toxicant reaching its primary site of action and thus account for their lack of action in some plants.

5.6 SELECTIVITY DUE TO LIMITED ACCESS TO SITE OF ACTION

Atrazine and simazine seem to effect higher plants in a similar manner. The principle mode of action of atrazine seems to be the inhibition of the Hill reaction (124).

Shimabukuro (156) has reported that detoxication of atrazine occurs in two possible ways in higher plants. Non-enzymatic dechlorination of atrazine and simazine in corn by the conversion of the S- chlorotriazines to their 2-hydroxy derivatives is an important detoxication mechanism as described previously. N- dealkylation of atrazine which forms partially detoxified 2-chloro-4 amino-6-ethyl-amino-s-triazine (Compound II) is another detoxication mechanism present in higher plants (Fig.14).

All higher plants seem to metabolise atrazine by N-dealkylation to some extent (98). While species such as corn and wheat which contain benzoxazinone utilize hydroxylation as well (79). The ability to metabolize atrazine may not necessarily render a plant resistant to atrazine, since resistant. intermediately susceptible and susceptible species all seem capable of detoxifying atrazine at different rates (79,142.133).

Moreland and Hill (126) showed that the Hill reaction in isolated chloroplasts from resistant and susceptible plants were equally inhibited by simazine. Thus there seems to be no difference to the chlorotriazine sensitive site in the susceptible and tolerant species.

If metabolism of atrazine is the basis for selectivity then:

1. Phytotoxicity difference between metabolite and parent must be evident.

2. Metabolism must alter physical properties of the herbicide, such as solubility which prevents penetration of the active molecule to the sensitive site in the chloroplast.

If recovery of photosynthesis with the concomitent metabolism of atrazine in Sorghum (168), clearly implicates metabolism as an inactivatory process. Metabolic inactivation of atrazine restored the photochemical activity in illuminated leaf discs of resistant sorghum plants.

The above observations suggest a probability of selective exclusion of the herbicide metabolites from the active centre brought by transformation of the structure of the herbicide.

Shimabukuro and Swanson (157) implied that an interaction between three factors exist in the cells of leaf tissue in influence atrazine activity.

The factors are:

1. Rate of atrazine metabolism and the nature of the derivatives formed.

2. Concentration of atrazine in the chloroplasts.

3. Rate of recovery of photochemical activity in the chloro-chloroplasts.

Atrazine must first penetrate the chloroplast and the active site involved in the photo-oxidation of water to produce the inhibitory effect. Once it reacts at the active site it must then meet the steric requirement for active inhibition. Thus partitioning behaviour, electron distribution and steric factors all seem to come into play in order for the herbicide to perform as a toxicant.

Thus changes in any of the above properties may reduce the activity of a herbicide.

Good (77) reported the reduction of activity of substituted phenyl urea herbicides when polar groups were added to the herbicide molecules. It was then postulated that a reason for the reduction in activity could be that an alteration of the partitioning and penetrating property must have occurred. It was pointed out that the more polar derivative may fail to penetrate the lipid rich chloroplasts in any significant amounts. Although the s-triazines are chemically very different from the substitute phenyl ureas, one could infer that the solubility of atrazine and its metabolites may also be an important factor in the penetration of lipophilic chloroplasts.

Working with isolated chloroplasts, Izawa and Good (89) consider that two factors influence the absorption process into the chloroplast.

They are:

1. Partitioning behaviour of atrazine between biological and aqueous solvent phases.

2. The concentration of the inhibitor in the medium which is influenced by the absorbed atrazine in the chloroplast. This absorbed atrazine is in two forms:

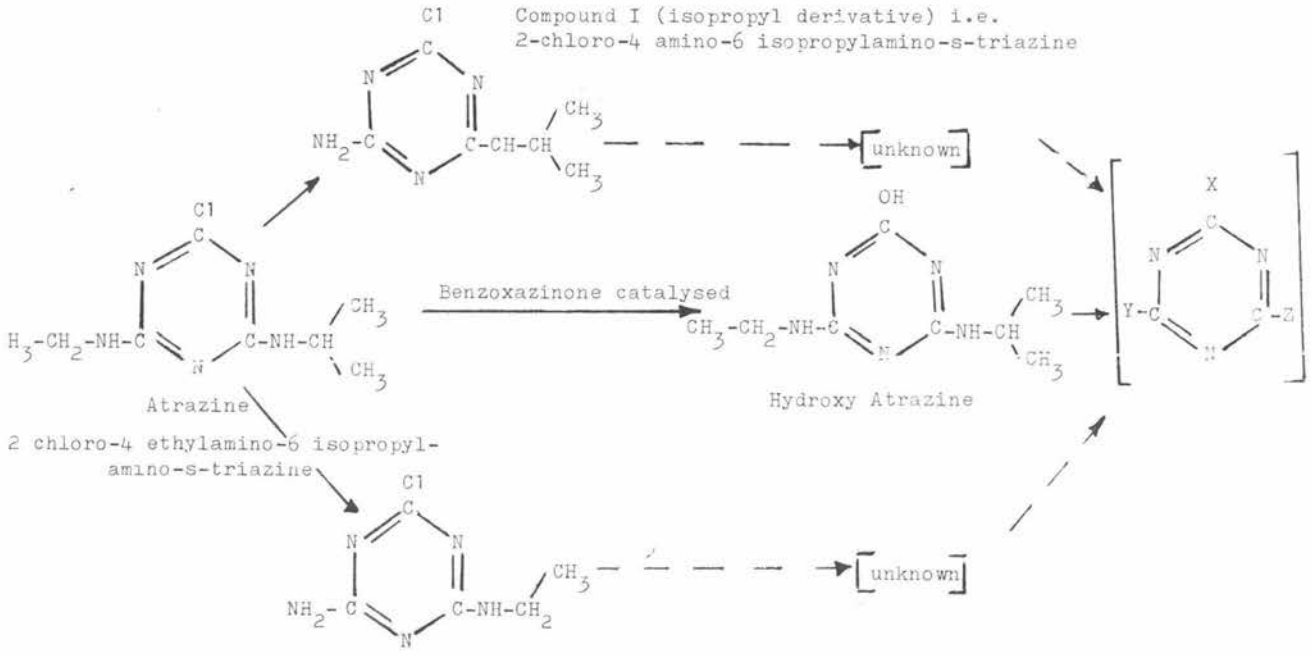
   (a) A low concentration of strongly bound inactive atrazine.

   (b) An active reversibly bound concentration which is in equilibrium with the external solution. The inhibitory concentration could be removed by washing the chloroplasts. Recovery of photochemical activity by the washing of chloroplasts has been demonstrated with simazine (126) and monuron (102).

Shimabukuro and Swanson (157) have proposed a model system based on the information obtained from isolated chloroplast system to explain the selective action of atrazine in living plant cells. For

Fig.14

Dealkylation and hydroxylation pathways for atrazine detoxication in higher plants. Broken lines signify unknown portions of pathways. Several unknown intermediates are present before formation of insoluble residue (56).



this mechanism of selectivity it is assumed that cells in the leaf of a plant will absorb atrazine from either the vascular system or the leaf surface if atrazine is applied on the foliage. Atrazine in the cell will then penetrate and accumulatate in the chloroplasts until the equilibrium concentration is attained between the chloroplasts and cytoplasm. It is assumed that the cytoplasm is equivalent to the medium in an isolated system. Based on the assumption that reversibly bound atrazine is the active form, it is then assumed that the degree of photosynthesis inhibition is proportional to the equilibrium concentration of reversibly bound atrazine in the chloroplast. Then the equilibrium concentration will be directly proportional to the rate of metabolism outside the chloroplasts. Metabolism has a "washing" effect on the chloroplasts and thus the atrazine concentration is reduced. It is evident from these proposals of Shimabukuro and Swanson (157) that the dynamic changes affecting such an equilibrium in a living cell will over a period of time result in a recovery of photosynthesis from its inhibited rate. Reduction of atrazine concentration is chiefly a result of metabolism of atrazine to form the water soluble derivative and its incorporation into insoluble residues.

According to the cellular model of Shimabukuro and Swanson (157) an equilibrium exists between atrazine concentration in chloroplasts and the rest of the cell. Metabolism then reduces the concentration of atrazine in the cell but the equilibrium relationship however, is maintained. Results from the intact leaf (157) indicate that concentration of a chloroform soluble compound, in other words, lipophilic compounds (atrazine and Compound I) in the chloroplasts and supernatent of Sorghum and pea appear to have a definite equilibrium relationship. This equilibrium seems to be maintained while atrazine concentration in the chloroplasts is progressively reduced; owing to the metabolism of atrazine. Absence of water soluble compounds in the chloroplasts except for a small amount in Sorghum chloroplasts at the 168 hour period was noted by Shimabukuro

and Swanson (157). This suggests that metabolism may occur outside the chloroplasts and the polar compounds formed do not readily penetrate chloroplast membrane. This metabolism of atrazine to a more hydrophilic compound and to insoluble residues is a highly effective process for reducing atrazine concentration in the chloroplasts. However, work of Shimabukuro and Swanson (157) has shown the lipophilic N- dealkylated derivative, Compound I, readily penetrates pea chloroplasts and is in apparent equilibrium with the supernatent concentration. Though Compound I is less active than atrazine its action seems to be similar. Steric changes or other factors are thought to have caused the decrease in activity as compared to parent atrazine since penetration is not affected.

If total inhibitory activity by unchanged atrazine and Compound I occurs in the chloroplasts, then the reason that pea is intermediately susceptible to atrazine, as reported by Shaimabukuro (154) is conceivable.

In a highly susceptible species such as soybean where very little N- dealkylation of atrazine occurs (156) the inhibibitor present in chloroplast can be expected to be predominately unchanged atrazine.

The basis for resistance in corn may be the same as for Sorghum; hydroxyatrazine is predominately water soluble metabolite. The partitioning of these compounds between the chloroplast and supernatent of corn has not been shown, but the changes in activity due to transformation suggest a similar process occuring in corn.

Atrazine solubility in chloroform is 0.24 moles per litre, while the hydroxymetabolites are insoluble in chloroform (155).

The atrazine sensitive site seems to be the same for resistant as for the susceptible plant. Thus the selective mechanism involved here seems to be an exclusion of atrazine from the site through transformation reaction which renders the chemical less lipophilic than parent atrazine.

5.7 ANATOMICAL AND PHYSIOLOGICAL MECHANISMS INVOLVED IN SELECTIVITY

Phenoxyacetic herbicides have been reported by several workers (187,52,95,10) to cause growth effects including cell proliferations. Several instances, for example the evidence of Srivastava and Sharma (162) also suggest that differential tissue proliferation and resulting vascular disorganization partially determine selectivity to phenoxyacetic compounds.

2,4-D greatly increased cell division in the cambial and phloem regions of stem in Sonchus arvensis and Convolvulus arvensis (178). Watson (187) reported 2,4-D treated bean leaves exhibited extensive division of spongy mesophyll which leads to obliteration of intercellular spaces. Distribution of phloem strands due to proliferation of parenchyma cells in Cyprus and beans was noted by Eames (52) and Kiermeyer (95) has shown that this distribution is due to crushing of the companion cells and sieve tubes by the extensive division of phloem parenchyma cells.

Muni (130) attributed the differential effect of 2,4-D on dicotyledonous and monocotyledonous species to "the lack of protection to the phloem resulting from an early killing of the cortex". In monocotyledons the phloem in each bundle is protected by sclerenchyma and is consequently less susceptible to direct exposure to herbicide.

This is a classical case of selectivity due to comparative difference in anatomical features in susceptible and tolerant species.

Other physiological features may also contribute to selective action of herbicides. King (96) has suggested a mechanism where "a block" to translocation appears at the point of insertion of leaf into Node in 2,4-D resistant variety of corn while such a block is not apparent in the susceptible variety.

Crafts (44) maintains that in addition to anatomical barriers, some physiological barriers do absorb and bind phenoxy compounds. He maintains that such a mechanism partly explains the selective action of some herbicides. Linder et.al. (107) and Crafts alleged

that high concentration rapidly killed phenoxyacid treated leaf regions and damages the energy requiring transport system so that translocation to the susceptible regions is prevented. Robertson and Kirkwood (144) have cited several papers to provide evidence that phenoxyacid compounds reduce their own movement by inhibiting the energy requiring process associated with translocation.

Thus although there are both direct and indirect evidence for physiological basis for selectivity, none of the suggested aspects has been investigated conclusively. It is difficult to separate the various physiological and biochemical systems without causing some other effects.

Chapter 6

MATERIALS AND METHOD

6.1 PLANT MATERIAL

Solanum nigrum and Solanum sarachoides plants grown for these investigations were raised from seeds collected by Mr E. Wallace at the Henry & York trial grounds, Hastings.

Seeds were germinated in petridishes in the dark and on germination transplanted in 4 inch ACE pots. Four seeds to each pot were raised in a glasshouse and later thinned to 2 plants.

The potting medium was river sand washed 4 times with tap water. Plants were fed with the NCSU phytotron nutrient mixture at the rate of 20 ml three times per pot per day.

6.2 RELATIVE INHIBITION OF ISOLATED CHLOROPLASTS FROM THE TWO SPECIES

Chloroplasts were isolated from both Solanum nigrum and Solanum sarachoides as described previously (3.1.3). The plants were 10 weeds old. Concentrations of SENCOR were 0, 10^{-8}, 10^{-6} and 10^{-4}M.

6.3 STUDIES ON UPTAKE, DISTRIBUTION AND METABOLISM

6.3.1 Radiochemical purity

SENCOR-5-^{14}C was supplied by Chemagro. The specific activity was 1.43 mc/mM. Thin layer chromatography on silica gel developed in chloroform : dioxane (9:1.V) established the radiochemical purity at 99.3%. The band under ultra violet light was superimposed on that of standard SENCOR.

6.3.2 Dose preparation

1.5 mg of SENCOR-5-C^{14} was weighed into a 5 ml flask, 3 ml of methanol was added and the flask agitated until the material

dissolved. This was transferred into a 200 ml flask. The 5 ml flask was rinsed several times with warm water. The 200 ml was then made up with warm water. The flask was gently agitated for several minutes.

6.3.3 Dose administration

Plants which had been removed from the glasshouse a week earlier and grown outdoors were treated with the radioactive materials. 20 ml/pot of the SENCOR-5-C^{14} solution was applied to the soil. Each pot contained two plants and five pots each of Solanum nigrum and Solanum sarachoides were treated. The SENCOR solution was dispersed using a pipette, no solution being allowed directly on the plant. The pots were then watered three times a day with 20 ml of nutrient solution, starting 6 hours after application of labelled SENCOR.

6.3.4.1 Autoradiographic studies

Plants which had been exposed to the labelled material for periods of 1 day and 7 days were collected.

They were cut into root and shoot portions. The roots were teased on a glass plate and rinsed several times with water and finally placed on blotting paper (8 x 10 inches) and teased out. The shoot portions were spread on 8 x 10 inches blotting paper. Several cuts had to be made in order to fit the plant on the paper. It was also expected that sectioning would prevent any continued translocation. Crafts and Yamaguchi (45) had suggested that translocation of material could occur while pressing the plant. The berries were pressed several times between layers of blotting papers, care was taken not to allow the sap to spread significantly. The barriers were then mounted close to their original point of attachment.

The mounted plant materials were then pressed between several layers of blotting paper, interposed between two chipboards (12 x 12 inches). The boards were damped together with bolts and winged nuts. The papers were changed frequently during the first two days, as the sap dried off less changes were necessary. The

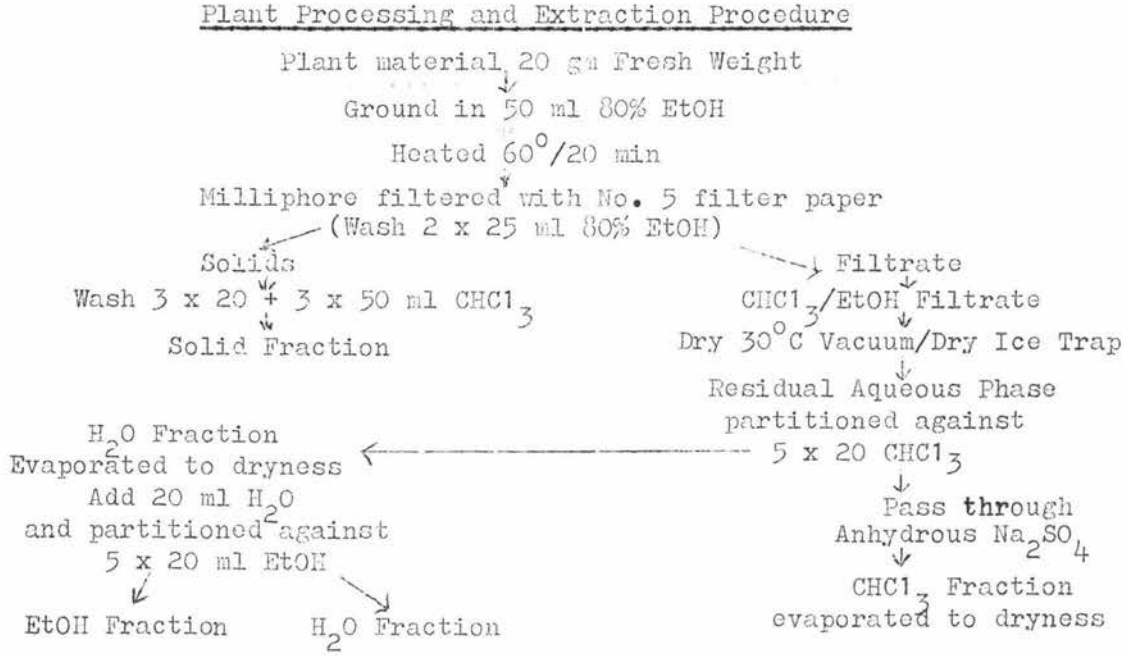
plants were pressed for seven days and were sufficiently dry within this period. Loss of some radioactivity in the sap which was squeezed out of plant sections probably did occur, however, contamination was prevented by frequent changes of paper and the loss of activity is of no consequence here since quantitative measurement of radioactivity was not intended.

The pressed plant portions were mounted on a card (8 x 10 inches) with plastic glue. The x-ray films (Kodirex) were placed in direct contact with the mounted plant material. These were then placed in the envelopes provided with the film and kept in the dark. The 1 day treated plants were exposed for 9 days and the 7 day treated plants for 7 and 4 days.

Films were processed in recommended solutions after the exposure period being processed for 4 minutes at 20°C (68°F). Usual dark room precautions for handling x-ray film were observed.

6.3.4.2 Phase distribution studies

A modified processing and extracting procedure based on that of Robinson et.al. (140) was devised and is summarized in the flow diagram below.



The activity in each fraction was counted using Packard Tri-Carb Liquid Scintillation Spectrometer and external standards.

Ethyl acetate, chloroform and water samples were counted in an emulsion cocktail (136).

The solid samples were counted using digestion-solubilization procedure (112), modified to permit counting of acid labile carbon-^{14} constituents (76). The emulsion cocktail used, however, differs from that used by Gornberg and co-workers (76). The adopted procedure is presented below.

Procedure for solid fraction counting

1 gm placed in scintillation vial
↓
Vial partially immersed in dry ice-acetones
↓
0.5 ml 70% perchloric acid added
↓
00.5 ml 30% Hydrogen peroxide added
and vial immediately sealed
↓
Heated at 80°C for 30 min
↓
Vial cooled and placed in liquid air
↓
0.5 ml Ammonium hydroxide added
↓
2 phenethylamine trapping solution added
↓
Reseal vial and stand at room temp 30 min
↓
5 ml cellosolve (ethylene glycol monomethyl ether)
↓
add 10 ml scintillation fluid and count for 10 min

6.3.4.3 Study on rate of metabolism

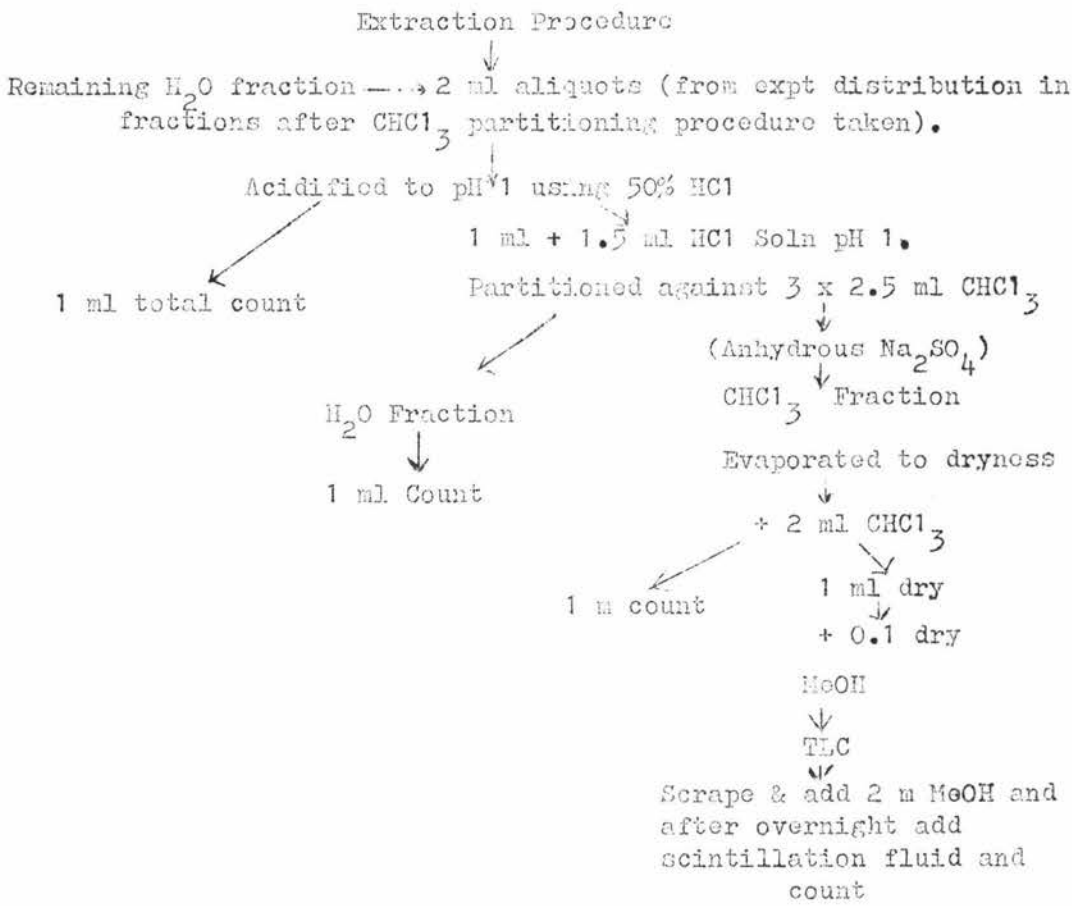
The ethyl acetate fraction was spotted on silca gel (F_{254}) plates together with standard SENCOR and its metabolites. The plates were then developed in chloroform: dixane (9:1). The retention factor for SENCOR was 0.55, DA 0.40 and DK/DADK 0.18. The areas of chromatogram corresponding to the designated spots were marked under ultraviolet light and then placed in scintillation vials. These were then eluted with 2 ml of methanol for 24 hours. Scintillation fluid was added after this 24 hour elution period and radioactivity was counted. The amount of silica-gel powder in the vials was approximately equal

and since it did not exceed 4 sq.cm, the estimation of activity should have a high efficiency (34,194).

An attempt was made to release the triazinone metabolites from its water soluble conjugates. In this experiment only samples from the 5 day and 6 day treated plants were used. The method most successful was a modified form of that report by Morgan (128). This is present as a flow diagram.

The chloroform extract was then applied in a single band 2 cm from the base of TLC plates and developed 15 cm. The chromatogram was marked and treated as above.

Study on Rate of Metabolism



6.4 RATE OF TRANSPIRATION STUDY

Plants were grown in the glasshouse, the treatments including:

1. 3 replicates of *Solanum nigrum*)
2. 3 replicates of *Solanum sarachoides*) placed in outside conditions
3. *Solanum nigrum* and *Solanum sarachoides* plants were placed in the dark but not replicated.
4. *Solanum nigrum* and *Solanum sarachoides* plants were allowed to wilt before treatment and then exposed to outdoor conditions. There was no replication.

The pots were placed in plastic bags and after treating the soil with 20 ml SENCOR solution (or 1 mg/pot) the bags were sealed with plastic glue and weighed immediately. The weighing was repeated every four hours for the next thirty hours. At the 12th hour, after the weight was recorded, the pots wer immediately watered, using a hypodermic syringe with 10 ml of water and the increased weight recorded immediately. The experiment was continued without further watering.

In this experiment the difference in weight is assumed as the approximate amount of water lost due to transpiration.

6.5 QUALITATIVE OBSERVATION OF STOMATAL APERTURE

Having referred to Williams (1973) paper on the method for preparing plastic epidermal imprints, some locally available materials were tried out (88). Plastic glue (Salleys Product, Auckland) gave excellent imprints.

During the above experiments on the rate of transpiration, imprints from leaves of the same plants were taken from abaxil surfaces.

An imprint was prepared using a drop of plastic glue, spread with the finger, so as to form a thin film. After drying, this was stripped off without damaging the leaf tissue and mounted on a slide. Photographs of representative plastic strips were taken. Studies of the conditions of the stomatal aperature were made both directly under the microscope and from photographs.

Chapter 7

RESULTS AND OBSERVATIONS ON THE VARIATION OF EFFECT OF SENCOR ON THE TWO SOLANUM SPECIES

7.1 RELATIVE INHIBITION OF ISOLATED CHLOROPLASTS

In Table V the percentage of inhibition measured as rate of DPIP reduction is expressed in arcsine values. The transformed values were then statistically analysed in a completely randomized block design.

Table V

Inhibition of isolated chloroplasts from *Solanum nigrum* and *Solanum sarachoides* by SENCOR

Species	10^{-4}M	10^{-6}M	10^{-8}M
Solanum nigrum	90	56.79	41.55
Solanum sarachoides	90	56.17	40.98

It is obvious from the above results that both the susceptible (*Solanum nigrum*) and tolerant (*Solanum sarachoides*) respond to SENCOR to the same extent and each of the levels of concentrations tested. No significant difference (at 1% level)could be established between the two species.

7.2 STUDIES ON UPTAKE, DISTRIBUTION AND METABOLISM

7.2.1 Autoradiographic studies

The x-ray films demonstrate that the radioactivity was translocated to various parts of the plant via the xylem. The extent of distribution as well as the intensity of the radioactivity varied

Plate 1

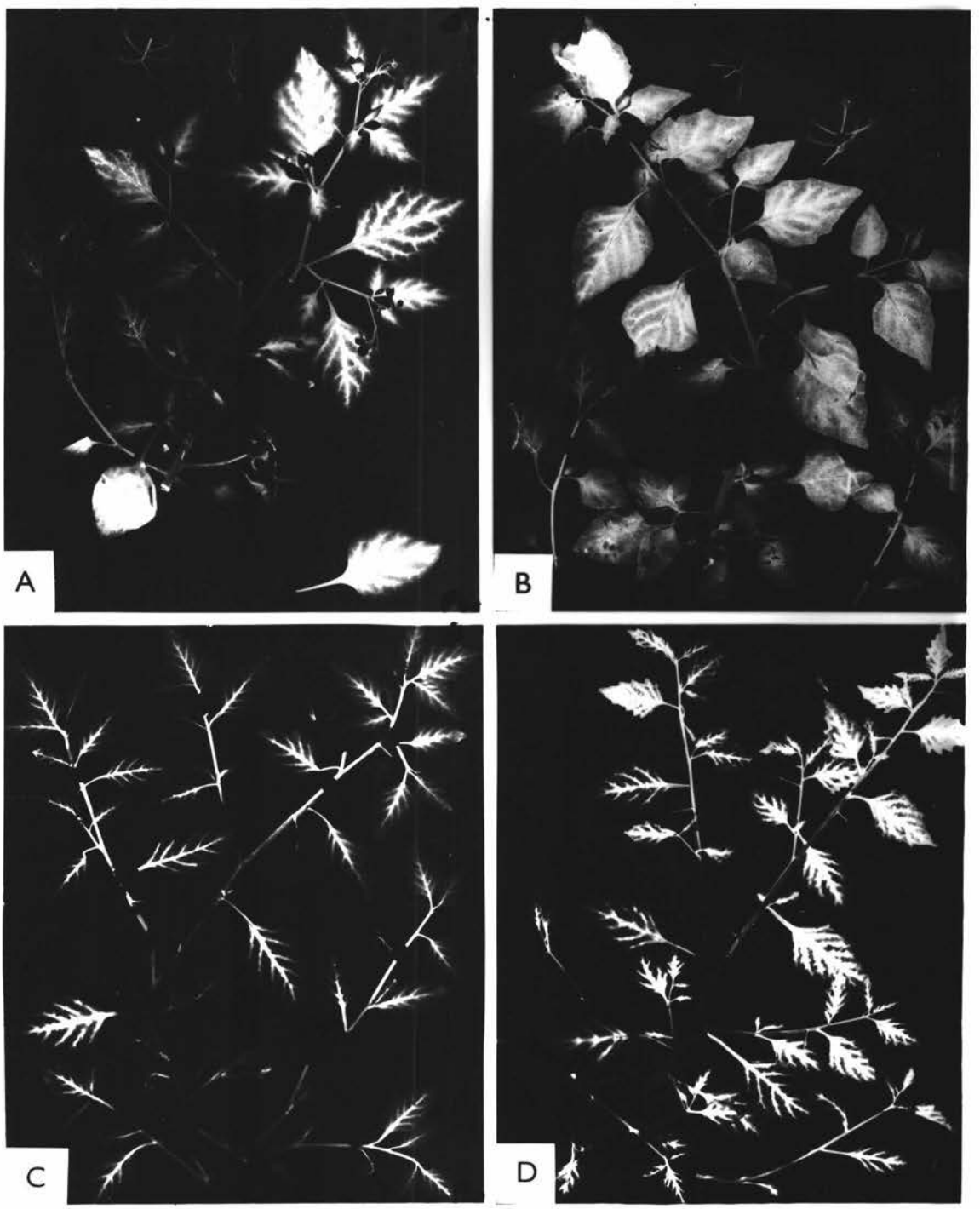
Autoradiographs

A. *Solanum nigrum* treated for 1 day.

B. *Solanum nigrum* treated for 7 days.

C. *Solanum sarachoides* treated for 1 day.

D. *Solanum sarachoides* treated for 7 days.


A
B
C
D

between the two species at the two different sampling periods. (Plate 1).

Control plants were also sampled for autoradiography, but no radioactivity could be observed, showing that untreated plants do not contain any substance which may affect the x-ray film during the adopted procedure.

On the one day sample the intensity of the image on the autoradiograph films did not vary between the species. In both the plants intense activity collects predominantly in the vascular tissue.

On the seventh day samples, both the intensity of the image as well as the distribution of the radioactivity differs between the two species. While most of the activity is confined to the vascular systems in the tolerant Solanum sarachoides plants, in the susceptible Solanum nigrum the material is diffused throughout the leaf lamellae and the image of the whole plant appears on the films.

No difference is evident in the intensity of the root images, both between the species and the sampling times.

Though the images of the fruit stalks appeared on the x-ray films, images of the berries did not develop. This observation was consistent between the two species and the two sampling times.

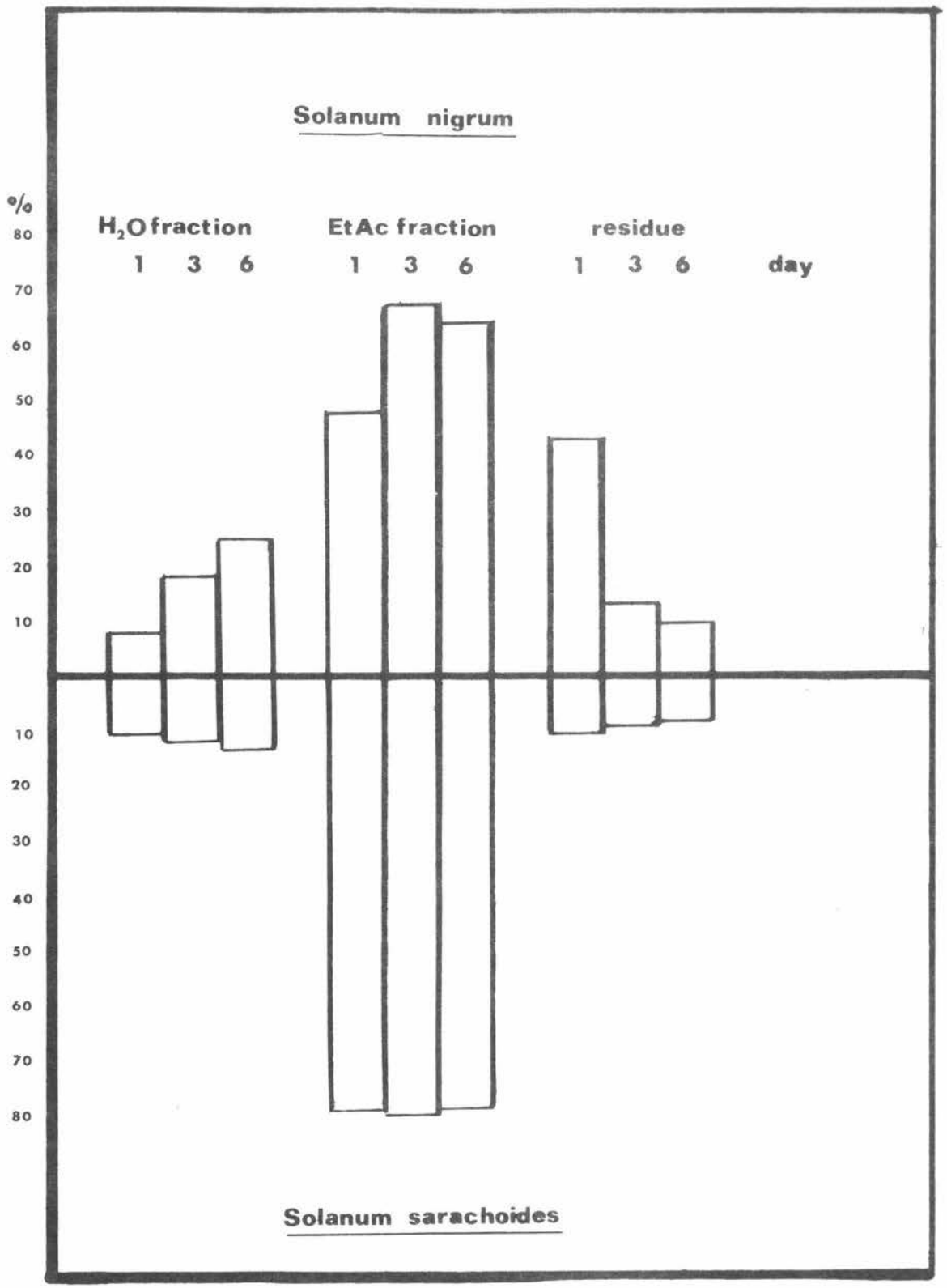
7.2.2 Phase distribution studies

Quantities of the redissolved fractions were counted in the liquid scintillation spectrometer with external standards. The counts per minute (cpm) were corrected for efficiency and quenching. The cpm were then transformed logarithmically for statistical and graphical analysis. These studies were conducted as factorial arrangements of a split-split plot design.

From the table of analysis of variance, the applied F test indicates that there is a highly significant difference between the two species in their response to SEMCOR. Figure 16 which expresses the cpm in each fraction as percentage of the total count of the various fractions indicates that there is an interaction between the various fractions extracted from Solanum nigrum plant. Such

Fig. 16

Comparison of levels of radioactivity distributed among various fractions in the two species.



interactions are not evident for Solanum sarachoides.

Table VI compares the total counts of both species at the three different sampling periods.

Table VI

Total counts in each species at the three sampling stages transformed to 100 Log cpm

Species	1 day	3 day	6 day
Solanum nigrum	381.1	441.0 ¦	467.0
Solanum sarachoides	391.7	465.3 ¦	466.7

Bar (¦) indicates means are significantly different at 5% confidence level

It is evident, that there is no significant difference between the two species in the level of total uptake at the 1 day and 6 day stages at which the plants were analysed. However, from the 3 day sample difference between the species, at the 5% confidence level is evident. Solanum sarachoides has a higher total count.

From Figure 15 it is apparent that the concentrations of labelled material partitioning into each fraction varies. The SENCOR equivalents are distributed mainly in the water soluble and non-extractable fractions. Very little of the activity is in organosoluble form. There is a much reduced increase in concentrations of labelled material in any of the fractions between the 3rd and 6th day. This is supported by Table VII. In fact no significant increase occurs in the Solanum sarachoides species. It is also evident that very little additional absorbed SENCOR equivalents are fixed in the non-extractable fraction of Solanum nigrum after the first day period.

Fig.15

~~Percentage~~ distribution of SENCOR equivalents in fractions at 1, 3, 6 day sampling periods.

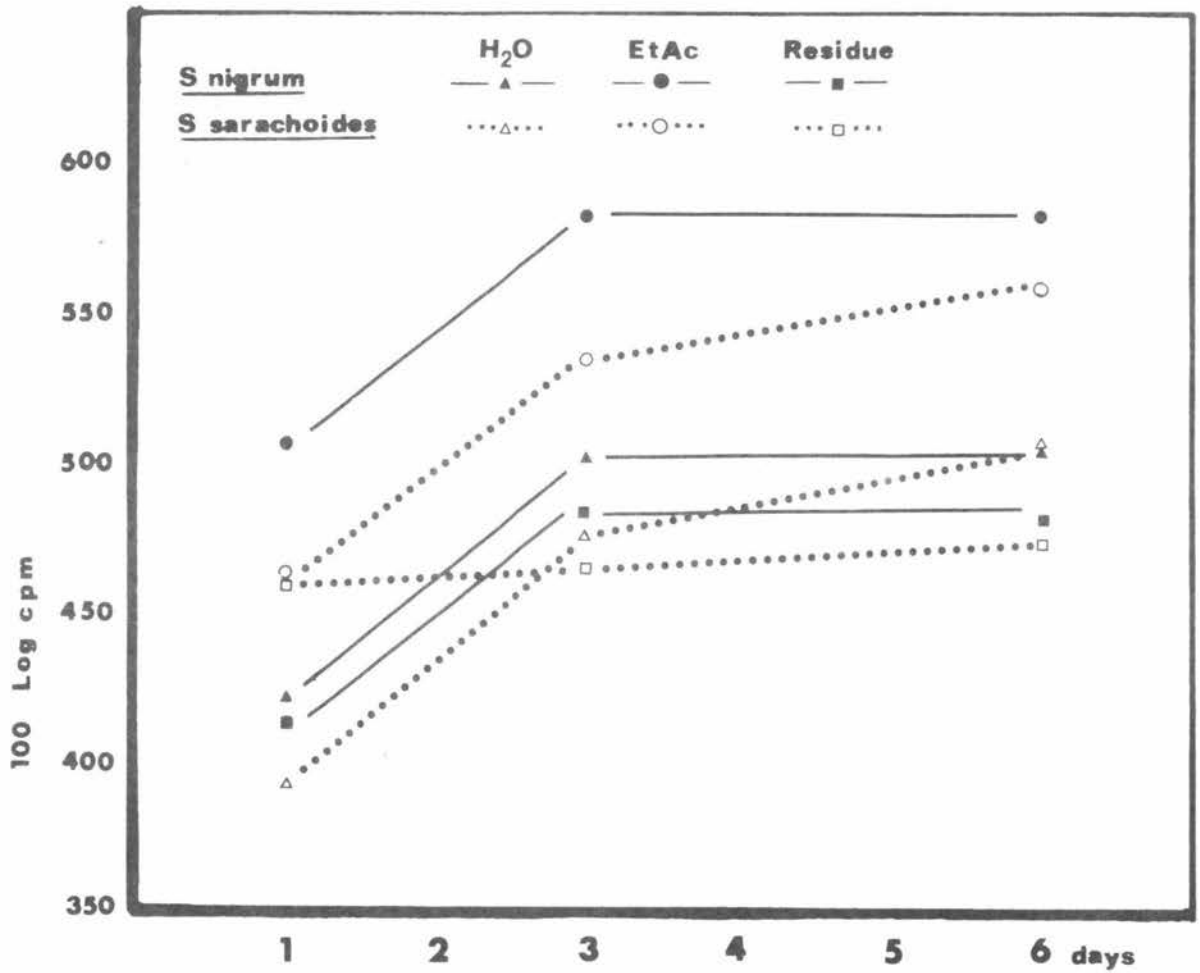


Table VII

Distribution of SENCOR equivalents
in the various fractions

	Solanum nigrum				*Solanum sarachoides*			
day	water phase	EtAc phase	Non-extractable residue	Chloroform phase	water phase	EtAc phase	Non-extractable residue	Chloroform phase
1	389.7	466.0	461.0	207.7	524.3	512.3	422.7	207.3
3	481.0	537.3	467.3	278.3	500.0	583.0	485.0	293.0
6	514.0	555.7	474.7	283.7	506.0	581.6	480.7	298.3

(Bar (|) indicates means are not significantly different at 5% level as measured by Duncan's multiple range test.

From Table VIII it is evident that *Solanum sarachoides* has higher levels of counts in the water soluble fractions. More radio active material appears in the non-extractable residue of *Solanum nigrum* at the 1 day period, however, this highly significant difference is reduced through the period such that on the 6th day sample no difference is evident between the species.

Table VIII

Comparison between species of the distribution of SENCOR equivalents in various fractions

Day	Species	Fractions			
		H_2O	EtAc	non-extractable	$CHCl_3$
1	S. nigrum	389.7	466.0	461.0	207.7
	S. sarachoides	424.3	512.3	422.7	207.3
2	S. nigrum	481.0	537.3	467.3	278.3
	S. sarachoides	500.0	585.0	485.0	293.0
3	S. nigrum	514.0	555.7	473.7	283.7
	S. sarachoides	506.0	581.6	480.7	298.3

Bar (|) indicates means are not significantly different at 5% level and (¦) indicates means are not significantly different at 1% level.

7.2.3 Study on rate of metabolism

Results obtained from various attempts to release the SENCOR equivalents from 'conjugates formed' indicated that it was more difficult to break the conjugates formed in Solanum sarachoides. Even in the finally attempted procedure, the results recorded (Table IX) indicate that a high number of counts were retained near the origin. However, the metabolites and SENCOR that moved off the origin, separated well on the chromatograms.

Fig.17

Comparison of rate of metabolism of SENCOR.

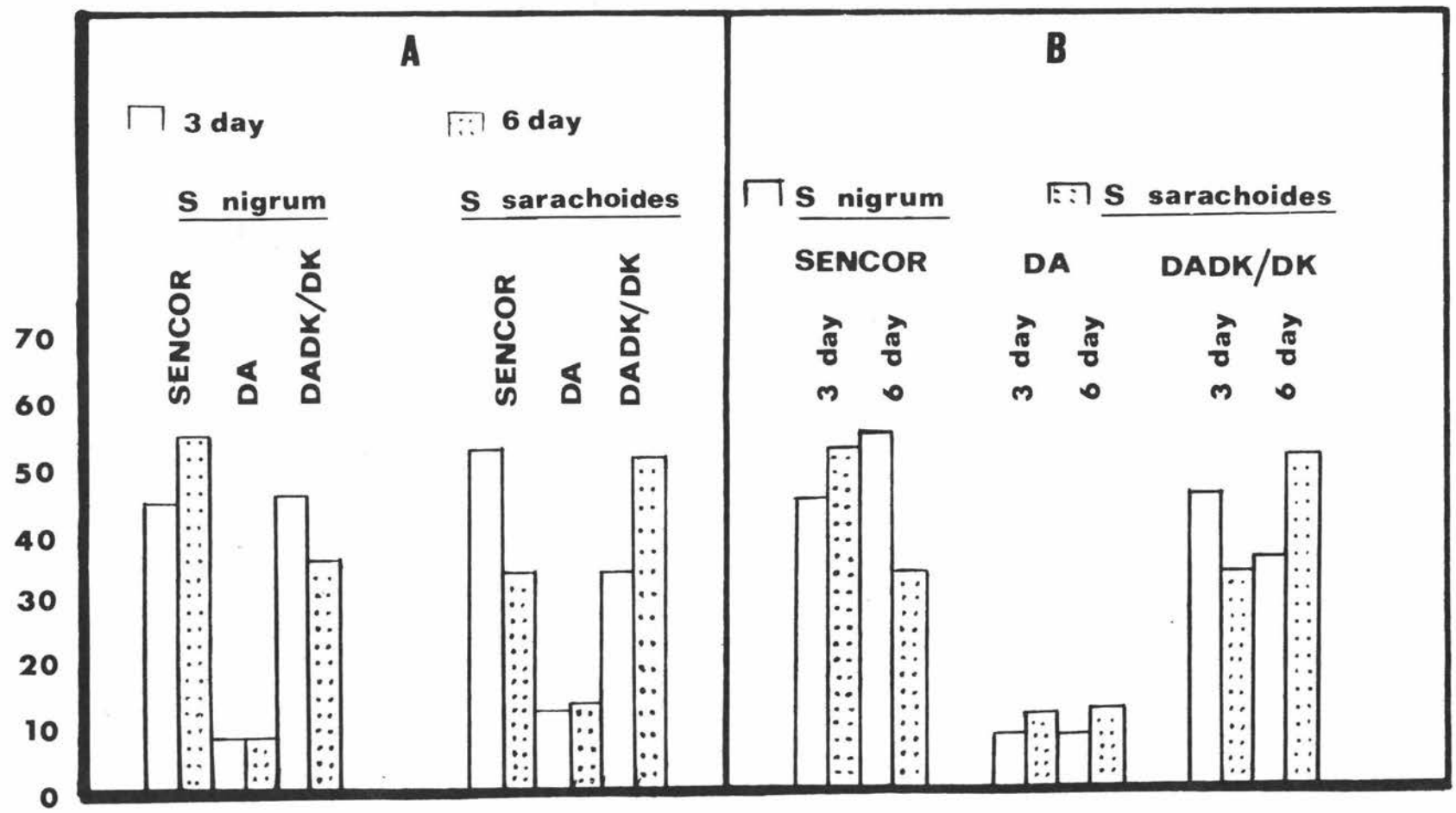


Table IX

Distribution of total radiocarbon (in cpm) between SENCOR and its metabolites

	3 day samples		6 day samples	
	Solanum nigrum	Solanum sarachoides	Solanum nigrum	Solanum sarachoides
x*	955	5186	4434	4141
SENCOR	11078	35853	29459	24912
DA	2283	8969	4871	10059
DARK/DK	11318	22958	19244	38075
Origin	13938	61433	26992	82977

x* is the total value of strips of the remaining chromotogram after the strips representing SENCOR, DA, DADK/DK and origin had been scrapped off.

With the solvent system used in this study it was not possible to separate the DADK and DK metabolites. The standard DADK and DK spotted on the same plate as the samples also failed to separate, their bands under U.V. were superimposed.

It must be noted here that due to lack of material, the experiment was not replicated. In other TLC separations of SENCOR equivalents attempted, the radiocarbon seldom moved more than 2 cm from the origin. Further attempts were not possible due to exhaustion of samples.

However from the best chromatogram obtained there are indications that a higher rate of metabolism of SENCOR may occur in Solanum sarachoides. This is illustrated in Figure 17 and Table X.

It is again emphasized that one can only indicate that there is any difference in the rate of metabolism of SENCOR by the two species. There is no statistical proof attempted in this study.

Table X

Percentage distribution of radiocarbon between the SENCOR equivalents: SENCOR, DA and DADK/DK

	SENCOR		DA		DADK/DK	
	3 day	6 day	3 day	6 day	3 day	6 day
S. nigrum	45%	55%	9%	9%	46%	36%
S. sarachoides	53%	34%	13%	13%	34%	52%

7.3 RATE OF TRANSPIRATION

The reduction in the weight of treated plants compared to those of control is expressed as percent inhibition of transpiration. These figures were then transformed to arcsine and were then analysed in a split plot design basis.

Results in Table XI indicate that there is a highly significant difference (at the 1% confidence level) between the two species in their response to SENCOR, transpiration in Solanum nigrum being inhibited more markedly than in Solanum sarachoides.

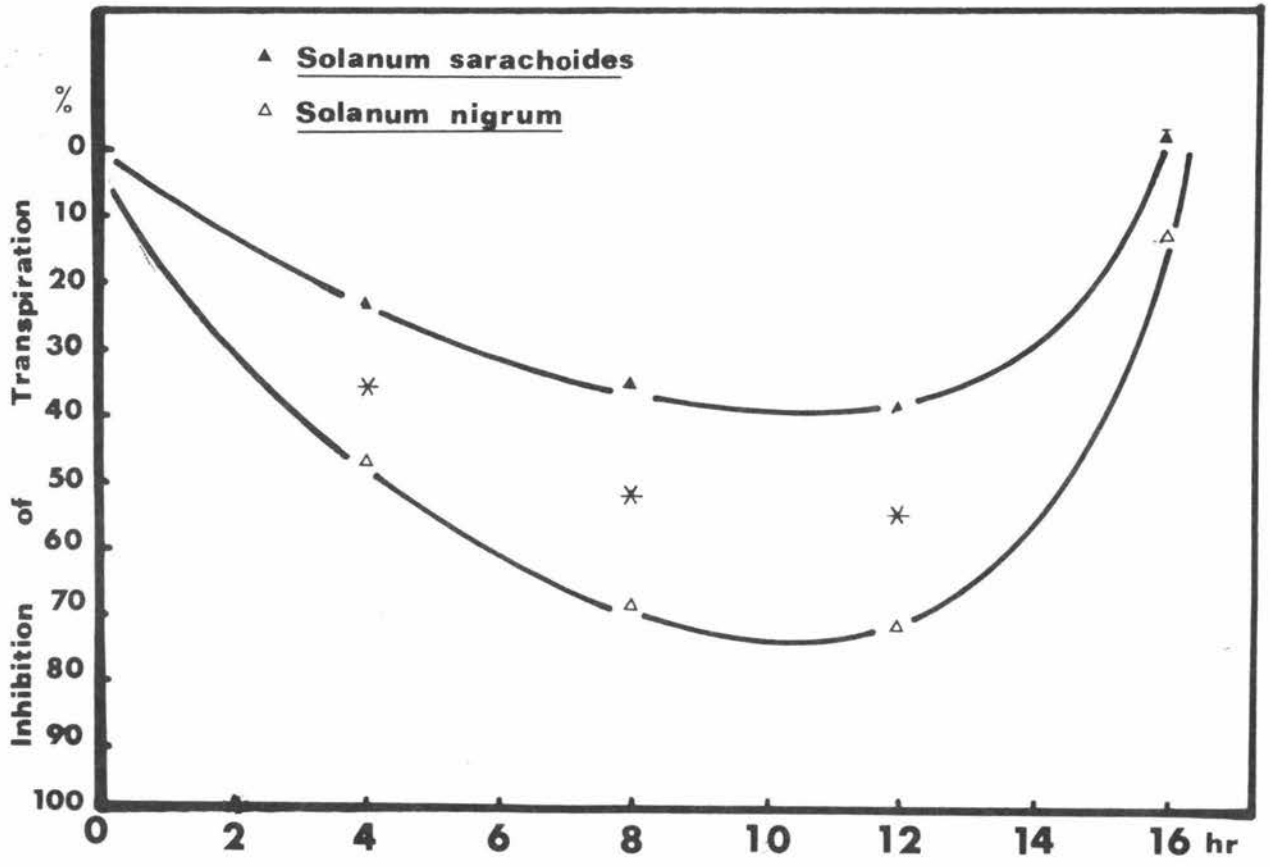
Table XI

Per cent inhibition of transpiration by SENCOR for total period of 16 hours

Species	Mean
Solanum nigrum	54.77
Solanum sarachoides	37.85

Fig.18

Inhibition of rate of transpiration. Response of the two species.



* difference between mean values highly significant.

Comparison of the levels of transpiration of the two species at the same period of time (Table XII and Fig.18) indicates that transpiration in Solanum nigrum is more drastically reduced than in Solanum sarachoides. This phenomena is evident throughout the day light period, however, at night no statistical difference is evident in the response of the two species to SENCOR - and there is no difference in the amount of water loss between treated and control plants at this stage.

Table XII

Comparison of level of inhibition of transpiration of the two species at the same period of time

Species	10 am	2 pm	6 pm	10 pm
Solanum nigrum	53.4	66.9	68.1	30.8 |
Solanum sarachoides	34.7	20.6	19.5	21.7 |

Bar (\|) indicates no significant difference between mean values.

7.4 QUALITATIVE OBSERVATIONS OF STOMATAL APERTURES

The stomatal imprints observed under the microscope and from photographs indicate that the treated plants of Solanum nigrum reduces its aperture within 2 hours of application of SENCOR to the roots. The stomata do not recover even after 30 hours. In the Solanum nigrum, however, no difference in the apertures of treated and control plants could be observed.

Sample photographs of stomatal imprints are presented here (Plate 2) to illustrate the observed phenomena and the difference in the response of the two species to SENCOR.

Plate 2

Imprints of the stomata

A. Solanum sarachoides, control plant at 10 am.

B. Solanum sarachoides, treated plant at 10 am.

C. Solanum sarachoides, control plant at 10 pm.

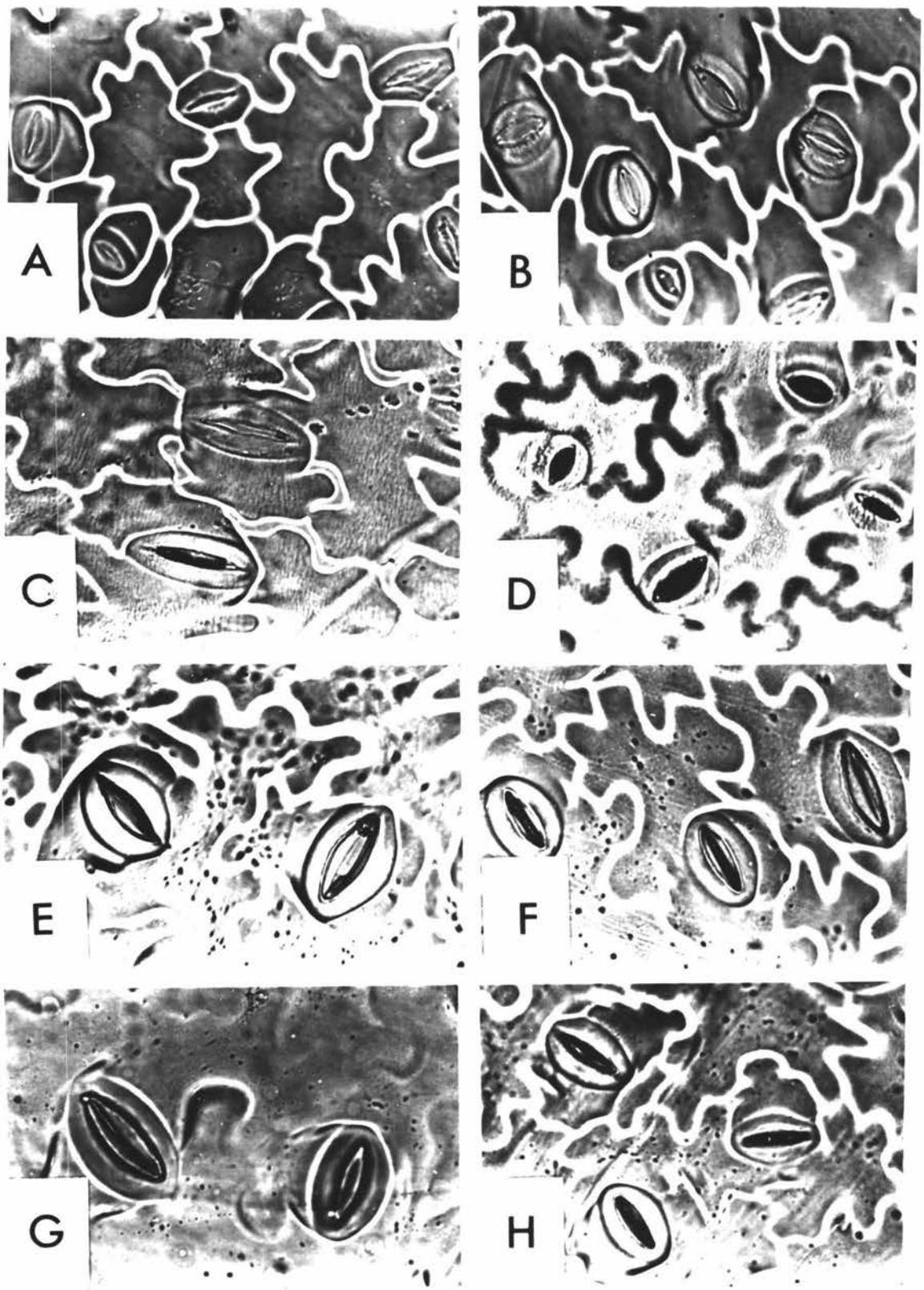
D. Solanum sarachoides, treated plant at 10 pm.

E. Solanum nigrum, control plant at 8 am.

F. Solanum nigrum, treated plant at 8 am.

G. Solanum nigrum, control plant at 10 pm.

H. Solanum nigrum, treated plant at 10 pm.


A
B
C
D
E
F
G
H

7.5 DISCUSSION

From the results and observations reported in this study, it is evident that the variation in the response of Solanum nigrum and Solanum sarachoides to SENCOR as observed in the field, is in fact due to species selectivity factors.

Mechanism of resistance is not due to a differential effect directly on the Hill reaction but rather involves the physiological and biochemical mechanisms.

Table V illustrates that chloroplasts of Solanum sarachoides (resistant plants) are as sensitive as those of Solanum nigrum (susceptible species) to SENCOR. Work by others on known photosynthetic inhibiting herbicides, simazine (126), pyrazon (63), sinduron (131), pentanochlor (40), also illustrates that influence of herbicides on the chloroplast activity bears no relationship to the phenomena of selectivity. Based on the reviews and evidence of several workers (13,28,72,135,149), it is evident that chloroplasts are surrounded by a pore-less double membrane and contains a complex of subunits bearing lipoprotein membrane system embedded in a hydrophilic proteinaceous matrix or stroma. Functional difference, i.e. the ability to perform (PSII) and(PSI) activities though evident in different chloroplasts, it cannot be attributed to the general structure and components of the chloroplast membrane. The difference is soley dependent on the arrangement of the basic subunits the 'thylakoids'. From logical deductions itself one cannot expect selective action of systemic herbicides in plants to be purely a result of one fundamental scientific and systematic design. Several factors within the plants environment will influence the final concentration of SENCOR reaching its primary site of action. the Hill reaction centre. Physiological and biochemical activities such as uptake, distribution and metabolism will determine the ultimate concentration of SENCOR around the chloroplasts. The phytocidal effect can only partly be derived from the effect at the chloroplast level. Thus the work with chloroplasts does not help

significantly. More fundamental plant physiology and experiments with various intact plant species are necessary.

Accordingly experiments were carried out using SENCOR labelled in the ring to determine whether there were differences such as restrictions to the uptake of the herbicide by the roots and its translocation to the xylem sap and leaves, and whether extensive breakdown of SENCOR in the tolerant Solanum sarachoides compared to the susceptible Solanum nigrum occurred.

From the autoradiographic studies it is evident that SENCOR behaves similar to other herbicides that are absorbed through the roots. Autoradiographs of leaves and plants treated with root applied herbicides show a similar pattern of distribution ofactivity (15,45,158). The difference in the distribution of radioactivity in the leaves between the two species may reflect the observed difference in susceptibility of Solanum nigrum and Solanum sarachoides in the field. While the activity is concentrated in the veins throughout the sampling periods in the tolerant Solanum sarachoides, the radioactivity had spread throughout the leaf tissue in the susceptible Solanum nigrum as observed in Plate 1b. From this study it is evident that some physiological and biochemical barrier mechanism operates in preventing SENCOR reaching its site of action, the chloroplasts of the tolerant Solanum sarachoides. There is no explanation for such a barrier mechanism in the literature. Perhaps the most plausible hypothesis would be that the susceptible spacies forms a weakly bound water soluble SENCOR conjugate which is distributed via the xylem. The toxicant is then released from the water soluble conjugate and penetrates the cellular membranes and accumulates at its site of action. There are several reports that SENCOR forms conjugates (36,128,129,145), however, the above proposal must remain a hypothesis since no proof of a reversible conjugation was obtained in this study or from the literature.

It is noted here that barrier to translocation of activity from the plant tissue to the fruit seems to exist in both the species. This implies that for adequate control of the two species from season to season, timing of spray application will be an important factor. The weeds should be killed before the seeds mature.

From total uptake and phase distribution studies it is evident that, differences between the two species in their total uptake is not highly significant. The slightly lower radioactivity recorded on the third day for the susceptible species could be explained as a reflection of inhibition of uptake. Lesser levels of reducing sugars reaching the roots due to inhibition of photosynthesis may be responsible for the reduced active uptake in the root system. It is also possible that the water soluble conjugates formed involve sugars and thus saturation of the available sugars could have occurred. This would lead to a reduced level of SENCOR within the plants. However it is difficult to credit that such reduction in the sugar levels could occur, since stored carbohydrates are not exhausted within this period. It is more acceptable that inhibition of photosynthesis leads to reduction in transpiration rate thus resulting in a reduction of uptake.

From the phase distribution studies it is evident that SENCOR equivalents are distributed mainly in the water soluble phase. Conjugation of SENCOR with sugars, nucleic acids or soluble proteins transforms the organosoluble SENCOR to a water soluble equivalent.

It is evident that treatment of plants with metribuzin (SENCOR) leads to transformation of proteins to soluble forms and an increase in levels of amino acids in susceptible plants (59). This provides support for the assumption that the levels of radioactivity in the non-extractable phase do not increase in the susceptible speices with time due to solubilization of the non-extractable components in the affected plants. This also accounts for the increase in levels of counts in the water soluble fractions.

From the study on rate of metabolism the indications are that the tolerant species metabolizes the toxicant to the non-active

forms at rates relatively higher than in the susceptible Solanum nigrum. However, a substantial proportion of the labelled material in the plant was present as the parent compound.

Studies on uptake, distribution and metabolism, indicate that there is a restriction to movement of SENCOR from the xylem to the mesophyll cells in the tolerant Solanum sarachoides. It is suggested that this restriction combined with partial breakdown of the herbicide may be responsible for the tolerance of Solanum sarachoides to SENCOR.

Herbicides are known to decrease the transpiration rate. It has been suggested that inhibition of photosynthesis by the herbicide brings about stomatal closure through production of excess CO_2, but this is somewhat controversial (74,160,179). From the scheme proposed and discussed for the mode of action of Hill reaction inhibitors (Fig.7) in this study, it is also possible, that production of ABA may lead to closure of stomata and thus result in reduction in transpiration rate. However, whatever the mechanism of inhibition of transpiration, it seems reasonable to infer that the herbicide must penetrate the mesophyll and reach the neighbourhood of the stomata for any effect to occur.

Accordingly in this study the effect of SENCOR on transpiration by both the susceptible Solanum nigrum and the tolerant Solanum sarachoides were examined.

Tables XI, XII and Figure 18 provide evidence that the transpiration rate is inhibited to a greater extent in the susceptible species. Also Plate 2 provides evidence for the effect of SENCOR on the stomata. Thus these results and observations on the effect of SENCOR on transpiration and on the stomata support the evidence from other experiments in this study that, there is a restriction to the movement of herbicide from the xylem into the mesophyll in the tolerant Solanum sarachoides.

CONCLUSION

From studies on isolated chloroplasts, it has been established that SENCOR is a Hill reaction inhibitor. The toxophore is the thiosulphate group.

From the results and observations in Part II study it is evident that variation in the response of Solanum nigrum and Solanum sarachoides to SENCOR as observed in the field, is in fact due to species selectivity.

SENCOR appears to be metabolized to a greater extent in the tolerant Solanum sarachoides. However a substantial proportion of the labelled material in the plant was present as the parent compound. Autoradiographic studies on distribution of SENCOR in the tolerant Solanum nigrum indicates that there is a restriction to movement of the herbicide from the xylem to the mesophyll. This was confirmed by the studies on the inhibition of rate of transpiration and from effects on stomata. It is suggested that this restriction combined with partial breakdown of the herbicide in the plant may be responsible for the tolerance of Solanum sarachoides to SENCOR.

SUGGESTIONS FOR FURTHER WORK

In addition to studying systems in isolation, studies on effect of SENCOR on the photosynthetic activity of intact plants is necessary. As selectivity can only be studied adequately with statistical design, it is necessary to obtain information on the performance of SENCOR under field conditions. In addition rapid turnover of chlorophyll molecules in the resistant plant may provide a basis for resistance to SENCOR. Further work with intact plants may be necessary for further elucidation of such mechanism.

Further studies on rate of metabolism of SENCOR and on possible subsequent recovery of the plants is highlighted. Intact plants, leaf discs and leaf homogenates should be used to determine if one

plant degrades SENCOR at rates higher than others. It is also necessary to identify the relative amounts of DK and DADK metabolites at several sampling periods since it is possible that the tolerant species may first remove the toxophore (the $-SCH_3$ group) before deaminating the SENCOR molecules in its metabolic sequence.

The identity of the conjugates and the ease with which they are reversible needs to be established. Enzymic breakdown or mild release of the components requires further work to establish or confirm the components as sugars, proteins and lipids, etc.

The relative rate of translocation of radioactive SENCOR to the mesophyll tissue may be examined by removing leaf discs between the veins at suitable sampling periods. The rate of arrival of radioactive SENCOR equivalents at the site of action may then be measured by employing either the digestion/solubilization or cobustion techniques.

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